Recycling of Agro-industrial Wastes by Vermiculture

by

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Abstract

Earthworms *Eisenia foetida* and *Pheretima asciatica* could live on a wide spectrum of agro-industrial wastes such as coconut waste, malt waste, soybean waste, spent mushroom compost, pig manure, cattle manure and digested sludge. These waste materials varied widely with their chemical properties in terms of nutrient and heavy metal contents. When fed with the various organic wastes, *E. foetida* and *P. asciatica* gained the greatest weight from soybean waste, pig manure and digested sludge, and had protein contents ranging from 51 to 67% for *E. foetida* and 55 to 71% for *P. asciatica*.

In a 60-day culture trial, *E. foetida* and *P. asciatica* obtained the highest growth rate of 945% and 2900% respectively from soybean waste, which were significantly higher ($P<0.05$) than those of pig manure and digested sludge. The reproductive rates for *P. asciatica* ranged from 3720% to 4670%, and were significantly higher ($P<0.05$) than those of *E. foetida* which were about 1900%. The feed conversion efficiency of *P. asciatica* was also higher than that of *E. foetida* from the corresponding waste substrate. *P. asciatica* was superior to *E. foetida* for vermiculture in terms of growth rate, reproduction potential and feed conversion efficiency.

E. foetida consisted of 65.4-67.7% crude protein and 6.25-7.10% crude fat, while *P. asciatica* had 66.2-68.3% crude protein and 5.58-8.95% crude fat. Both earthworms contained high proportions of amino acids in their tissues which were comparable to that of fish meal, except that lysine and methionine

were lower in some worm meals. Of the worm meals from the three organic wastes, those from pig manure were the most contaminated, especially with Cu, which were up to 131 μg/g and 152 μg/g for *E. foetida* and *P. asciatica* respectively. Worm meals from soybean waste contained little metals and could be taken up in larger amount without any possible health risk.

In the feeding trial using worm meal as the sole protein source for fish, growth rates ranged from 728 to 1180% which were significantly lower than that of the control (2190%). The composition of fish tissues varied slightly when fed with various dietary protein, with protein ranged from 72.1 to 79.8%, and crude fat content from 12.0 to 18.0%. Heavy metals of Cd, Cu, Pb and Zn were found deposited in the fish carcasses in most of the treatments. Fish from diets containing worm meals generally contained higher contents of heavy metals, and Zn was the only metal found to retard fish growth.

In the greenhouse pot experiment, potting mixes containing spent beddings from various sources resulted in lower shoot yields when compared with the control of unused bedding. Fertilized treatments gave higher yields than those without additional fertilizer. Potting mixes containing spent beddings from pig manure contained elevated concentrations of Cu, Zn and other soluble salts and gave the lowest plant yields. Heavy metals were not accumulated in plant tissues to elevated levels, especially those in aerial part, except those of Zn from most treatments and Cu from soil mixes with spent beddings from pig manure.

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Chapter 1. INTRODUCTION

1.1 Waste Management in Hong Kong

Environmental pollution has become an increasing problem in the past decades both globally and locally. Many countries implemented a series of management planning and policy, trying to protect the environment and reduce pollution. However, great expense is usually required for the effective pollution control in order to achieve higher environmental quality. Environmental protection has always been neglected in developing countries, in which investment focused very much on economic development, and this in turn resulted in more serious environmental problems.

Hong Kong suffers from all kinds of pollution, and solid waste is one of the sources of pollutants. With a population of close to six million, Hong Kong generates huge quantities of solid wastes from municipal and agricultural activities, which are mainly disposed of at landfills or burnt at incinerators (Table 1.1). In 1992, an average daily total of 7,930 tonnes of municipal (domestic, industrial and commercial) solid wastes were collected, and over 80% were disposed of by landfilling (EPD, 1993). About 2,000 tonnes of livestock waste were generated daily (PELB, 1989), and only a small amount was collected and composted (EPD, 1993); others were either recycled as fertilizer for cropland and fish ponds or discharged into the nearest water

Table 1.1 Waste types and their potential management methods in Hong Kong (PELB, 1989).

Waste type	Management method
Household	pulverisation/incineration/landfill
Marine collected	landfill
Commercial	landfill
Industrial	landfill
Construction	landfill
Dredged & excavated materials	marine dumping/public dump
"Difficult"	
Sewage sludge	landfill/screening plants
Water works sludge	discharge to water course/submarine outfall
Sewage works screenings	landfill
Excremental waste	landfill/sewage works/screening plants
Livestock waste	composting/recycling/discharge to water course
Animal carcasses	cremation/landfill/reprocessing
Abattoir waste	landfill/reprocessing
Condemned food	landfill/reprocessing
Chemical	discharge to drains/landfill
Clinical	landfill/incineration
Pulverised fuel ash	lagooning/recycling
Furnace bottom ash	recycling
Secondary	
Incinerator ash	landfill
Pulverised waste	landfill

course. Moreover, 45,000 tonnes/year of sewage sludge from the eight sewage treatment plants were disposed of by landfilling and ocean dumping (EPD, 1993).

In Hong Kong, incineration is being phased out owing to air pollution problems. Landfilling is the major and will be the sole waste disposal method in Hong Kong. Not only the non-processed wastes are directly dumped into landfill sites, some processed wastes such as sludge from sewage works and ash residue from incinerators are finally deposited in landfills. There are now 13 landfills in the territory, occupying a total land area of approximately 280 hectares (EPD, 1993). With increasing waste arisings, most of the old landfills have reached site capacity, and the government is facing the problems of looking for new landfill sites and restoring completed landfills. Three big strategic landfills located in the New Territories have been designated to accommodate all the municipal solid wastes generated in the next two to three decades. Landfill gas (mostly methane) and leachate which are both potentially hazardous are the main problems arised from landfills. Although Hong Kong government has worked out some control strategies to deal with these problems, including landfill gas extraction and leachate treatment (EPD, 1993), landfilling is still not an environmental friendly option to deal with the ever-increasing generation of solid wastes.

To minimize the quantity of wastes for disposal, waste reduction, reuse and recycling are actively taking place for some materials, most of which are export-oriented. Over one million tonnes of the wastes of wood, paper, glass, plastics, ferrous metals and steel, non-ferrous metals and textile fibre are exported every year, and the total volume of these wastes has been increasing in these years. The value of these materials has been over two million Hong Kong dollars since 1988 (PELB, 1989). Some other recycling activities were carried out only to a limited extent, such as pulverised fuel ash as building material or livestock wastes for application to both cropland and fish ponds. Most of the biodegradable waste materials which are organic in nature are recycled in a minimal scale, probably due to the limitation of their usage, availability of manufactured commodities from virgin materials and the cost for the conversion of wastes into useful products. However, recycling of organic wastes has become more and more attractive in conserving our finite resources and reducing environmental pollution.

1.2 Sources of Organic Wastes and Their Characteristics

In Hong Kong, municipal wastes including refuse and sludge, and agricultural wastes such as animal manure are the main categories of organic wastes which are biological in origin. Unsorted refuse, though a massive untapped resource, is difficult and expensive to recycle because of its heterogeneity and contamination. For successful recycling, separation at source or intensive post-collection segregation by physical means is a prerequisite, and the sorted refuse can then undergo composting or anaerobic digestion. However, low cost-effectiveness is the usual barrier and recycling is only limited to materials such as paper and metals which have a market for the recycled products.

1.2.1 Animal manure

Animal wastes have been blamed as the major source of pollutants which deteriorate our water quality since 1970s (Binnie and Partners, 1974). They were usually discharged directly into rivers without treatment, though a small fraction is recycled as fertilizers in agricultural land and fish ponds.

Although there is a change in the land use pattern in Hong Kong and animal farms have been forced to either stop operation or move to mainland China because of the tighter pollution control, animal manure from existing local farms is still the cause of river pollution. Animal manure is usually high in BOD and nutrient contents and its discharge to water bodies often results in oxygen depletion and eutrophication.

Nutrient contents of animal manure are usually affected by the animal feed composition. Of the total input feed nutrients, there are about 72-79% of N, 61-87% of P, and 80-82% of K remained in their wastes (Edwards, 1980). Of the most commonly raised animals, poultry manure has the highest concentration of N, P and K followed by that of pig and cattle (Table 1.2). However, most of the protein in manure is generally of a non-digestible nature; for instance, of the 25% crude protein in chicken manure, only 10% is digestible and available for assimilation (Little and Muir, 1987). The utilization

Table 1.2(a) Composition of livestock wastes (in dry weight basis) (Little and Muir, 1987).

	Pork pig	Laying hens	Feedlot beef	Sheep	Cattle
COD:BOD	3.3	4.3	5.7	12.8	7.2
N (%)	5.6	5.9	7.8	4.0	4.0
P (%)	1.1	2.0	0.5	0.6	0.5
K (%)	1.2	1.7	1.5	2.4	1.4

Table 1.2 (b) Mineral composition of manure (in dry weight basis) (Martin *et al.*, 1983a).

	Poultry	Broiler	Dairy cow	Beef cattle
Al (%)	0.07-0.20	0.03-0.09	-	0.17-1.56
Ca (%)	5.50-12.3	1.60-6.07	2.3-4.9	0.87-3.02
Fe (%)	0.15-1.22	0.05-0.08	-	0.16-0.65
Mg (%)	0.20-0.77	0.26-0.54	0.42-1.03	0.38-0.63
K (%)	1.72-3.30	1.54-1.88	0.81-1.75	1.10-3.00
Na (%)	0.26-0.96	0.21-0.54	-	0.26-0.91
Cd (ppm)	0.58-1.30	0.42-1.3	-	0.24-1.3
Cr (ppm)	-	-	-	20-31
Cu (ppm)	18-179	31-127	-	19.5-40
Pb (ppm)	3.45-5.80	2.1-2.5	-	2.1-12.7
Mn (ppm)	233-468	166-321	-	111-222
Zn (ppm)	147-713	133-273	-	79.2-150

of animal manure depends very much on its characteristics, although the nutrient composition of livestock waste will be more predictable when methods of intensive animal rearing become standardized (Tunney, 1980).

1.2.2 Sewage sludge

Sludge, which is the settled residue from the treatment of sewage, varies greatly in chemical, biological and physical properties. Their characteristics depend on the sources of sewage, treatment system, and the management of treatment system. It is of interest that domestic sewage fluctuates in chemical composition with time of the day, while those from industries change with season of the year (Sabey, 1980).

Table 1.3 lists the typical composition of sewage sludge (Dowdy *et al.*, 1976). Generally, sewage sludge is rich in organic matter and possesses relatively high concentrations of total metals. Sewage sludge contains most of the nutrients required by animals and plants, and they are the beneficial factors for the economic value of sewage sludge. On the contrary, toxic substances, e.g. heavy metals and organics, limit the exploitation of sludge. Therefore, the degree of contamination of sludge is of primary concern with recycling of sludge for agricultural purpose to prevent the contaminants from entering the food chains.

1.2.3 Food waste

Table 1.3 Elemental composition of sewage sludge (in dry weight basis) (Dowdy *et al.*, 1976).

Component	Minimum	Maximum	Median
		Concentration (%)	
Organic C	6.5	48.0	30.4
Inorganic C	0.3	43.0	1.4
Total N	<0.1	17.6	3.3
NH_4-N	<0.1	6.7	1.0
NO_3-N	<0.1	0.5	<0.1
Total P	<0.1	14.3	2.3
Inorganic P	<0.1	2.4	1.6
Total S	0.6	1.5	1.1
Ca	0.1	25.0	3.9
Na	0.01	3.1	0.2
K	0.02	2.6	0.3
Mg	0.03	2.0	0.4
		Concentration (ppm)	
B	4	757	33
Cd	3	3,410	16
Cr	10	99,000	890
Cu	84	10,400	850
Mn	18	7,100	260
Ni	2	3,515	82
Pb	13	19,730	500
Zn	101	27,800	1,740

Data compiled from over 200 samples from eight states in U.S.

Food production, food processing and other agro-industrial activities produce vast quantities of by-products which are organic in nature. The most common wastes are slaughterhouse wastes, meat and fish processing wastes, wastes from brewing industry, residues from fruit canneries and packaged drink industries, and plant residues from crop production. Generally, these wastes are more predictable in volume and relatively uniform in composition (Wilson and Lemieux, 1980). Regarding the composition of over 100 waste products studied, meat process residues and plant seeds are usually protein rich, and wastes from processed sugarcane and plant hulls are high in fibre contents (Table 1.4).

1.3 Organic Waste Recycling

Residues from farms, food processing industries and sewage treatment plants are usually organic and biodegradable; therefore, they can to be converted by physical, chemical and biological processes into fuel, animal feed, organic fertilizer and other beneficial products (Polprasert, 1989). If such residues, rather than being regarded as wastes, are seen as valuable material sources, it will be possible both to reduce undesirable environmental impacts and conserve global finite resources.

1.3.1 Organic wastes for land application

Cropland in continuous cultivation is usually deficient in organic matter. The cultivation process speeds up the decay of soil organic matter, and soils

Table 1.4 Chemical composition (%) of food processing wastes (Wilson and Lemieux, 1980).

	Fish (meal residue)	Grain (brewers' dried grains)	Oysters (meat residue)	Peanut (meal and hulls)	Soybean (hulls)	Sugarcane (bagasse)
Crude protein	73.2	28.3	52.6	46.9	12.4	1.7
Crude fibre	-	16.1	9.9	14.0	36.1	48.6
Ca	-	0.30	0.51	0.11	0.59	-
P	-	0.53	0.83	0.65	0.17	-
Ash	24.0	4.2	-	5.6	4.2	3.1

which lack organic matter are likely to suffer from poor physical structure. Organic wastes supply available nutrients in a slow-release manner such that excessive loss through leaching can be reduced. The addition of organic wastes to soil also replenish soil organic matter which is depleted by plant growth and microbial activities.

1.3.1.1 Animal manure

Traditionally, organic wastes such as livestock waste are amended to soil to improve soil fertility and crop yield. Some soils may show deficiencies of certain nutrients, and application of these elements are necessary for healthy plant growth. For example, peat soils are often Cu-deficient, and B must be added to many soils for healthy sugar beet production (Tunney, 1980). Optimal application of agricultural wastes which contain these trace elements can correct their deficiencies in these soils. Although the application of agricultural wastes as fertilizer has long been recognized, there is no evidence existing in scientific literature to support the view that plant growth can be significantly improved by animal wastes. The feasibility, in terms of economic value, of replacing chemical fertilizers with animal wastes remains unknown (Nakamoto *et al.*, 1993). Chemical fertilizers are easier to handle than animal wastes. Meanwhile, chemical fertilizers are inexpensive, and the application of chemical fertilizers rather than animal wastes has increased since formulated fertilizers are readily available in market (Tunney, 1980).

Compared with chemical fertilizer, animal wastes usually fluctuate in their composition, and cannot supply correct proportion of NPK for many crops. For instance, the wastes from pig and poultry usually contain high concentrations of P (Table 2.1), and Cu additive in pig feed results in high Cu content in pig manure. Plants can gain benefits from animal wastes only when the optimal quantities are applied. Traditionally, the normal rate of application was between 25 and 50 t/ha/annum in a dry weight basis (Tunney, 1980). Excessive animal waste application can inhibit plant growth, contaminate surface and underground water and lead to various environmental problems (Edwards *et al.*, 1993).

1.3.1.2 Sewage sludge

The value of sewage sludge amended to agricultural land as fertilizer or soil conditioner is well documented (Cavallaro *et al.*, 1993; Hall and Cokes, 1983; Sabey, 1980; Vigerust, 1983). The benefits contributed by sewage sludge are the improvement of soil physical and chemical properties.

Addition of sewage sludge in soil increased porosity, reduced bulk density and improved plant yields (Hall and Cokes, 1983). As sewage sludge contains considerable amount of NPK and other nutrients, it is undoubtedly that sludge can increase available macro- and micro-nutrients in soil (Dannberg *et al.*, 1983; Sabey, 1980), but they are not in the optimal proportion for all plants. Thus, nitrogen concentration in sludge usually determines the rates of application, because N is the most important element for plant growth. P and K may be other two factors governing the rate of application of sludge to land once the patterns of nitrogen released are determined (Sabey, 1980). Sometimes additional fertilizers are required to obtain a balanced nutrient composition for maximum yields.

Despite the improvement of physical and chemical properties contributed by sludge, the application of sludge to agricultural land is restricted because of actual potential hazards due to the presence of heavy metals, organics and pathogens (Danneberg *et al.*, 1983). Of these side effects, heavy metal contamination to soil is the most concerned. The fate and effects of sewage sludge constituents in a soil-plant system are influenced by factors such as climate, sludge composition and soil management (irrigation, drainage, liming, fertilization and addition of amendments). In addition, soil properties including pH, organic matter content, cation exchange capacity, texture, aeration and water availability affect the reactions and the resultant uptake of sewage sludge constituents by plants (Sommers *et al.*, 1983).

Many sewage sludges contain elevated contents of heavy metals, e.g. Cd, Cr, Cu, Ni, Pb and Zn, which are potentially toxic to plants, animals, or humans. It is of interest that some metals (e.g. Cu and Zn) which can be accumulated by plants cause phytotoxicity before the concentrations in plants become toxic to humans (Sabey, 1980). Cadmium, though not very phytotoxic, is highly toxic to animals (Page *et al.*, 1981). This makes Cd a metal of great concern in considering the loading rates of sludge to agricultural land to prevent the transfer from plants to animals. Other metals, though of some concern, are considered less of a problem than Cd. For instance, Cr is usually in the Cr(III) form, which is not available to plants; Pb in phosphate form is generally rendered unavailable to plants (Sabey, 1980). However, it is very important to apply sludge in optimal rates which are both satisfactory for plant growth and environmental quality.

1.3.1.3 Plant residue

Plant residue has been disposed of via soil application, which increases the content of organic matter and inorganic nutrients in the soil. When compared with animal wastes, the risk of contamination of cropland by heavy metals from plant residues is of less concern. However, the direct application of plant residues to soil as fertilizer is not common, because application of plant residues to soil usually cause high C/N ratio, and thus additional N, about 30 kg/ha, is recommended to be supplied to the soil for proper plant growth (Tunney, 1980).

Occasionally post-harvested residues are returned to cropland directly, which supply considerable quantities of essential nutrients after mineralization by soil micro-organisms. Dry plant residues are sometimes burned or mechanically chopped into small pieces prior to soil application. In comparing the economic value of plant residues, most have higher values as feed than as fertilizer (Wilson and Lemieux, 1980). Exceptions are those which contain low protein or assimilable energy content which are preferred to be recycled as fuel, such as sugarcane bagasse, rice hulls and other fibrous wastes.

1.3.2 Composting

Composting of organic wastes is usually accomplished by placing the wastes in open heaps or closed reactors in which the wastes decomposed under controlled conditions of ventilation, moisture and temperature. Many biodegradable organic wastes are amenable to composting which converts the wastes into composts, the products for safe emission to environment (CS/LU Staff Report, 1979; Vallini *et al.*, 1984).

Mature composts after stabilization are in general superior to raw materials before composting for plant growth (Hirai *et al.*, 1986). Composting prior to land application has the benefits of volume reduction of wastes, ease of storage and handling, freedom from pathogens, stabilization of material, elimination of objectionable odors and pollution reduction.

Compost should be looked upon as soil conditioner rather than fertilizer. Compared to readily available commercial fertilizers, the NPK values of composts, even those derived from sludge and manure, are relatively low (Hileman, 1982). Composts contain very high humus content, which will not only improve the structure of the soil but will also hold the water and nutrients for plant uptake (Hughes, 1980).

However, the most important contribution of compost is its long term effect on soil status, especially on accumulated fertility. Soil humus content exerts a balancing effect by retaining excess nutrients and keeping them available to plant roots, while the incorporation of fresh wastes or green manure may disturb the soil balance until the raw waste has been decomposed and digested by soil micro-organisms (Steffen, 1979).

High concentrations of heavy metals and soluble salts in composts are the two major limitations to their application. The uptake of heavy metals and their phytotoxicity depend very much on the compost properties, especially the pH values and metal contents. Maintaining adequate levels of organic matter and high pH of soils are the normal cultural practices recommended to minimize metal uptake for optimal plant growth (Gouin, 1982). High soluble salt content is generally encountered in compost (Adams, 1993; Pessarakli and Tucker, 1985; Shiralipour *et al.*, 1993) Shanks and Gouin (1984) suggested leaching by spraying water prior to cultivation can prevent the plants from salt damage.

1.3.3 Biogas production

Anaerobic digestion has been widely used to treat sewage sludge and animal wastes because it can accomplish a high degree of stabilization of organic wastes and is more energy efficient than alternative aerobic processes (Hawkes and Hawkes, 1987). The anaerobic treatment of organic residues by bacteria produces a combustible gas (methane) which is usually utilized as an alternative fuel. This waste-to-energy option is commonly carried out in the rural areas of developing countries to improve living standards and increase the efficiency of resource utilization (El-Halwagi, 1984). Biogas production not only supplies an energy source for lighting, cooking and other energy-consumed activities but the digested waste provides an upgraded fertilizer for agricultural land, and therefore, contributes very much toward solving the problems of waste disposal. Other advantages of anaerobic digestion include lower microbial growth yields, which therefore reduces sludge disposal problem, and lowers nutrient requirements (Eckanfelder *et al.*, 1985; Vochten *et al.*, 1988).

Animal manure, sewage sludge and municipal refuse are the common substrates for biogas production (Badawi *et al.*, 1992; El-Halwagi, 1984; Kang and Weiland, 1993; Singh and Anand, 1994; Xavier and Nand, 1990). Plant residues are sometimes used for biogas production to adjust the C/N ratio (Trujillo *et al.*, 1993). However, plant materials for biogas production may decrease gas yield and produce excessive scum in the digester (Polprasert *et al.*, 1986).

Factors, such as gas yield, operation cost, disposal of digested slurry and the collection, storage and transportation of the gas product, should be considered for the production of biogas. However, it is still a good method for energy recovery, especially in rural area where energy shortage is a common problem.

1.3.4 Organic wastes as feedstuffs or feed supplements

Organic wastes can be recycled as animal feed by completely or partially replacing conventional feed or supplementing to a diet as protein or energy source. The wastes can be grouped into three categories of feedstuffs according to their protein, lipid and fibre contents: (a) protein feeds, (b) energy feeds, (c) and forages (Martin *et al.*, 1983a). Utilization of wastes as animal feedstuffs depends primarily on their contents of available nutrients, assimilable energy and dietary fibre, especially in relation to alternative conventional feedstuffs and their cost (Owen, 1980). Other factors such as palatability, toxicity, pathogenicity and anti-nutritional factors should also be considered.

1.3.4.1 Animal manure

Animal wastes contain considerable nutrient contents which can be utilized as animal feedstuffs. Most of the animal wastes, such as broiler litter, beef cattle manure and dairy cattle manure, are regarded as forage due to their high-fibre contents (Martin *et al.*, 1983a). Therefore, ruminants are the most desirable species for feeding animal wastes because of the rumen microflora which can utilize nonprotein nitrogen for amino acid synthesis (Martin, 1980; Martin *et al.*, 1983a). Animal manure is not commonly used as direct feed for animals due to their low palatability and pathogenicity (Martin *et al.*, 1983b); even very low replacement ratio, decreased feed efficiency and growth rate (Martin *et al.*, 1983b and c). Prior to feeding, animal manure is usually dehydrated and fermented to improve the texture and nutrient contents and reduce odor problems (Biely and Stapleton, 1976; Jakhmola *et al.*, 1983; Martin and Loehr, 1983). However, their incorporation into diets at levels higher than 10-20% usually caused growth depression (Biely and Stapleton, 1976; Martin *et al.*, 1983b and c; Sehgal and Makkar, 1994), while low ratios of inclusion in the diets did not adversely affect animal growth performance in some cases (Biely and Stapleton, 1976; Martin, 1980).

1.3.4.2 Sewage sludge

Research on using sewage sludge as a supplementary feed has been conducted so as to find an outlet to reduce disposal pressure. The value of sludge as a protein source has been the main focus. On the other hand, problems of heavy metals and toxic organic compounds are barriers to the application of sludge, especially when they possess the opportunity to enter the food chains (Chishti *et al.*, 1992; Lau, 1981; Smith, 1981). Therefore, utilization of sludge as direct feed is rare in practice. The feasibility of protein extraction from activated sludge has been demonstrated by using physico-chemical methods (Lau, 1981; Stafford *et al.*, 1979).

Results of culturing tilapia (*Oreochromis mossambicus*) in sewage sludge as supplementary diets showed that the higher the portions of sludge incorporated into the diets, the higher the concentrations of trace metals in the tested fish tissues and the lower the growth rates (Wong and Chiu, 1993). In another experiment, sewage sludge supplemented over 5% in the diet depressed chick growth (Wong and Leung, 1979). Generally, sewage sludge as direct feed for fish and livestock is not appreciated because of their potential hazards and poor growth performance in high rate inclusion. Similar to animal manure, pretreatment by dehydration or anaerobic digestion may help to increase the value of sewage sludge as animal feed.

1.3.4.3 Plant residue

Compared with animal wastes and sludge, plant residues are regarded as better feedstuffs because of their innocuous nature and better palatability in most of the cases. Some wastes, such as soybean meal and plant seeds, contain considerable concentrations of protein which are potential protein sources for animals.

Owing to the diversity of the sources of plant residue, it is difficult to make specific recommendations for all types of livestock operations using wastes as feedstuff (Wilson and Lemieux, 1980). Most of these wastes are fed to animals directly in 100% inclusion in feedstuffs. Wastes as direct feed are less expensive than the processed ones. However, it cannot provide balanced nutrients for animals and it reduces waste utilization efficiencies. An exception is in swine feeding operations which are ideal for utilizing these edible wastes because the nutritional requirements of swine are very similar to those of humans. Approximately 1 million hogs, or about one percent of the nation's production are raised on waste-feeding operations in the United States and Puerto Rico annually (Price *et al.*, 1985). In these practice operations, food waste is usually supplemented with bakery wastes and small amount of grain, although it may not be necessary.

Nowadays, intensive crop production and food industries are performed by large farms and centralized companies. Vast quantities of by-products are produced and separated according to their sources, which make the wastes relatively uniform in characteristics. Therefore, it is possible to formulate the feedstuff containing balanced and completed nutrients by integrating the wastes with other conventional nutrients in optimal ratios. The preparation of balanced and completed diets for aquaculture from a variety of unconventional sources has proven potential on a large centralized scale (Little and Muir, 1987). However, successful examples are rare in experimental feeding trials; fishes seem to prefer fish meal rather than other common protein sources. Replacement of fish meal by less expensive food wastes has generally resulted in reduced growth and poor feed efficiency (Davies *et al.*, 1990; El-Sayed, 1990; Ng and Wee; 1989; Shiau *et al.*, 1989).

1.3.4.4 Biological products from waste conversion

Most wastes, in particular municipal wastes, are not readily be considered as valuable feedstuff due to their low nutrient contents, poor palatability and non-consistent quality. Some organic wastes can be upgraded to better quality by physical and bio-chemical methods. It is of much more interest if a completely different product with concentrated protein can be produced from the wastes (Kirsop and Hilton, 1981).

Single cell protein

Single cell protein is used for either animal and human consumption, and this imposes particular limitations on the production processes. Firstly, the substrates from wastes must be sterilized to prevent the products from contamination by pathogenic micro-organisms. Secondly, the whole production process must be under strict controls without the introduction of any other organisms. Finally, the products must be acceptable to regulatory authorities as food or a component of diet for animals and humans (Kirsop and Hilton, 1981).

A wide ranges of wastes have been used as the feedstocks in single cell protein production. The production of single cell protein from hydrocarbon wastes has been studied extensively in laboratory and pilot scale, but interest fades out due to the unguaranteed absence of carcinogens escalating price of substrates and increasing demand for other competing uses (Hamdan and Senez, 1992). Some carbohydrate-rich wastes, such as sugarcane bagasse and paper solid wastes, are innocuous and are more suitable for single cell protein production (Echevarria *et al.*, 1991; Samman *et al.*, 1993; Sandhya *et al.*, 1990). Wastes, such as starch wastes, which contain high molecular weight polysaccharides are more difficult to handle than those containing simple sugars, and require a more sophisticated approach, since it is necessary to break them down to assimilable monosaccharides and disaccharides before they can be metabolized (Gray and Berry, 1980).

A great variety of micro-organisms have been studied as possible organisms for biomass production. These include bacteria, fungi and algae. Among them fungi (including yeasts) are the most widely used, such as *Aspergillus niger*, *Candida krusei*, *Candida utilis*, *Saccharomyces chevalierie*, *Saccharomyces rouxii*, *Saccharomycopsis lipolytica* and *Torula utilis* (Echevarria *et al.*, 1991; Gharsallah, 1993; Martin *et al.*, 1993; Samman *et al.*, 1993). It is no doubt that through single cell protein production, the wastes are upgraded to contain higher protein contents and more balanced amino acid profiles (Martin *et al.*, 1993; Ofuya and Nwajiuba, 1990).

The growth response of different animals to single cell protein as feed is conflicting. Results depend on the experimental animal, the species of micro-organism used and the inclusion ratio which is the most important. Promising result was obtained in *C. utilis* as supplementary diet for rainbow trout (Martin *et al.*, 1993). On the contrary, in a feeding trial of 4, 8 and 16% replacement of fish meal by two bacterial strains *Brevibacterium lactofermentum* (PL) and *Bacterium glutamaticum* (PR) for rainbow trout, the single cell protein produced did not significantly influence specific growth rate, feed efficiency, carcass yield (Kiessling and Askbrandt, 1993); however, higher dietary levels of PL and PR caused a marked reduction in growth rate and feed efficiency. Generally, single cell protein could replace small amounts of fish meal in aquaculture, but in high inclusion rates, fish growth was retarded. Nevertheless, single cell protein may not be a good protein source for primates because of the high nuclei acid content which can result in urate precipitation in tissues and joints, formation of renal stone and hyperuricemic nephropathy (Keilin, 1959).

Invertebrates

Invertebrates have been used as animal feed. In China, wild snails are collected to feed poultry or livestock, and silkworms are harvested for aquaculture. Most of these practices are carried out by extensive individuals or family farms. Some invertebrates, such as earthworm and chironomid larvae, can utilize waste materials and convert them into protein-rich biomass (Edwards *et al.*, 1985; Shaw and Mark, 1980).

The bioconversion of wastes into invertebrate biomass provides a new dimension in waste recycling. The harvested invertebrates can be used as animal feedstuff and the residual medium can be exploited for agricultural purposes, mainly for crop production (Fig. 1.1) (Little and Muir, 1987). The invertebrates can be fed to animals both in dried or fresh forms, or integrated with other nutrients to make a complete diet. Of the invertebrates studied, earthworm which demonstrates a great ability to convert a wide spectrum of organic wastes into earthworm biomass appears to be a very good candidate (Edwards *et al.*, 1985; Loehr *et al.*, 1985b).

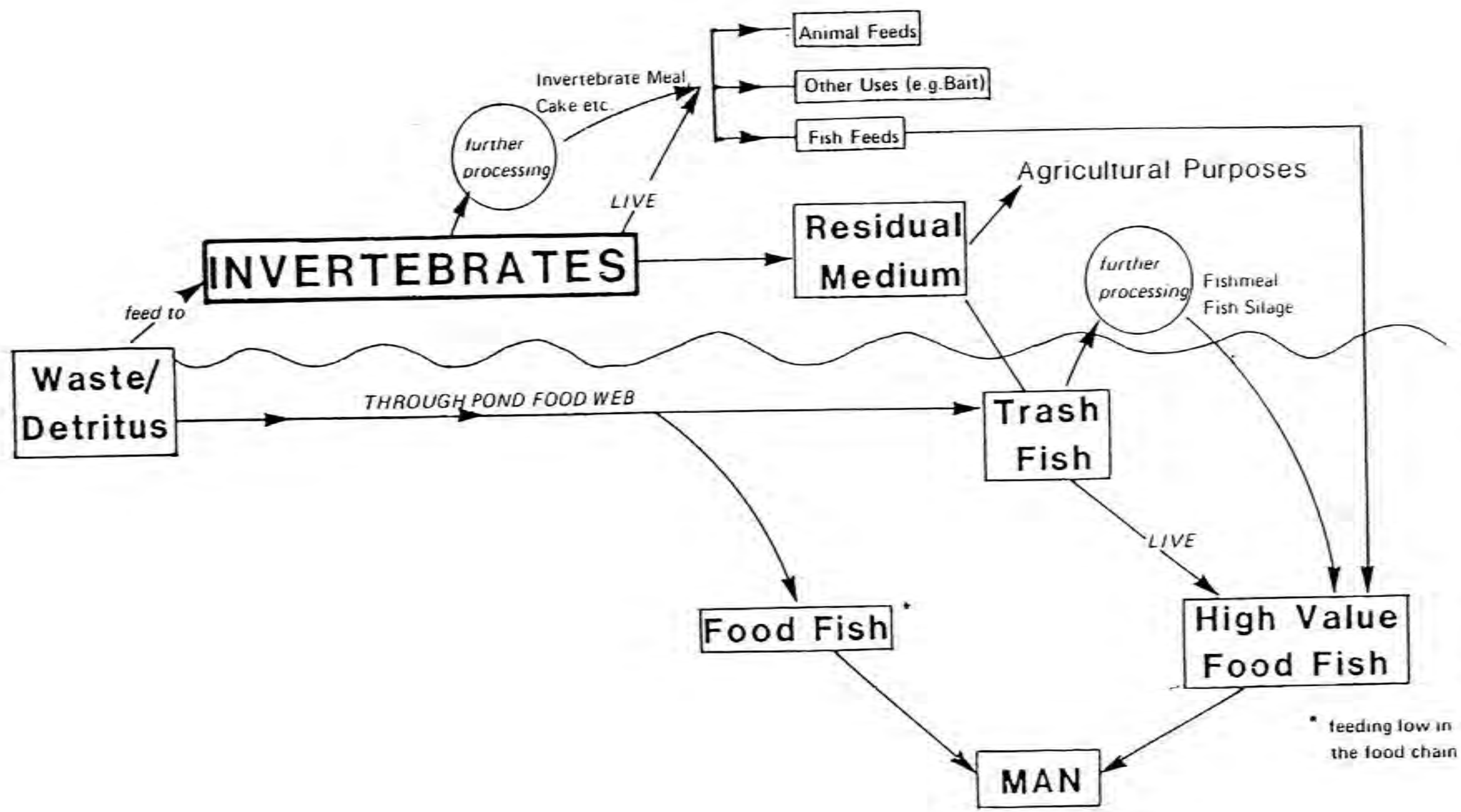


Fig. 1.1 Recycling of organic wastes by invertebrates (Little and Muir, 1987).

Earthworms are soil living organisms. They mainly live on plant litter, animal manure or other decaying matter. Their feeding and burrowing activities enhance ventilation and microbial activities (Chan and Griffiths, 1988; Wallwork, 1983), enabling their usage in stabilization organic wastes (Hartenstein *et al.*, 1984; Loehr *et al.*, 1985 a and b). These, combined with their rapid growth rate and high reproductive potential (Huang, 1982), allow the culture of earthworm on organic wastes for protein production. Earthworms which contain 54-64% protein with high proportions of amino acids can be potential protein sources for animals (Huang, 1982).

Earthworm may not be suitable for all animals in terms of palatability, and growth response is different among animals. In a rat feeding trial, worm meal of *Eisenia foetida* was comparable to casein in terms of growth response, reproduction and lactation performance (Ibanez *et al.*, 1993). However, results of fish feeding trials were controversial, which depended very much on the earthworm species and inclusion ratios (Bouguenec, 1992; Hilton, 1983; Stafford and Tacon, 1984; Tacon *et al.*, 1983).

1.4. Objectives and Outlines of the Present Study

Vermiculture is a cultural practice in which earthworms are fed with organic matter for the production of worm biomass. In order to reduce production cost and conserve material, the organic matter used is usually biological wastes of various origins. By recycling these organic wastes, there were three benefits contributed by vermiculture: firstly, reduce the environmental impacts by the recycling of organic wastes; secondly, supply a plant growth medium from the worm-worked bedding; and finally, provide a protein source for animals.

In order to study the feasibility of recycling various agro-industrial wastes through vermiculture, four experiments were conducted, each for the purpose of accessing the suitability of various wastes for vermiculture, the quality of the worm meals in terms of nutrient contents and heavy metal contents, the feasibility of using worm meals from organic wastes as protein source for fish culture and the value of worm-worked bedding as potting medium for plants.

Earthworms are known to live on a variety of organic matter, however, not much is known about their nutritional requirement. The organic wastes used in the present study came from various sources, with different chemical composition, which may influence their suitability for worm culture. With the first earthworm culture trial, properties of the organic wastes were analyzed and growth status, in terms of growth rates, of the two earthworm species was assessed to indicate their ability for biomass production. The relationship between the waste properties and earthworm growth were determined.

The second experiment was a follow up study of the first one in vermiculture. In this experiment, the two earthworm species were subjected to the three wastes which supported the greatest growth in the first culture trial.

The earthworms were evaluated for their chemical composition, growth response in terms of growth rate and reproductive rate, and their abilities to convert organic wastes into protein in terms of feed conversion efficiency and protein yield.

The earthworm tissues from the second culture trial were mixed with other nutrients to form a complete diet for aquaculture. The effects of worm meals on fish were determined by their growth response as well as the whole fish analysis of nutrients and heavy metal contents.

In the last experiment, the worm-worked beddings from vermiculture were mixed with a sandy soil to grow a Chinese vegetable (*Brassica chinensis*). It aimed to investigate the feasibility of using the spent bedding as a cultivating medium for plants. The soil mixes and plant tissues harvested were also analyzed for their chemical composition to assess the fertility of the soil mixes and their effects on plant growth.

It is hoped that through vermiculture, organic wastes can be recycled. Worm biomass thus produced can be used as a supplementary feed and spent bedding from vermiculture can be utilized as a soil medium for plant growth.

Chapter 2. VERMICULTURE OF *EISENIA FOETIDA* AND *PHERETIMA ASCIATICA* ON DIFFERENT AGRO-INDUSTRIAL WASTES

2.1 Introduction

In the nineteenth century, Darwin realized the value of earthworm in improving the fertility of soil, and described worms as "nature's ploughshare". More recently, scientists and farmers have begun to manage earthworms to increase the productivity of agricultural land (Curry and Boyle, 1987). New ways to exploit worms were developed, particularly in waste disposal.

Hartenstein *et al.* (1984) examined the trickling filters in U.S., and found that 31% of the filters studied contained *Eisenia foetida*. With their presence, a clearer effluent with less pathogens and lower BOD were obtained. Loehr *et al.* (1985a) evaluated the performance of earthworms in liquid sludge stabilization, and found that earthworm was a key component in the process. Earthworms are also used in vermicomposting of organic wastes which stabilize the wastes and at the same time convert the wastes into worm biomass. The stabilized vermicompost can be used as a fertilizer or soil conditioner for agricultural or horticultural purpose (Edwards *et al.*, 1985).

These exploitation of earthworms were all derived from the unique characteristics of this soil-living organism. Earthworms are, for the most part, saprophages. They feed mainly on organic detritus, usually decomposing plant materials and animal bodies. This suggests that earthworms can feed on anything they can find in the environment that is organic in nature (Wallwork, 1983). The detritivorous feeding mode, rapid growth rate and high reproductive potential allow the culture of earthworms in organic wastes which are otherwise an important source of environmental pollution. Earthworm culture (vermiculture) has the benefit of producing worm biomass with a high protein content, which can be a very good animal feed.

Earthworms show three distinct "lifestyles" (Knight, 1989; Wallwork, 1983). First are the large, deep-burrowing types aneciques, such as *Lumbricus terrestris*. This worm comes to the surface to feed, dragging food down into its permanent burrow. Second are the small burrowing species endoges, such as *Allolobophora chlorotica*, which eat their way through a substratum that contains organic and mineral particles in an advance state of decomposition. Finally, there are deeply pigmented earthworms such as the red worm epiges, *Lumbricus rebellus*, which feed on the surface.

Epige and endoge, which live on decaying detritus lying on the soil surface, are subject to much greater fluctuations in temperature and moisture than in the deeper soil layer. The population of these worms undergoes erratic changes in size in an opportunistic manner. Reproductive rates are high to counteract high juvenile mortality. Small individual body size, large numbers of offspring and rapid development to maturity are other characteristics commonly found in these worms

(Wallwork, 1983). On the contrary, anecique earthworms are less affected by unpredictable environment. Their development to maturity is long as individual body size tends to be large.

Attributing to their life style, worm species of epiges benefit from food rich in organic matte and can reach pest population in arable and organic rich soils. They exhibit greater potential than endoges and aneciques for worm population exploitation, since they produce many more cocoons individually (Table 2.1). Epiges are therefore suitable earthworm species which can be exploited in vermiculture. Anecique species, with deep burrowing habits and large body size, are more useful in improving soil structure rather than vermiculture.

Vermiculture can be simply conducted by introducing epige earthworms to the bio-degradable materials, such as animal manure, sewage sludge or other decomposable organic matter. There are several environmental factors, namely temperature, moisture and pH, which influence worm growth. Some kinds of bedding material is required to provide a soil-like environment, and to control pH and moisture content to the optimal levels.

Earthworms can reach the maximum growth and reproduction under suitable environmental conditions; under these optimal conditions, vermiculture systems can process organic waste rapidly and convert the wastes into soil-like material for cultivating plants and worm biomass for animal consumption (Knight, 1989). Scientists have shown that 1800 or so species of earthworms are superbly designed

Table 2.1. Number of cocoons produced in a year by various species of British lumbricids, based on the output from one individual in each case (Evans & Guild, 1948)

Earthworm species	Number of cocoons
Epige	
Lumbricus rebellus	79
Lumbricus castaneus	65
Dendrobaena subrubicunda	42
Endoge	
Allolobophora caliginosa	27
Allolobophora chlorotica	25
Anecique	
Octolasion cyaneum	13
Allolobophora nocturna	3
Allolobophora longa	8

machine for converting decaying materials into worm protein which is a potential protein source for animals.

Earthworms *Eisenia foetida*, *Dendrobaena veneta*, *D. subrubicunda*, *Lumbricus rebellus*, *Eudrilus eugeniae*, *Perionyx excavatus* and *Pheretima hawayana* have been investigated for their growth response to various agricultural wastes and their ability in breaking down organic matter (Edwards *et al.*, 1985; Loehr *et al.*, 1985b). Among all these earthworms studied, *E. foetida* appears to be a suitable earthworm to process organic wastes to high grade protein. This species growes well in a wide range of agricultural wastes including manure and slurries from pig and cattle, and wastes from laying chickens and broilers. However, not all wastes are readily acceptable to the earthworm or are equally productive in terms of biomass.

Based on the agro-industrial wastes available in Hong Kong, an earthworm culture trial was conducted to investigate the suitability of these wastes for vermiculture. The experiment aimed to evaluate the possibilities of growing earthworms in organic wastes from agro-industrial activities and to identify the type of wastes which can support the best worm growth in terms of biomass production. It is hoped that such culture system can reduce the disposal pressure of some agro-industrial wastes and produce a protein source which can be used as high grade feed for fish, poultry and livestock.

2.2 Materials and Methods

2.2.1 Collection of materials

Horse manure compost, which is a mixture of horse manure, straw and sawdust after composting, was bought from a composting plant in Ngau Tam Mei, New Territories, and was used as the bedding material for worm culture.

Nine agro-industrial wastes were used for growing worms. Food processing wastes including soybean waste, coconut waste and malt waste were obtained from beancurd factory, fruit-squeezing shop and brewing plant respectively. Pig and cattle manure were collected from a pig farm and dairy farm respectively. Spent mushroom compost was collected from a local farm which grows straw mushroom. Dewatered digested sludge was collected from the Shueng Shui sewage treatment plant which receives domestic sewage mixed with some industrial discharge.

After collection, the wastes were separated and packed into small volumes with cling wrap, weighed and stored in a cold room at 4°C.

2.2.2 Preparation of earthworms

Two earthworm species, *Eisenia foetida* and *Pheretima asciatica*, were investigated in this project. *E. foetida* was bought from the Department of Biology, South China Normal University, Guangzhou, China, and *P. asciatica* from the Department of Botany, University of Hong Kong, which originally came from the Philippines Earthworm Center. They were kept in 31 cm x 24 cm x 12 cm plastic boxes filled with bedding material and fed with vegetable slurry daily for one month before the culture trial commenced.

2.2.3 Culture trial

Polystyrene boxes of 15.5 cm in length, 10 cm in width and 6.5 cm in height were used for the culture trial (Plate 2.1). Earthworms with body length ranging from 2 to 2.5 cm were sorted by hand from the stocks, cleaned and rinsed with tap water. Ten worms were randomly selected, blotted dry and weighed before putting into each box. Wastes were added into the culture boxes, and mixed with the bedding material. The control group received no additional wastes but only bedding material. The boxes were placed in randomized blocks in an air-conditioned room with temperature maintained at 24-28°C. Each treatment was replicated three times.

The boxes were illuminated with cool fluorescent tubes to prevent the earthworms from escaping. The beddings were watered when necessary to keep the moisture content from 70 to 80% according to Edwards *et al.* (1985) and Huang (1982). The wastes were added as soon as they had been consumed by the earthworms.

2.2.4 Harvesting of earthworms

Plate 2.1 Vermiculture of *Eisenia foetida* and *Pheretima asciatica* in boxes arranged in randomized blocks in a temperature-controlled room.

The earthworms were harvested after 30 days. They were sorted out from the bedding material, washed with distilled water, blotted dry and weighed. The worms were homogenized by an electric tissue homogenizer, freeze dried and stored at 4°C before chemical analysis.

2.2.5 Chemical analysis of bedding material, wastes and worm tissues

The bedding material and wastes were air-dried for two weeks, ground and sieved (2 mm) before being analyzed for pH (pH meter, sample:distilled H_2O = 1:10 (w:v)), organic carbon (Walkley and Black, 1934), total nitrogen (semi-micro Kjeldahl digestion followed by salicylate nitroprusside method using a Lachat QuickChem AE Automated Ion Analyzer), total phosphorus (semi-micro Kjeldahl digestion followed by molydenum blue method using a Lachat QuickChem AE Automated Ion Analyzer), total mineral contents (K, Na and Mg) and total heavy metal contents (Cd, Cr, Cu, Ni, Pb and Zn) by 70% nitric acid digestion followed by determination using a Hitachi Polarized Zeeman Atomic Absorption Spectrophotometer Model Z-8100 (Allen *et al.*, 1974), crude protein (total N x 6.25), and crude fat (extraction with diethylether for 24 hours and evaporation of the solvent before weighing).

The freeze-dried earthworms were determined for their total nitrogen content and crude protein content using the same methods as described above.

2.2.6 Statistical analysis

The data were subjected to analysis of variance and significant differences between the means were calculated according to the Fisher PLSD test. The regression analysis using the F-test at 90-95% interval was performed where necessary.

2.3 Results and Discussion

2.3.1 Chemical properties of organic wastes and bedding material

Earthworm, which are saprophages, are primary consumers in the detritivorous food chain. They convert the organic material into biomass. However, little is known about the compositional requirements of organic matter in relation to worm growth. It is believed that earthworms derive most of the nutrients from the organic matter ingested, but is hard to know if they feed on those organic matter directly or live on the microorganisms growing on the decomposing matter.

Bedding material and the organic wastes studied varied widely in their chemical properties (Table 2.2). The bedding used was a carbon rich but nutrient poor compost. It is commonly used as a soil conditioner in horticulture and landscape projects. The three food processing wastes studied were all plant materials (seed or fruit) by origin, but with different nutritional composition. Pig manure and cattle manure, though both are animal excreta, differed in chemical

Table 2.2 Chemical properties of bedding material and organic wastes (means of triplicates)

	Bedding	CW	MW	SW	SMC	CM	PM	DS
pH	6.2 (0.03)	5.6 (0.03)	5.6 (0.03)	6.9 (0.00)	7.0 (0.06)	8.6 (0.09)	8.4 (0.06)	6.5 (0.00)
Organic C (%)	46.6 (1.30)	63.9 (1.73)	51.3 (0.93)	53.3 (0.56)	29.3 (0.65)	40.3 (0.18)	35.1 (0.38)	25.8 (0.59)
Total N (%)	1.56 (0.05)	1.95 (0.10)	4.51 (0.34)	9.19 (0.14)	3.76 (0.15)	1.95 (0.08)	3.90 (0.09)	8.04 (0.36)
C/N ratio	29.9	32.8	11.4	5.80	7.79	20.7	9.00	3.21
Total P (%)	0.47 (0.01)	0.14 (0.00)	0.53 (0.00)	0.38 (0.00)	0.54 (0.05)	1.04 (0.02)	3.01 (0.08)	1.40 (0.02)
Total K (%)	0.36 (0.00)	0.36 (0.01)	1.19 (0.01)	1.51 (0.04)	0.65 (0.02)	0.50 (0.01)	1.40 (0.03)	0.26 (0.01)
Total Na (%)	0.13 (0.00)	0.07 (0.00)	0.67 (0.01)	0.03 (0.00)	0.03 (0.01)	0.17 (0.00)	0.31 (0.00)	0.05 (0.00)
Total Mg (%)	0.27 (0.01)	0.12 (0.00)	0.25 (0.00)	0.28 (0.00)	0.00 (0.00)	0.00 (0.00)	0.02 (0.00)	0.05 (0.00)
Crude protein (%)	9.75 (0.29)	12.2 (0.50)	28.2 (2.14)	57.4 (0.88)	23.5 (0.95)	12.2 (0.49)	24.4 (0.54)	50.3 (2.27)
Crude fat (%)	0.53 (0.05)	29.8 (0.67)	2.27 (0.02)	11.5 (0.08)	0.60 (0.04)	0.63 (0.02)	1.70 (0.04)	2.08 (0.50)

Bedding = horse manure compost, CW = coconut waste, MW = malt waste, SW = soybean waste, SMC = spent mushroom compost, CM = cattle manure, PM = pig manure, DS = digested sludge.

Figures within brackets are standard errors.

properties which were related to the animal's nutritional mode and ration composition.

The bedding material and the various organic wastes were moderately acidic to alkaline (pH 5.6 to 8.6) and were within the pH range suitable for worm growth (Edwards *et al.*, 1985).

High organic C was found in coconut waste, malt waste and soybean waste which are of plant origin. The organic C content of animal manure, which was influenced by their feedstuffs, was generally lower than those of plant materials. Cattle manure contained 40.3% organic C which was higher than that of pig manure. This was probably due to the higher fibre content in forage for cattle. Spent mushroom compost and digested sludge contained the lowest organic C. The organic matter which was used up by mushroom growth may be attributed to the low organic C in spent mushroom compost, while that in the digested sludge was reduced as a result of anaerobic decomposition.

The highest total N content of 9.19% was found in soybean waste, which was followed by digested sludge, malt waste and pig manure. This is not suprising as soybean is a legume rich in plant protein. The bedding material, coconut waste and cattle manure had the lowest of $< 2\%$. Digested sludge contained very high concentration of total N, and thus resulted in high crude protein content. However, it was uncertain that how much total N was associated with proteinaceous material in the wastes. The animal manure and digested sludge contained the highest total P, while relatively low P was found in the plant materials such as coconut waste and soybean waste.

Soybean waste, pig manure and malt waste were rich in K. Na was high in malt waste and pig manure. Mg was high in bedding material as well as in soybean waste, malt waste and coconut waste which are plant residue in nature.

Coconut waste contained extremely high crude fat content of 29.8% which was more than twice as much as that of soybean waste, as coconut meat is a common source of plant oil. The lowest crude fat contents were found in bedding material, spent mushroom compost and cattle manure which were only 0.53%, 0.60% and 0.63% respectively.

2.3.2 Heavy metal contents in bedding material and organic wastes

Agro-industrial wastes like animal manure and sewage sludge were usually contaminated with heavy metals to various extent, whilst most of the food processing wastes were not contaminated or at least to a lesser extent.

The bedding material, coconut waste, malt waste and soybean waste were low in heavy metal contents when compared with animal wastes and digested sludge (Table 2.3). Cu and Zn were the two heavy metals found to be high in spent mushroom compost, which were 104 μg/g and 232 μg/g respectively. Cd and Ni could not be detected in the bedding material, spent mushroom compost and all the food processing wastes used.

Table 2.3 Total heavy metal contents ($\mu g/g$) of bedding material and organic wastes (means of triplicates)

	Bedding	CW	MW	SW	SMC	CM	PM	DS
Cd	ND	ND	ND	ND	ND	0.71 (0.00)	8.04 (0.34)	2.14 (0.00)
Cr	5.64 (0.17)	5.08 (0.00)	4.14 (0.08)	0.80 (0.07)	7.24 (0.12)	1.80 (0.05)	12.8 (0.90)	49.3 (1.13)
Cu	21.4 (0.52)	10.8 (0.20)	20.3 (0.25)	13.2 (0.06)	104 (2.35)	85.5 (1.37)	2060 (18.3)	235 (2.05)
Ni	ND	ND	ND	ND	ND	26.4 (0.88)	24.5 (0.54)	53.0 (0.68)
Pb	4.30 (0.40)	0.70 (0.00)	0.70 (0.00)	ND	26.1 (0.35)	8.05 (0.36)	85.7 (2.86)	137 (0.75)
Zn	109 (1.48)	19.7 (0.20)	8.25 (1.58)	38.2 (1.86)	232 (6.18)	139 (1.99)	2400 (116)	1480 (6.29)

Bedding = horse manure compost, CW = coconut waste, MW = malt waste, SW = soybean waste, SMC = spent mushroom compost , CM = cattle manure, PM = pig manure, DS = digested sludge.

Figures within brackets are standard errors.

ND means not detectable.

All the six heavy metals tested could be detected in relatively low levels in cattle manure, except that of Zn which was 139 μg/g. Pig manure was seriously contaminated with Cu and Zn which were higher than 2,000 μg/g. The former is added as an artificial supplement to pig diets to promote weight gain and increase feed conversion efficiency, while the latter is added to eliminate any toxicity caused by Cu. Besides Cu and Zn, Cd concentration in pig manure was the highest among the various organic wastes including bedding material. Digested sludge was heavily contaminated with Cr (49 μg/g), Ni (53 μg/g), Pb (137 μg/g) and Zn (1480 μg/g) which was related to the industrial contribution to the sewage entering the treatment plant.

2.3.3 Effects of organic wastes on worm growth

E. foetida and *P. asciatica* could be categorized as epiges, which feed on the organic wastes on soil surface with excellent growth. No change in worm population was obtained in all treatments of both earthworms. *E. foetida* and *P. asciatica* could live on all the organic wastes, showing better growth performance in terms of biomass increase when compared with the control, i.e. bedding material only (Fig. 2.1). This could be due to the poor nutrient contents of the horse manure compost used. With organic wastes added as food for the earthworms, all treatments, except *P. asciatica* from spent mushroom compost resulted in significantly higher ($P < 0.05$) biomass increase than the control. *E. foetida* and *P.*

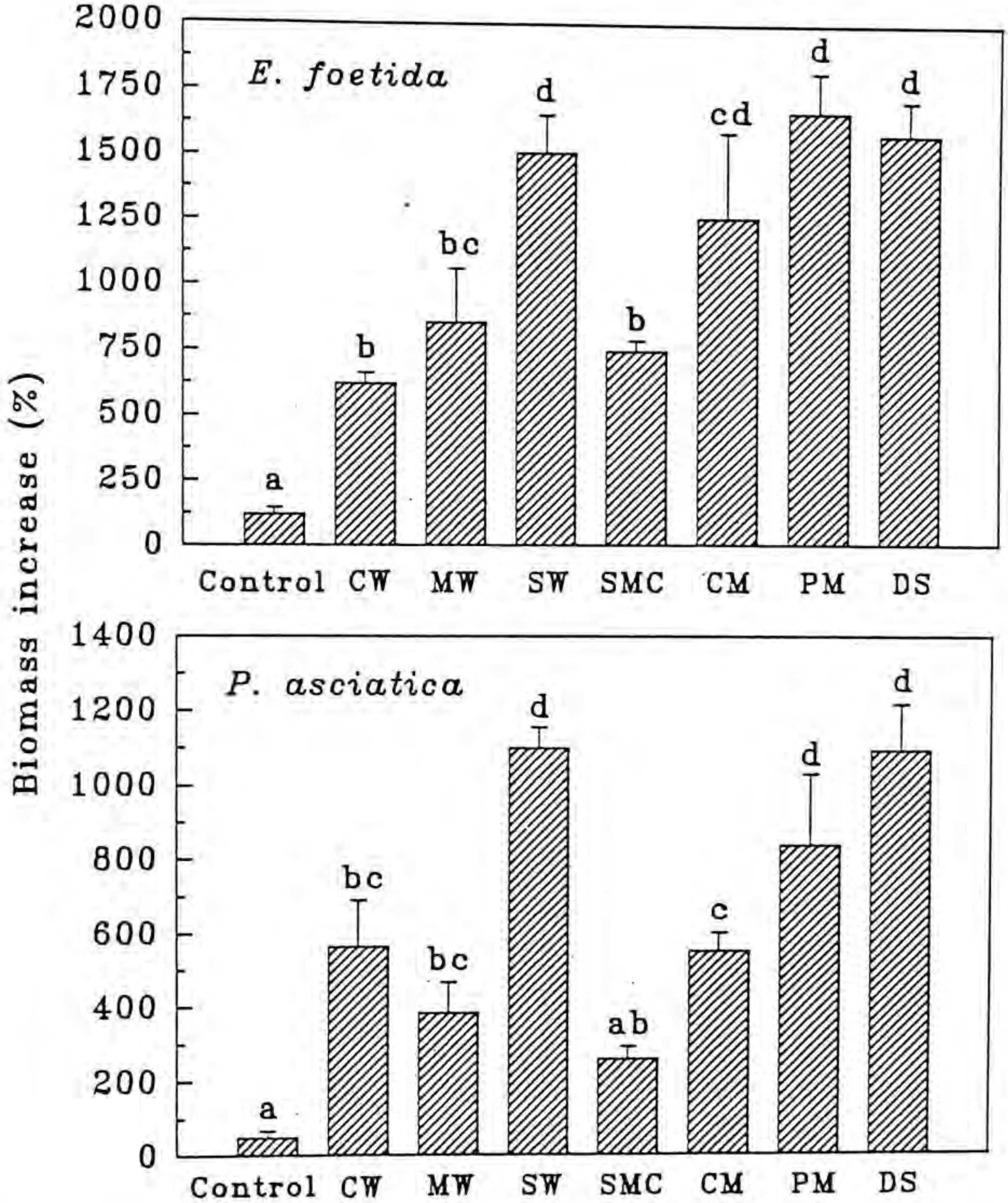


Fig. 2.1 Increase in biomass (dry wt.) in *E. foetida* and *P. asciatica* cultured in different wastes for 30 days (means of three replicates). Error lines denote standard errors. Bars having the same letter were not significantly different at 95% according to the Fisher PLSD test. CW = coconut waste, MW = malt waste, SW = soybean waste, SMC = spent mushroom compost, CM = cattle manure, PM = pig manure, DS = digested sludge.

asciatica showed very similar pattern for their ability to utilize various organic wastes. For instance, they gained the greatest growth from soybean waste, pig manure and digested sludge, and the lowest from coconut waste, malt waste and spent mushroom compost.

Soybean waste, pig manure and digested sludge supported the greatest growth in both species of earthworms. This could not be simply attributed to the nutrient contents of these three wastes, since only soybean waste was likely to supply adequate nutrients to earthworms, while pig manure was relatively low in protein content and digested sludge did not have enough organic matter and K. However they shared the same characteristic of having small particle size and low C/N ratios which were only 5.80 for soybean waste, 9.00 for pig manure and 3.21 for digested sludge. It was investigated by Neuhauser *et al.* (1980) about the influence of C/N ratios on worm growth, who suggested that materials supporting weight gain were most effective when present at low C/N ratios.

Although with low C/N ratio and quite balance nutrient contents, malt waste could not support a worm growth as good as soybean waste, pig manure and digested sludge. Malt waste was used as collected and its coarse particle size may hinder the ingestion by earthworms which do not have strong chewing mouthparts. It was found by Neuhauser *et al.* (1980) that the weight of *E. foetida* generally increase with a decrease in particle size of the substrate. Mechanical process like grinding, or biochemical pretreatment such as fermentation is required to reduce the particle size of malt waste before it can be fully utilized in vermiculture.

Coconut waste and cattle manure supported moderate increase in worm biomass when compared with soybean waste, pig manure and digested sludge. It was partly due to the unbalanced nutrient contents, high C/N ratios and coarse particle size of the two wastes.

According to Wallwork (1983), earthworm castings contain significantly higher counts of bacteria than does the surrounding environment. These microorganisms are capable of secreting digestive enzymes to break down ingested food, but it is uncertain if earthworms rely on gut symbionts for their supply of digestive enzymes. It was also found that the main dominant microorganism in *E. lucens* was *Streptomyces lipmanii* (Contreras, 1980). This suggested that the earthworm gut exerts a selective influence on *S. lipmanii*, favoring its development there (Wallwork, 1983). Significant weight could also be obtained when earthworm was fed with axenic cultured microorganisms (Neuhauser *et al.*, 1980)

These evidences imply that microorganisms play an important role in earthworm nutrition. The advantages of having wastes with low C/N ratios and small particle size on worm growth may be related to the microbial activities associated with the wastes in the digestive system of earthworm.

Heavy metals in high concentrations caused growth retardation of earthworms was an evidence (Gupta and Sundararaman, 1990; Malecki *et al.*, 1982; Ireland, 1983). Pig manure and digested sludge contained relatively high concentrations of heavy metals, especially Cu and Zn. It was supposed that worm growth would be depressed in these two wastes; however, on the contrary, pig manure and digested sludge could supported very good growth when compared with other wastes. It may be possible that the nutrient contents of the wastes are high enough to counteract the negative effect of matals on worm growth or the heavy metals in these wastes were not present in a form or level that could cause substantial depression in the biomass production which lasted 30 days.

It is assumed that worm growth was influenced by the contents of nutrients and toxic metals of the organic wastes, as well as the digestibility of the wastes. Of the chemical properties of the organic wastes studied, most of them were not related to the worm growth, except that total N content (crude protein content) was highly correlated ($P < 0.05$) with the growth of *P. asciatica* and to that of *E. foetida* to a lesser extent ($P < 0.1$), and C/N ratio was significantly correlated ($P < 0.05$) to the growth of *E. foetida* and to that of *P. asciatica* to a lesser extent ($P < 0.1$) (Table 2.4). Some other factors rather than chemical properties such as particle size and palatability may also affect worm growth. It suggested that chemical analysis alone cannot be used to predict the suitability of organic wastes for vermiculture. The effect on worm growth has to be assessed through experimentation.

2.3.4 Effects of organic wastes on protein contents and protein yields

Table 2.4 Relationship of worm growth rate and various chemical parameters of the organic wastes fed to the earthworms.

	Earthworm	Equation	R^2	P
pH	*E. foetida*	y=266x-781	0.33	0.14
	P. asciatica	y=93.2x-45.7	0.08	0.49
Organic C	*E. foetida*	y=-16.6x+1760	0.16	0.33
	P. asciatica	y=-5.77x+842	0.04	0.64
Total N	*E. foetida*	y=126x+493	0.43	0.08
	P. asciatica	y=110x+115	0.66	0.01
C/N ratio	*E. foetida*	y=-35.3x+1570	0.53	0.04
	P. asciatica	y=-21.5x+917	0.40	0.09
Total P	*E. foetida*	y=377x+689	0.41	0.09
	P. asciatica	y=169x+434	0.17	0.31
Total K	*E. foetida*	y=506x+649	0.22	0.24
	P. asciatica	y=282x+373	0.14	0.36
Total Na	*E. foetida*	y=-49.2x+1050	0.00	0.96
	P. asciatica	y=-363x+659	0.04	0.62
Total Mg	*E. foetida*	y=-1640x+1250	0.14	0.36
	P. asciatica	y=-391x+641	0.02	0.76
Crude protein	*E. foetida*	y=20.2x+492	0.43	0.08
	P. asciatica	y=17.6x+114	0.66	0.01
Crude fat	*E. foetida*	y=-8.22x+1090	0.02	0.72
	P. asciatica	y=3.74x+570	0.01	0.81

When fed with the various organic wastes, earthworm tissues had protein contents ranging from 51 to 67% for *E. foetida*, and 55 to 71% for *P. asciatica* (Fig. 2.2) which were comparable to that of fish meal (Edwards *et al.*, 1985). Such high protein contents suggested that worm biomass from the various wastes was a good protein source, and worm meal produced can be a potential animal feed in terms of protein content. The lowest protein content was found in both species of earthworms from coconut waste which may be due to the high crude fat content in the waste.

Variation in growth rate was greater than that in protein contents. Greater protein yields were obtained in earthworms fed with wastes which supported higher biomass production (Fig. 2.3), suggesting that the wastes which supported rapid worm growth could be a good implication of high protein production from vermiculture.

2.4 Conclusions

E. foetida and *P. asciatica* can both live on a wide range of agro-industrial wastes. Among these wastes, soybean waste, pig manure and digested sludge supported the greatest growth in terms of biomass. This may due to both the favourable chemical composition and particle size of these wastes. Unfortunately, pig manure and digested sludge contained relatively high concentrations of heavy metals, which possessed a risk of metal accumulation in worm tissues. This is

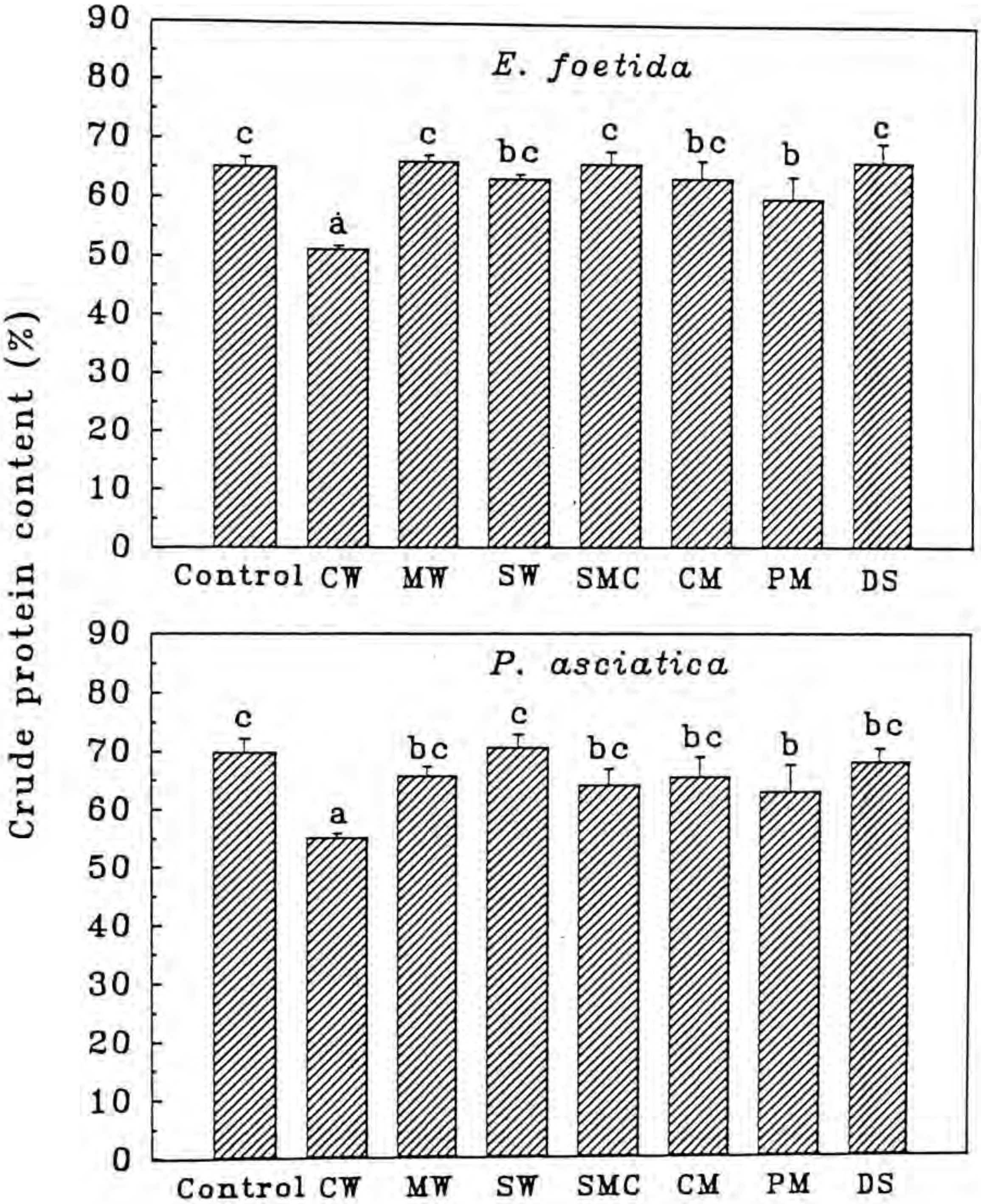


Fig. 2.2 Crude protein content in tissues of *E. foetida* and *P. asciatica* cultured in different wastes for 30 days (means of three replicates). Error lines denote standard errors. Bars having the same letter were not significantly different at 95% according to the Fisher PLSD test. CW = coconut waste, MW = malt waste, SW = soybean waste, SMC = spent mushroom compost, CM = cattle manure, PM = pig manure, DS = digested sludge.

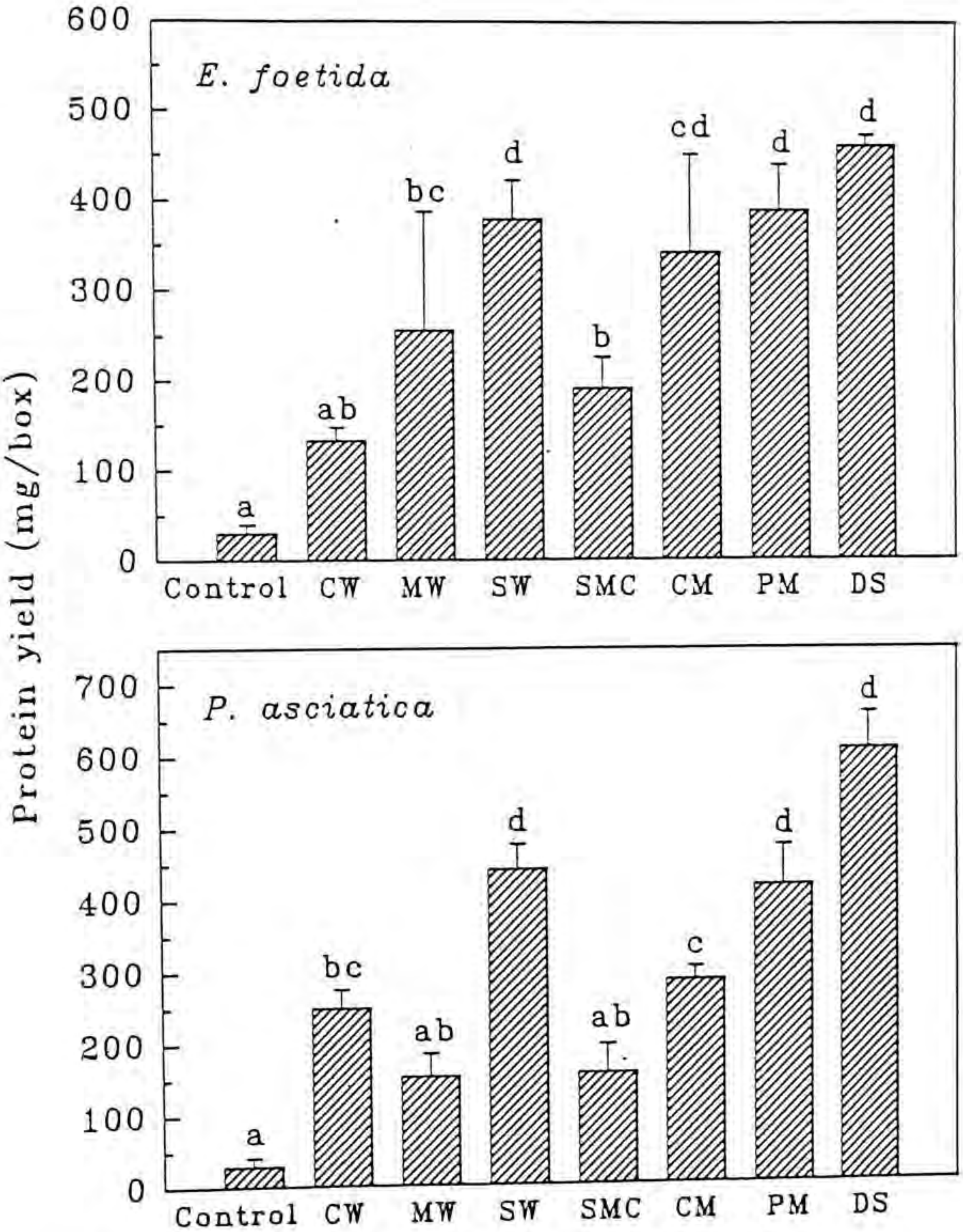


Fig. 2.3 Protein yields of *E. foetida* and *P. asciatica* cultured in different wastes for 30 days (means of three replicates). Error lines denote standard errors. Bars having the same letter were not significantly different at 95% according to the Fisher PLSD test. CW = coconut waste, MW = malt waste, SW = soybean waste, SMC = spent mushroom compost, CM = cattle manure, PM = pig manure, DS = digested sludge.

totally not acceptable if the worm meal is to be used as an animal feed. Further studies were needed to evaluate the quality of worm meal from soybean waste, pig manure and digested sludge (Chapter 3).

Chapter 3. EFFECTS OF SOYBEAN WASTE, PIG MANURE AND DIGESTED SLUDGE ON THE QUALITY OF WORM MEAL FROM *EISENIA FOETIDA* AND *PHERETIMA ASCIATICA*

3.1 Introduction

"Earthworm farming" or vermiculture, was borned with the increasing demand for bait as fishing became a popular sport in the 1930s and 1940s. Unfortunately, the most commonly cultured worm, *Eisenia foetida*, makes very poor bait. *E. foetida* earned its name from its defense mechanism; when threatened, as it is put on the end of hook for instance, it discharges a noxious yellowy, fetid-smelling fluid. Soil dwelling species, such as *Lumbricus terrestris*, make much better bait (Knight, 1989).

Although, some earthworms are unpalatable to fish, its studies on vermiculture for worm protein production are still going on. There are three reasons which account for this.

Firstly, earthworm can utilize a wide spectrum of organic wastes and convert 10% of them into protein (Edwards *et al.*, 1985). The production of worm protein by vermiculture is less expensive than other kinds of protein production.

Secondly, the nutrient content of worm meal is excellent, at least as good as meat and fish meal (Edwards *et al.*, 1985). It contains high concentration of protein with a high proportion of essential amino acids, and is also very rich in long-chain fatty acids, some of which fish cannot make for themselves. Vitamins, especially niacin, riboflavin and vitamin B_{12} were found sufficient in worm tissue. Huang (1982) summarized the nutrient content of six earthworm species (Table 3.1). The contents of crude protein, crude fat and ash ranged from 53.5 - 63.5%, 4.4 - 17.4% and 7.8 - 23.1% respectively. The nutrient contents differ from species to species, and environmental conditions or organic matter on which they thrive may also influence the nutrient content of the worm tissue.

Finally, feeding trials have been successfully conducted by feeding earthworm to fish and rat (Ibanez *et al.*, 1993; Tacon *et al.*, 1983). The unpalatability of *E. foetida* only happened to certain fish species, but not all other animals.

Unfortunately, earthworm are known to take up and accumulate in their tissues heavy metals when living both in contaminated and uncontaminated environment. This possesses the risk of introducing heavy metals into worm tissues, and extensively to the consumers of earthworm when worms are fed on organic wastes such as animal manure, digested sludge and other heavy metal contaminated wastes.

Heavy metal uptake by earthworms has been reviewed by Ireland (1983). The highest concentration factors were 151 for Cd, 1.3 for Pb, 18 for Zn and 1.5 for Cu at non-contaminated sites (Table 3.2), and 236 for Cd, 2.7 for Pb, 7.3 for Zn and 0.8 for Cu at contaminated sites (Table 3.3).

Table 3.1 Chemical composition of earthworms (Huang, 1982).

Earthworm	Dry matter (%)	Protein (%)	Crude fat (%)	Ash (%)
Pheretima guillelm	19.2	63.5	13.7	9.50
Drawida aisti	15.3	62.2	6.41	14.6
Eisenia foetida	11.0	62.2	4.36	7.77
Eisenia rosa	16.4	61.3	4.50	15.0
Lumbricus terrestris	17.4	53.5	6.07	23.1

Table 3.3 Concentration factors for cadmium, lead, zinc and copper at contaminated sites (Ireland, 1983)

Sites	Species	Cd	Pb	Zn	Cu
3-7 m from highway	mixed	10	0.6	3.2	--
pig waste amended soil	mixed	--	--	--	0.4-0.8
roadside soil	*L. terrestris*	17	0.4	7.3	0.6
lead mine complex	*L. terrestris*	--	0.03	0.02	--
sludge-amended soil	*L. terrestris*	26	0.4	--	--
sludge-amended soil	4 *Allolobophora* spp.	9-17	0.1-0.2	--	--
sludge-amended soil	*Aporrectodea tuberculata*	236	--	4.5	--
lead mine complex	*L. terrestris*	7.5	2.7	5.4	0.7
sludge	*E. foetida*	0.8	0.6	0.6	0.1
metal-enriched sludge	*E. foetida*	1.7	0.01	0.07	0.2

--: not determined

Table 3.2 Concentration factors for cadmium, lead, zinc and copper at non-contaminated sites (Ireland, 1983)

Sites	Species	Cd	Pb	Zn	Cu
soil	mixed	4.6	0.8	5.3	--
soil	mixed	16	0.2	7.4	--
compost	*Dendrodaena rubida*	--	0.8	0.7	--
soil	mixed	12	1.3	1.8	--
soil	mixed	--	--	--	1.5
soil	*L. terrestris*	27	0.5	18	0.8
soil	*L. terrestris*	58	0.04	--	--
soil	2 *Allolobophora* spp.	71-151	0.2-0.4	--	--
soil	*Aporrectodea tuberculata*	32	--	2.9	--
compost	*Eisenia foetida*	3.8	--	0.5	--
soil	*L. terrestris*	--	0.04	0.7	--
soil	5 *Allolobophora* spp.	16-33	0.3-0.5	3-10	--
	L. terrestris	15	0.3	5.2	--

--: Not determined

Of all the metals studied, cadmium appeared to accumulate in most species of earthworms at greater level than other metals. Cd was particularly concentrated in chloragogen cells in *L. terrestris*, where it was bound in the form of Cd-metallothioneins with small amount deposited in waste nodules (Anderson and Laursen, 1982). Some other metals, such as Zn, Mn and Fe, were shown to be excreted through the calciferous glands in *L. terrestris*.

Uptake and accumulation of heavy metals by earthworm was too complicated to be generalized. It varies with earthworm species, as well as the pH and organic matter content in their living environment (Ma, 1982; Ma *et al.*, 1983; Morgan & Morgan, 1988a, b, and c). Uptake, accumulation and excretion of heavy metals may happen at the same time in earthworm bodies. So, it is necessary to assess the extent of contamination of heavy metals in worm meals before using them as animal feedstuff.

Eisenia foetida and *Pheretima asciatica* have been successfully employed in vermiculture and exhibited excellent growth in terms of biomass increase when fed with soybean waste, pig manure and digested sludge (Chapter 2). To further study the feasibility of using these wastes in vermiculture, a second culture trial was carried out on the three wastes which gave the best worm growth to give more information on the growth rate, reproduction rate and feed conversion efficiency of earthworms. The contents of crude protein, crude fat, minerals, and heavy metals, as well as the amino acid profile of worm tissues were determined in order to assess the nutritional quality of the worm meals as animal feedstuff.

3.2 Materials and Methods

3.2.1 Collection of materials

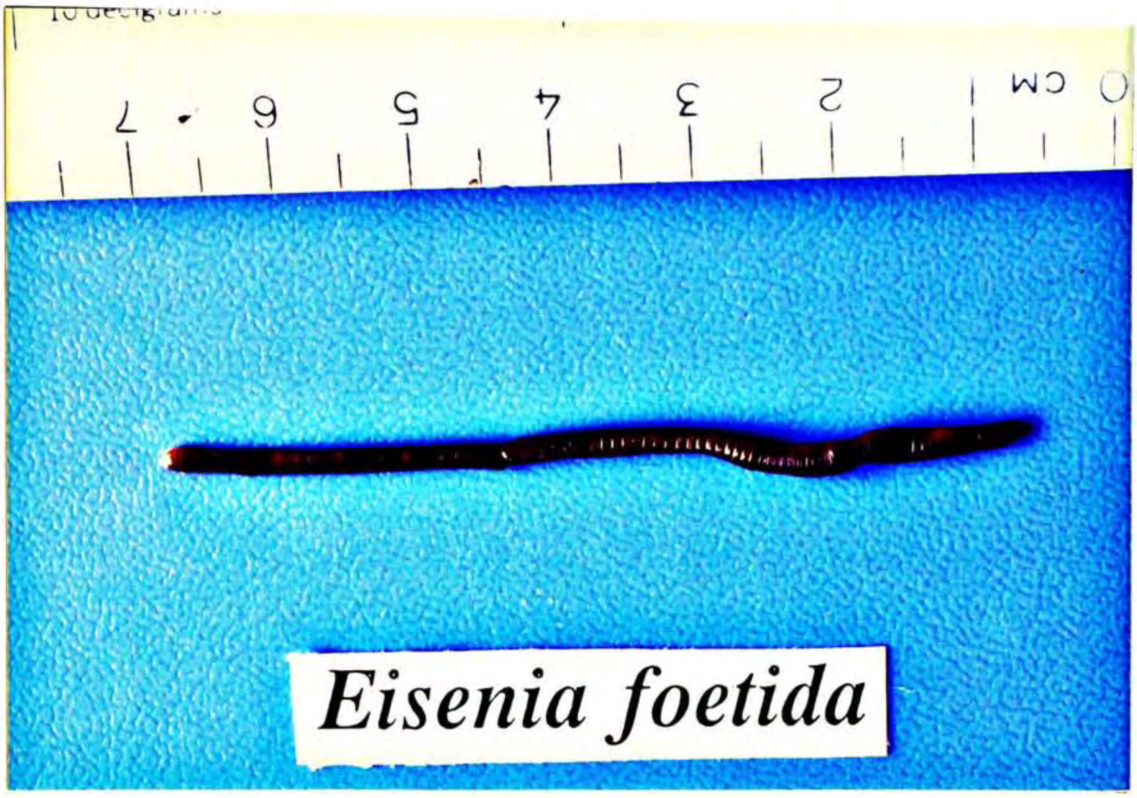
Bedding material was horse manure compost which had been used in the previous culture trial. The three wastes, including soybean waste, pig manure and digested sludge which supported the greatest worm growth were selected for further investigation on their effect on the nutritional value and heavy metal contents of worm meal so produced. The waste samples used were of the same batch as those used in the previous experiment. They were kept in a cold room at 4° C before being used for vermiculture.

3.2.2 Preparation of earthworms

Mature earthworms with clitellum (Plate 3.1), which measured about 7 cm for *E. foetida* and 15 cm for *P. asciatica*, were selected from the stock culture. Bedding materials were removed from the body and the worms rinsed with tap water, sorted, blot-dried and weighed.

3.2.3 Experimental design and setup

Earthworms were grown in 31 cm x 24 cm x 12 cm plastic boxes which contained the bedding material to a height of 10 cm. Soybean waste, pig manure



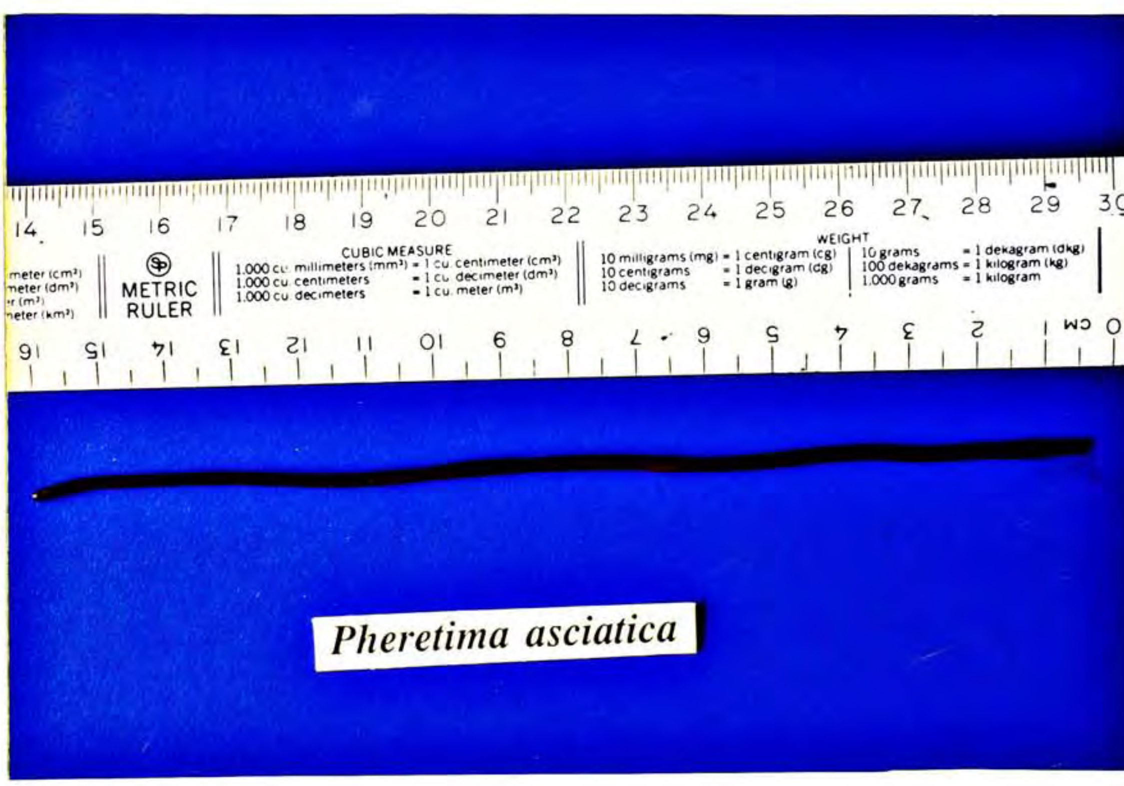


Plate 3.1 Earthworm species used in the experimental trial.

or digested sludge was added to each box and mixed with the bedding material. Thirty earthworms were transferred carefully to each box after weighing. Each treatment was replicated four times. The boxes were arranged in randomized blocks in growth chambers in an air-conditioned culture room with temperature controlled at 24-28°C (Plate 3.2). The boxes were illuminated with cool fluorescent tubes to prevent the earthworms from escaping. The bedding material was watered when necessary to keep the moisture content at 70-80%. The waste was added as soon as it has been depleted by the earthworms.

3.2.4 Harvesting of earthworms

Earthworms were removed from the bedding after growing for 60 days, washed with distilled water and blot-dried. Their number was counted and the worms were weighed. The earthworms were then smashed with an electric tissue homogenizer, freeze dried and kept in a freezer at -4°C before analysis.

3.2.5 Chemical analysis of worm tissues

The worm tissues were analyzed for the following items: total nitrogen (semi-micro Kjeldahl digestion followed by salicylate nitroprusside method using a Lachat QuickChem AE Automated Ion Analyzer), total contents of mineral (K, Na and Mg) and heavy metal (Cd, Cr, Cu, Ni, Pb and Zn) (70% nitric acid digestion followed by determination using a Hitachi Polarized Zeeman Atomic Absorption Spectrophotometer) (Allen *et al.*, 1974), crude

Plate 3.2 Setup for the earthworm culture.

protein (total N x 6.25), and crude fat (diethylether extraction for 24 hours, and evaporation of the solvent before weighing). For the determination of amino acids, 10 mg of samples were hydrolyzed with 6N HCl in an oven at 110°C for 24 h. The hydrolysate was filtered with 4.5 μm nylon filter. HCl in filtrate was evaporated using a Savant speed vacuum concentrator. The residue was dissolved in 0.5 ml Beckman Na-S buffer and diluted 50-fold, and the diluted sample was analyzed by a Beckman 6300 Amino Acid Analyzer.

3.2.6 Statistical analysis

The data were subjected to analysis of variance and significant differences between the means were calculated according to the Fisher PLSD test.

3.3 Results and Discussion

3.3.1 Effects of wastes on worm growth

For *E. foetida,* the highest growth rate of 945% was obtained from soybean waste, which was significantly higher ($P<0.05$) than those of pig manure and digested sludge (Fig. 3.1 and Table 3.4). Similarly, for *P. asciatica,* soybean waste gave the highest growth in term of biomass production, followed by pig manure and digested sludge (Fig. 3.1). The growth in soybean waste corresponded to a 2900% increase in biomass (Table 3.4). Statistical difference ($P<0.05$) was found among the growth rates of each of these two

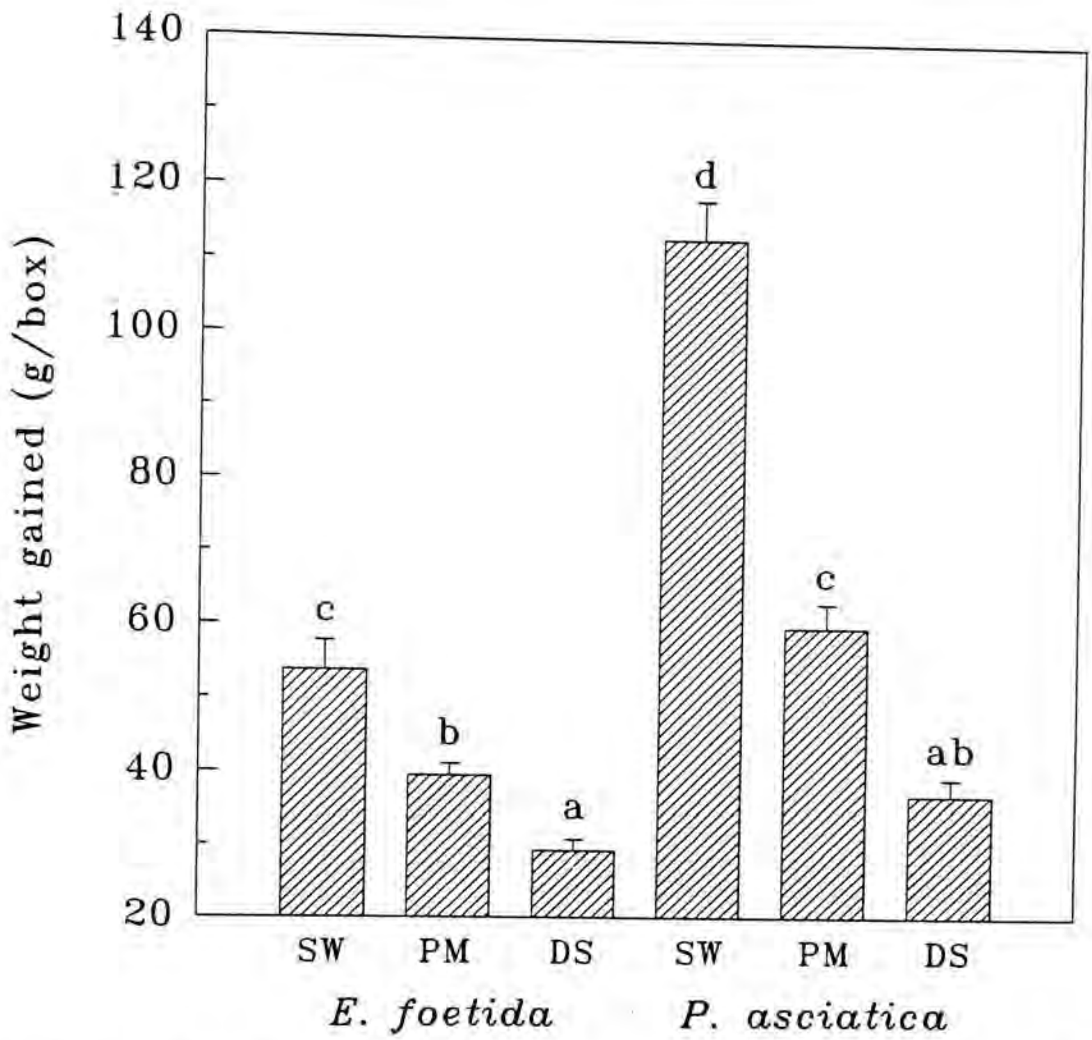


Fig. 3.1 Gain in fresh weight in *E. foetida* and *P. asciatica* cultured in soybean waste, pig manure and digested sludge for 60 days (means of four replicates). Error lines denote standard errors. Bars having the same letter were not significantly different at 95% according to the Fisher PLSD test. SW, PM and DS are abbreviations for soybean waste, pig manure and digested sludge respectively.

Table 3.4 Effect of soybean waste, pig manure and digested sludge on *E. foetida* and *P. asciatica* (means of four replicates).

	E. foetida			*P. asciatica*		
	SW	PM	DS	SW	PM	DS
Biomass increase rate (%)	945^{b} (75.5)	662^{ab} (13.1)	520^{a} (33.8)	2870^{d} (207)	1510^{c} (125)	918^{b} (29.6)
Reproductive rate (%)	1710^{a} (180)	1710^{a} (115)	2100^{a} (398)	4670^{b} (495)	3720^{b} (603)	4510^{b} (250)
Feed conversion efficiency (biomass gained/feed consumed, dry wt, %)	6.57^{b} (0.46)	4.99^{a} (0.10)	6.29^{ab} (0.32)	17.3^{d} (0.88)	8.27^{c} (0.44)	6.72^{b} (0.40)
Protein yield (dry wt, g/box)	5.30^{c} (0.40)	3.88^{ab} (0.16)	2.97^{a} (0.15)	13.8^{e} (0.64)	7.47^{d} (0.41)	4.67^{bc} (0.29)

Figures within a row followed by the same letter were not significantly different at 95% according to the Fisher PLSD test.

Figures within brackets are standard errors.

SW, PM and DS are abbreviations for soybean waste, pig manure and digested sludge respectively.

worms from soybean waste, pig manure and digested sludge. When comparing the same waste, *P. asciatica* had higher growth rate than *E. foetida*, and significant differences ($P<0.05$) were found between the worms from soybean waste and pig manure.

No significant difference ($P>0.05$) was found in the reproductive rates of *E. foetida* or *P. asciatica* from soybean waste, pig manure and digested sludge (Fig. 3.2). However, the reproduction rate of *P. asciatica* was significantly higher ($P<0.05$) than that of *E. foetida* from the same waste.

The growth and reproduction of earthworms were mainly influenced by the contents of nutrients and toxic substances present in the organic wastes on which the worms lived. Worm growth was closely related to the N content (thus crude protein content) and to a lesser extent the C/N ratio of the wastes (Chapter 2).

Pig manure and digested sludge contained relatively high concentrations of heavy metals which probably had a negative effect on worm growth. It has been well-documented that heavy metals will retard growth and reduce cocoon production (Gupta and Sundararaman, 1990; Malecki *et al.*, 1982; Ireland, 1983). Minimum concentrations of metals that significantly retard growth of *E. foetida* were 200 μg/g for Ni (as chloride), 100 μg/g for Cu (as nitrate), 2,000 μg/g for Zn (as chloride or nitrate) and 12,000 μg/g for Pb (as acetate) (Ireland, 1983). Cocoon production was totally inhibited by acetate of the five metals at

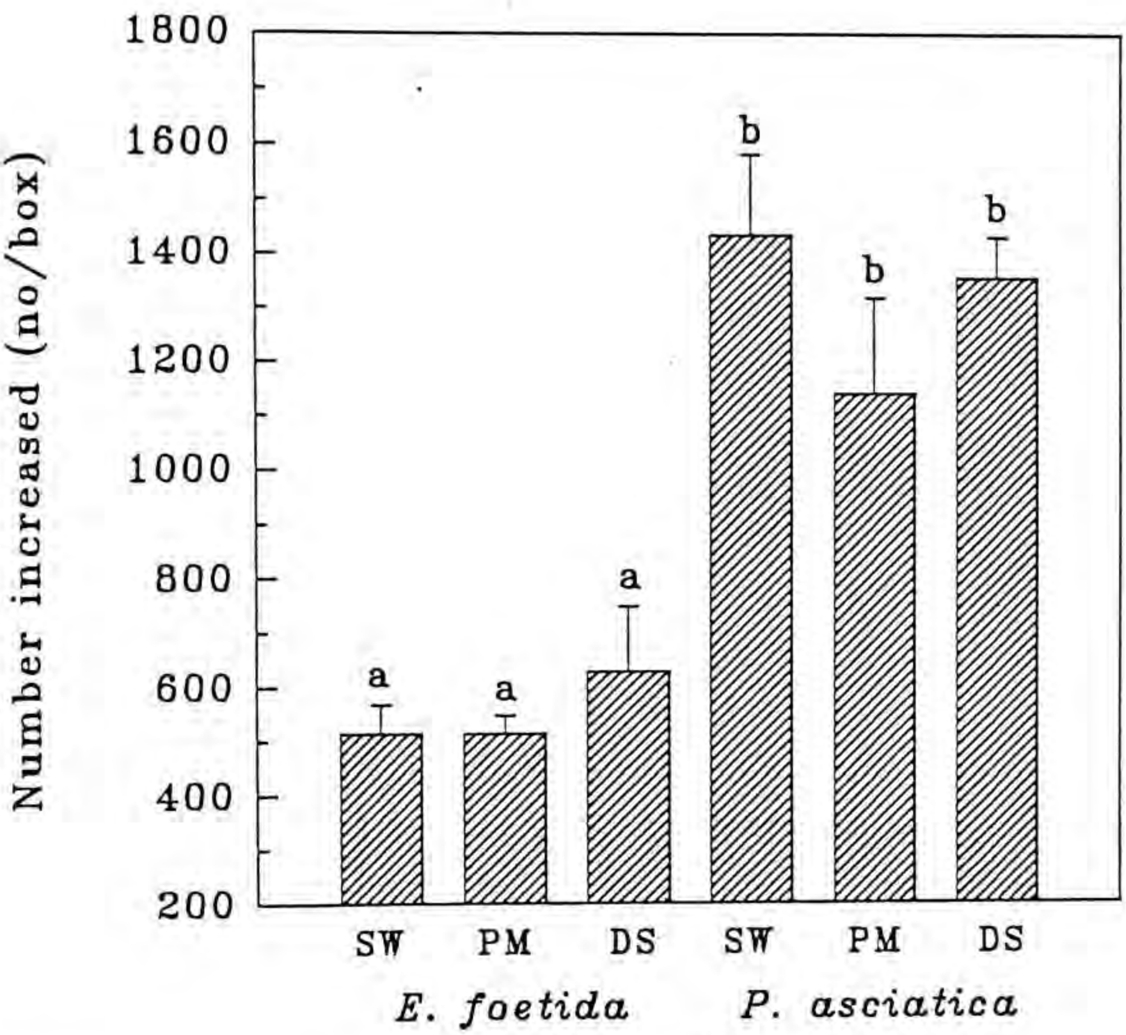


Fig. 3.2 Increase in number in *E. foetida* and *P. asciatica* cultured in soybean waste, pig manure and digested sludge for 60 days (means of four replicates). Error lines denote standard errors. Bars having the same letter were not significantly different at 95% according to the Fisher PLSD test. SW, PM and DS are abbreviations for soybean waste, pig manure and digested sludge respectively.

concentrations of 50 μg/g for Cd, 400 μg/g, 2,000 μg/g for Cu and 500 μg/g for Zn and Pb (Ireland, 1983).

With reference to the data mentioned above, pig manure contained remarkably high concentrations of Cu, Pb and Zn, while digested sludge contained relatively high concentrations of Cr, Cu, Ni, Pb and Zn. These metals might be present at levels high enough to retard growth but not enough to affect reproduction.

E. foetida can convert 10% of organic matter into worm protein (Edwards *et al.*, 1985). In this study, it converted 6.57% of soybean waste, 4.99% of pig manure and 6.29% digested sludge into worm biomass (Table 3.4). Feed conversion efficiency of *E. foetida* from soybean waste was significantly higher ($P<0.05$) than those from pig manure. *P. asciatica* appeared to be more efficient than *E. foetida* in feed conversion. It converted 17.3% of soybean waste into worm meal, which was statistically higher ($P<0.05$) than those of pig manure and digested sludge.

Among the three organic wastes, soybean waste was superior in terms of worm growth and reproduction, as well as feed conversion efficiency (Table 3.4). Besides the differences in worm tissues from different wastes, *E. foetida* and *P. asciatica* were found to be significantly different ($P<0.05$) in growth rate, reproductive rate and feed conversion efficiency. *P. asciatica* was better than *E. foetida* for vermiculture, at least, under the controlled environment used in the present study.

3.3.2 Nutrient contents of earthworms from different wastes

E. foetida consisted of 65.4 - 67.7% crude protein, 6.25 -7.10 % crude fat, 0.84 - 0.85% K, 0.83 - 0.87% Na and 0.20 - 0.24% Mg, while *P. asciatica* contained 66.2 - 68.3% crude protein, 5.58 - 8.95% crude fat, 0.94 - 1.05% K, 0.43 - 0.54 % Na and 0.18 - 0.25% Mg (Table 3.5).

The crude protein contents did not differ significantly ($P>0.05$) among worm tissues of the two worm species from the three wastes (Table 3.5). The crude protein contents of the two species were similar to those presented by Edwards *et al.* (1985). The crude fat contents were influenced by the organic wastes on which the worms were fed; the highest content was found in *P. asciatica* from soybean waste, and the lowest in *P. asciatica* from digested sludge. The mineral (K and Na) contents were related to earthworm species, but not very much to organic wastes. The K content in *P. asciatica* was higher than that of *E. foetida* when they grew in the same waste. On the contrary, Na content in *P. asciatica* was found lower than that in *E. foetdia.* It seems that some regulatory mechanisms may exist in earthworm for the homeostasis of tissue concentration of these macro-nutrients, especially for K and Na.

Tissues of *E. foetida* and *P. asciatica* contained high proportion of amino acids in their tissues, which were comparable to fish meal (Table 3.6). Of the essential amino acids, histidine was significantly higher ($P<0.05$) in almost all the worm tissues than that of fish meal. On the other hand, lysine of *P.*

Table 3.5 Nutrient contents (dry wt) of worm meals from soybean waste, pig manure and digested sludge (means of four replicates).

	E/SW	E/PM	E/DS	P/SW	P/PM	P/DS
Crude protein (%)	66.4^{a} (0.46)	65.4^{a} (0.53)	67.7^{a} (1.33)	66.2^{a} (1.97)	67.4^{a} (0.09)	68.3^{a} (0.63)
Crude fat (%)	6.93^{b} (0.16)	7.10^{b} (0.08)	6.25^{ab} (0.09)	8.95^{c} (0.88)	6.08^{ab} (0.12)	5.58^{a} (0.26)
K (%)	0.84^{a} (0.02)	0.85^{a} (0.02)	0.85^{a} (0.02)	0.94^{b} (0.01)	1.05^{c} (0.01)	0.98^{b} (0.01)
Na (%)	0.87^{d} (0.02)	0.86^{cd} (0.03)	0.83^{c} (0.01)	0.43^{a} (0.02)	0.54^{b} (0.00)	0.54^{b} (0.01)
Mg (%)	0.22^{c} (0.01)	0.24^{d} (0.00)	0.20^{b} (0.01)	0.18^{a} (0.00)	0.25^{d} (0.01)	0.21^{bc} (0.01)

Figures within a row followed by the same superscript were not significantly different at 95% according to the Fisher PLSD test.

Figures within brackets are standard errors.

E/SW, E/PM, E/DS, P/SW, P/PM and P/DS are abbreviations for worm meals of *E. foetida* from soybean waste (E/SW), pig manure (E/PM), digested sludge (E/DS), and *P. asciatica* from soybean waste (P/SW), pig manure (P/PM) , digested sludge (P/DS) respectively.

Table 3.6 Amino acid profiles of *E. foetida* and *P. asciatica* from soybean waste, pig manure and digested sludge (means of three replicates, amino acids are expressed as g amino acid/100 g dry meal.)

	Fish Meal	E/SW	E/PM	E/DS	P/SW	P/PM	P/DS
Essential amino acids							
Arg	3.46^{a}	3.68^{a}	3.60^{a}	3.50^{a}	3.64^{a}	3.49^{a}	3.68^{a}
	(0.33)	(0.23)	(0.24)	(0.26)	(0.18)	(0.10)	(0.20)
His	1.29^{a}	1.62^{b}	1.57^{b}	1.60^{b}	1.43^{ab}	1.55^{b}	1.57^{b}
	(0.10)	(0.03)	(0.06)	(0.11)	(0.05)	(0.03)	(0.09)
Ile	3.02^{a}	2.89^{a}	2.77^{a}	2.90^{a}	2.75^{a}	2.88^{a}	2.87^{a}
	(0.19)	(0.05)	(0.11)	(0.13)	(0.10)	(0.04)	(0.08)
Leu	5.03^{a}	4.93^{a}	4.75^{a}	4.90^{a}	4.58^{a}	4.77^{a}	4.83^{a}
	(0.32)	(0.10)	(0.19)	(0.22)	(0.16)	(0.10)	(0.17)
Lys	5.12^{b}	4.75^{ab}	4.55^{ab}	4.88^{ab}	4.16^{a}	4.48^{a}	4.48^{a}
	(0.31)	(0.09)	(0.19)	(0.32)	(0.16)	(0.05)	(0.16)
Met	1.78^{b}	0.88^{a}	0.73^{a}	0.79^{a}	0.71^{a}	0.84^{a}	0.85^{a}
	(0.19)	(0.02)	(0.05)	(0.09)	(0.01)	(0.05)	(0.04)
Phe	2.75^{ab}	2.69^{ab}	2.54^{a}	2.59^{ab}	2.84^{ab}	2.83^{ab}	2.97^{b}
	(0.24)	(0.06)	(0.15)	(0.11)	(0.10)	(0.07)	(0.12)
Thr	2.88^{a}	2.89^{a}	2.76^{a}	2.85^{a}	2.83^{a}	2.79^{a}	2.73^{a}
	(0.19)	(0.05)	(0.11)	(0.14)	(0.11)	(0.13)	(0.19)
Val	3.51^{b}	3.20^{ab}	3.12^{ab}	2.99^{a}	3.13^{ab}	3.08^{a}	3.22^{ab}
	(0.22)	(0.04)	(0.16)	(0.12)	(0.06)	(0.11)	(0.17)
Non-essential amino acids							
Ala	4.06^{b}	3.40^{a}	3.29^{a}	3.39^{a}	3.15^{a}	3.23^{a}	3.30^{a}
	(0.25)	(0.06)	(0.10)	(0.14)	(0.13)	(0.07)	(0.11)
Asp	5.93^{a}	6.30^{a}	6.07^{a}	6.23^{a}	6.01^{a}	6.24^{a}	6.28^{a}
	(0.36)	(0.11)	(0.25)	(0.30)	(0.21)	(0.13)	(0.25)
Cys	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.04^{b}	0.00^{a}
	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.02)	(0.00)
Gly	3.67^{b}	3.14^{a}	3.05^{a}	3.05^{a}	2.96^{a}	3.00^{a}	3.03^{a}
	(0.21)	(0.06)	(0.15)	(0.15)	(0.11)	(0.06)	(0.09)
Glu	8.60^{b}	8.47^{ab}	8.09^{ab}	8.33^{ab}	7.65^{a}	7.90^{ab}	8.03ab
	(0.39)	(0.19)	(0.35)	(0.36)	(0.18)	(0.21)	(0.30)
Pro	2.73^{b}	2.50^{ab}	2.24^{a}	2.38^{ab}	2.33^{ab}	2.59^{ab}	2.55^{ab}
	(0.19)	(0.12)	(0.10)	(0.03)	(0.04)	(0.23)	(0.24)
Ser	2.41^{a}	2.74^{a}	2.58^{a}	2.65^{a}	2.57^{a}	2.54^{a}	2.69^{a}
	(0.15)	(0.07)	(0.13)	(0.12)	(0.04)	(0.21)	(0.22)
Tyr	1.03^{a}	1.38^{a}	1.23^{a}	1.21^{a}	1.26^{a}	1.29^{a}	1.39^{a}
	(0.23)	(0.08)	(0.14)	(0.11)	(0.08)	(0.03)	(0.01)
Total	57.29^{a}	55.43^{a}	52.98^{a}	54.41^{a}	51.85^{a}	53.83^{a}	54.45^{a}
	(3.82)	(1.27)	(2.28)	(2.58)	(1.08)	(1.01)	(1.93)

Figures within a row followed by the same letter were not significantly different at 95% according to the Fisher PLSD test.

Figures within brackets are standard errors.

E/SW, E/PM, E/DS, P/SW, P/PM and P/DS are abbreviations for worm meals of *E. foetida* from soybean waste (E/SW), pig manure (E/PM), digested sludge (E/DS), and *P. asciatica* from soybean waste (P/SW), pig manure (P/PM), digested sludge (P/DS), respectively.

asciatica, methionine of both species, and valine of *E. foetida* from digested sludge and *P. asciatica* from pig manure were found to be significantly lower ($P<0.05$) than those of fish meal. For the non-essential amino acids, alanine and glycine of both species, glutamic acid of *P. asciatica* from soybean waste, proline of *E. foetida* from pig manure were found significantly lower ($P<0.05$) than those of fish meal. Cysteine was only found in trace amount in *P. asciatica* from pig manure. It is not sure whether the amino acid profile of worm meals was influenced by the type of organic waste fed to earthworms. There was no significant difference ($P>0.05$) between the total amino acid contents in the worm meals and that in fish meal.

3.3.3 Heavy metal contents in earthworm tissues

The metal concentrations of the bedding material, organic wastes and tissues of *E. foetida* and *P. asciatica* from these wastes are shown in Figs. 3.3-3.7.

Most of the metals in worm tissue did not reach the concentrations in the bedding material or organic waste on which the worms lived, which means that accumulation did not happen in most cases as what may be expected. If concentration factors greater than 1.0 indicated accumulation, only Cd was found to be accumulated in *E. foetida* from digested sludge and soybean waste, and Cu in *P. asciatica* from soybean waste.

Cd in *E. foetida* was higher than that in *P. asciatica* from the same organic wastes (Fig. 3.3). *E. foetida* showed Cd accumulation from digested sludge and surprisingly from soybean. Though pig manure was seriously contaminated, and Cd level in E/PM was remarkable, accumulation was not observed. This agreed with . that of Ireland (1979) who found that the concentration factor tends to fall as soil Cd levels rise.

Cr was taken up by *E. foetida* and *P. asciatica* to different extent (Fig. 3.4). Despite Cr contamination, particularly in digested sludge, Cr concentration in worm tissues remained at low levels. Although digested sludge contained about 50 μg/g Cr, the Cr concentrations in *E. foetida* and *P. asiciatica* were only 7% and 14% respectively of that in the sludge.

Uptake of Cu in *E. foetida* was lower than that in *P. asciatica* from the same wastes (Fig. 3.5). Pig manure was heavily contaminated by supplementary Cu in pig diets to promote growth. However, though Cu contents in worm meals from pig manure were high, no accumulation was observed. Similar pattern was noted for digested sludge, though to a lesser extent (Fig. 3.5). There may be a regulatory system which excretes excessive Cu from the earthworms.

Accumulation of Pb did not happen in most cases (Fig. 3.6). This was in line with the low Pb concentration factors as reviewed by Ireland (1983) which may be due to the low availability of Pb. The small amount of Pb in worm meals from soybean may come from the bedding material used.

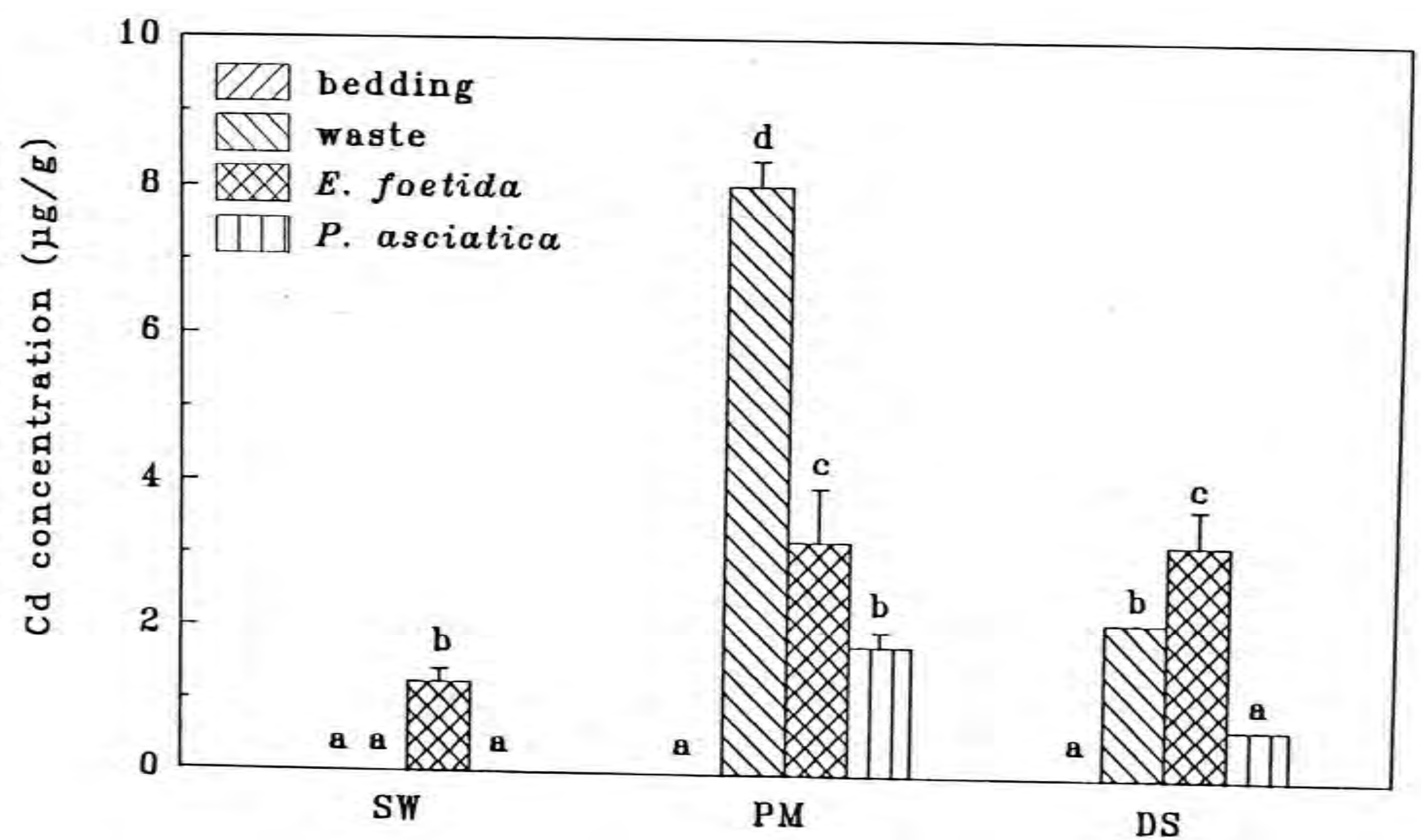


Fig. 3.3 Cd concentrations of bedding material, soybean waste, pig manure, digested sludge and tissue of worms cultured in these wastes (means of four replicates). Error lines denote standard errors. Bars within each waste group having the same letter were not significantly different at 95% according to the Fisher PLSD test. SW, PM and DS are the abbreviations for soybean waste, pig manure and digested sludge respectively.

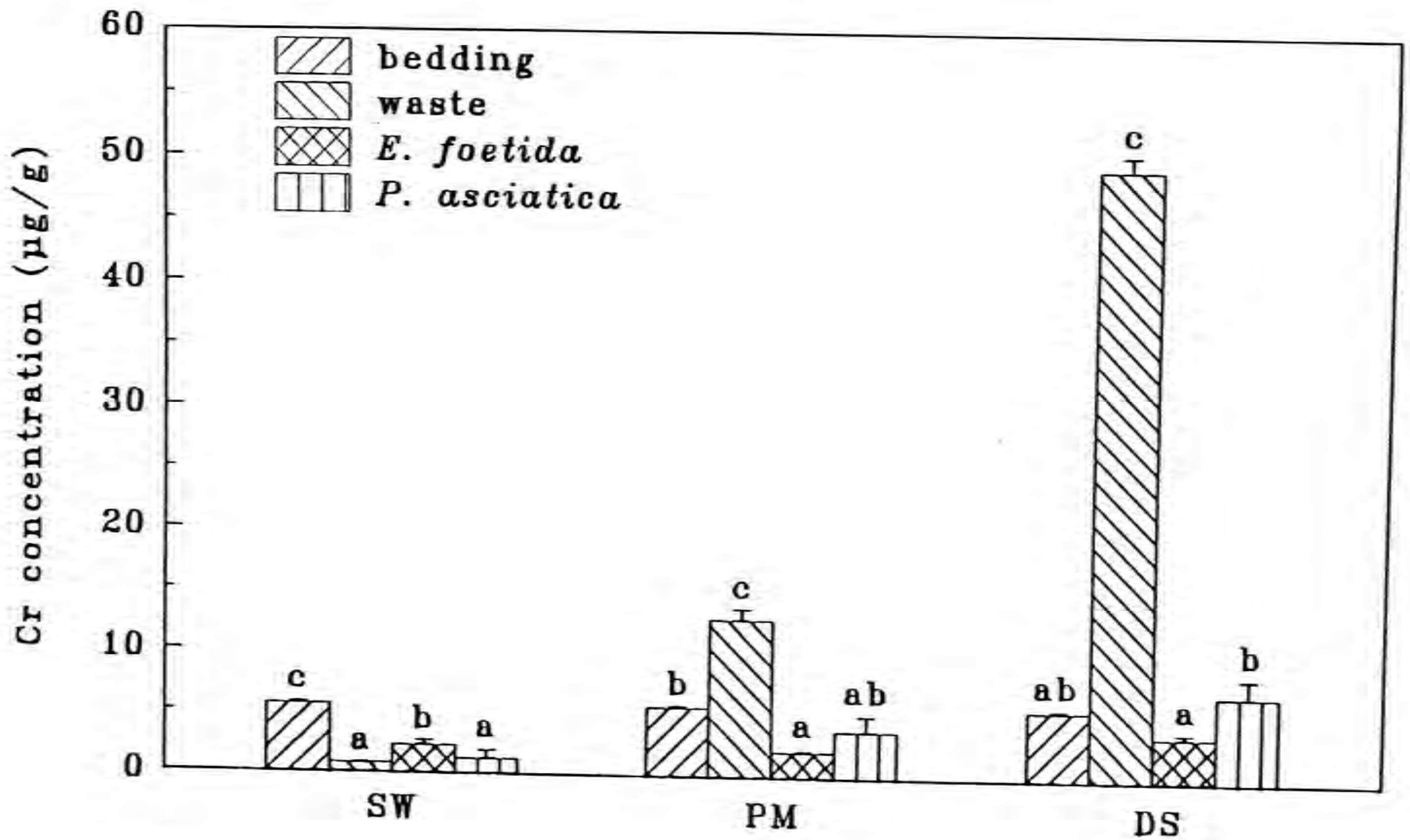


Fig. 3.4 Cr concentrations of bedding material, soybean waste, pig manure, digested sludge and tissue of worms cultured in these wastes (means of four replicates). Error lines denote standard errors. Bars within each waste group having the same letter were not significantly different at 95% according to the Fisher PLSD test. SW, PM and DS are the abbreviations for soybean waste, pig manure and digested sludge respectively.

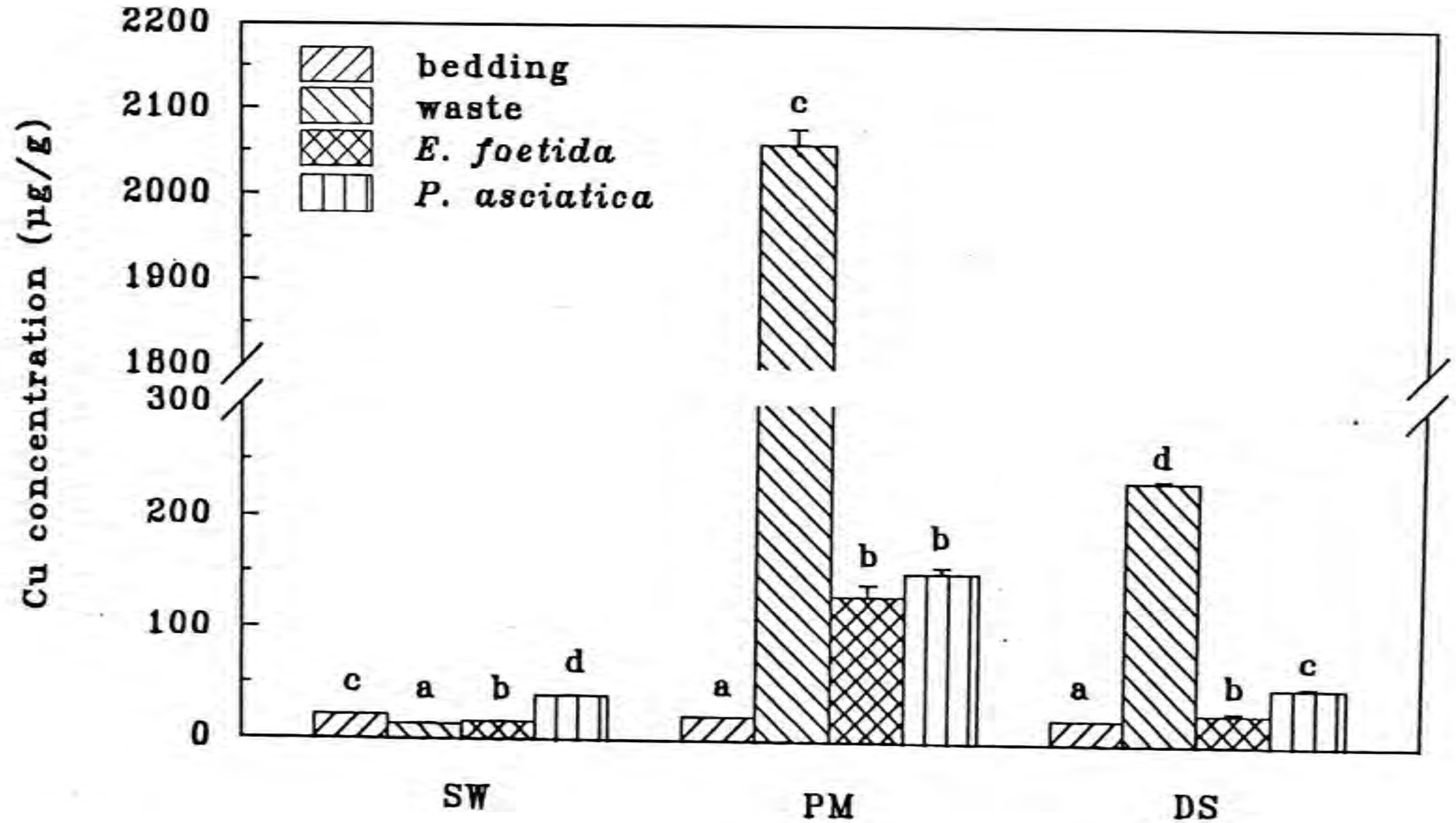


Fig. 3.5 Cu concentrations of bedding material, soybean waste, pig manure, digested sludge and tissue of worms cultured in these wastes (means of four replicates). Error lines denote standard errors. Bars within each waste group having the same letter were not significantly different at 95% according to the Fisher PLSD test. SW, PM and DS are the abbreviations for soybean waste, pig manure and digested sludge respectively.

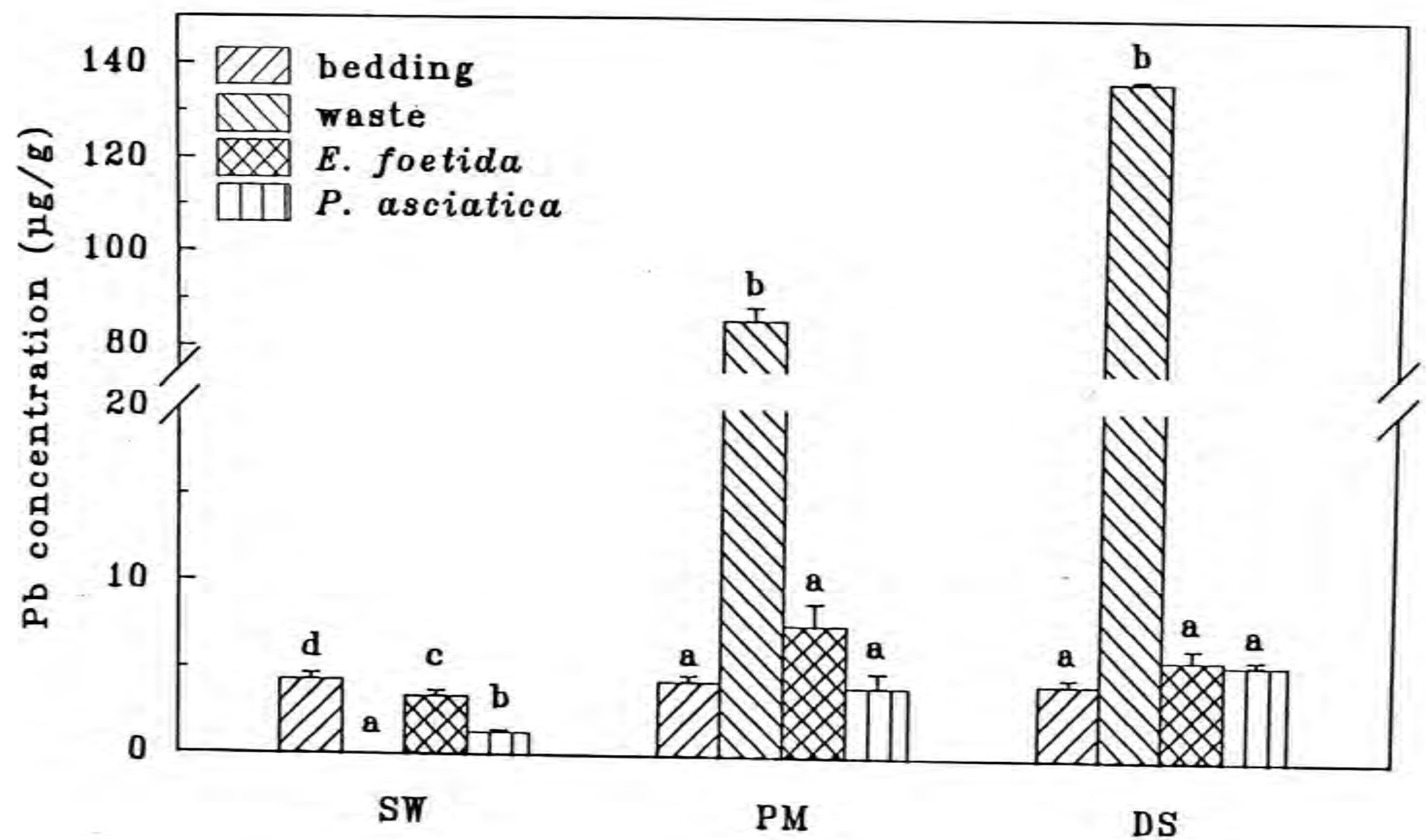


Fig. 3.6 Pb concentrations of bedding material, soybean waste, pig manure, digested sludge and tissue of worms cultured in these wastes (means of four replicates). Error lines denote standard errors. Bars within each waste group having the same letter were not significantly different at 95% according to the Fisher PLSD test. SW, PM and DS are the abbreviations for soybean waste, pig manure and digested sludge respectively.

Similar to Cd, higher levels of Zn were taken up by *E. foetida* than *P. asciatica* (Fig. 3.7). Concentration factor in earthworms tended to fall as concentration in wastes increased. Zn contents in the worm meals were only 6-10% of that in the corresponding wastes. Zn in worm meals from soybean waste may again be derived from the bedding used.

Ni was undetectable in most of the worm tissues, except that in *P. asciatica* from digested sludge which had a concentration of 8.38 μg/g. No matter accumulation happened or not, the hazardous of the deposited heavy metals in worm tissues depended on the actual concentrations in worm meals and their amount of ingestion by animals or humans.

The U.S. recommended dietary allowances of essential elements and the provisional tolerate intakes for toxic metals (Cd and Pb) for a 65 kg person are shown in Table 3.7 (NRC, 1989; WHO, 1987 and 1989). The maximum dietary allowance of worm meals for an average man per day were calculated assuming that worm meals were consumed as food by man. The intake of worm meals for humans was restricted to very small amount due to heavy metal contamination. Cr was the major factor which limited the maximum allowance of worm meal of *E. foetida* from pig manure to 2.21 g/day and that *E. foetida* from digested sludge to 3.62 g/day, and worm meal of *P. asciatica* from digested sludge to 7.00 g/day. Worm meals from soybean waste contained little heavy metals and can be taken in in larger amount without any health risk., though concentration factors were greater than 1 in some cases.

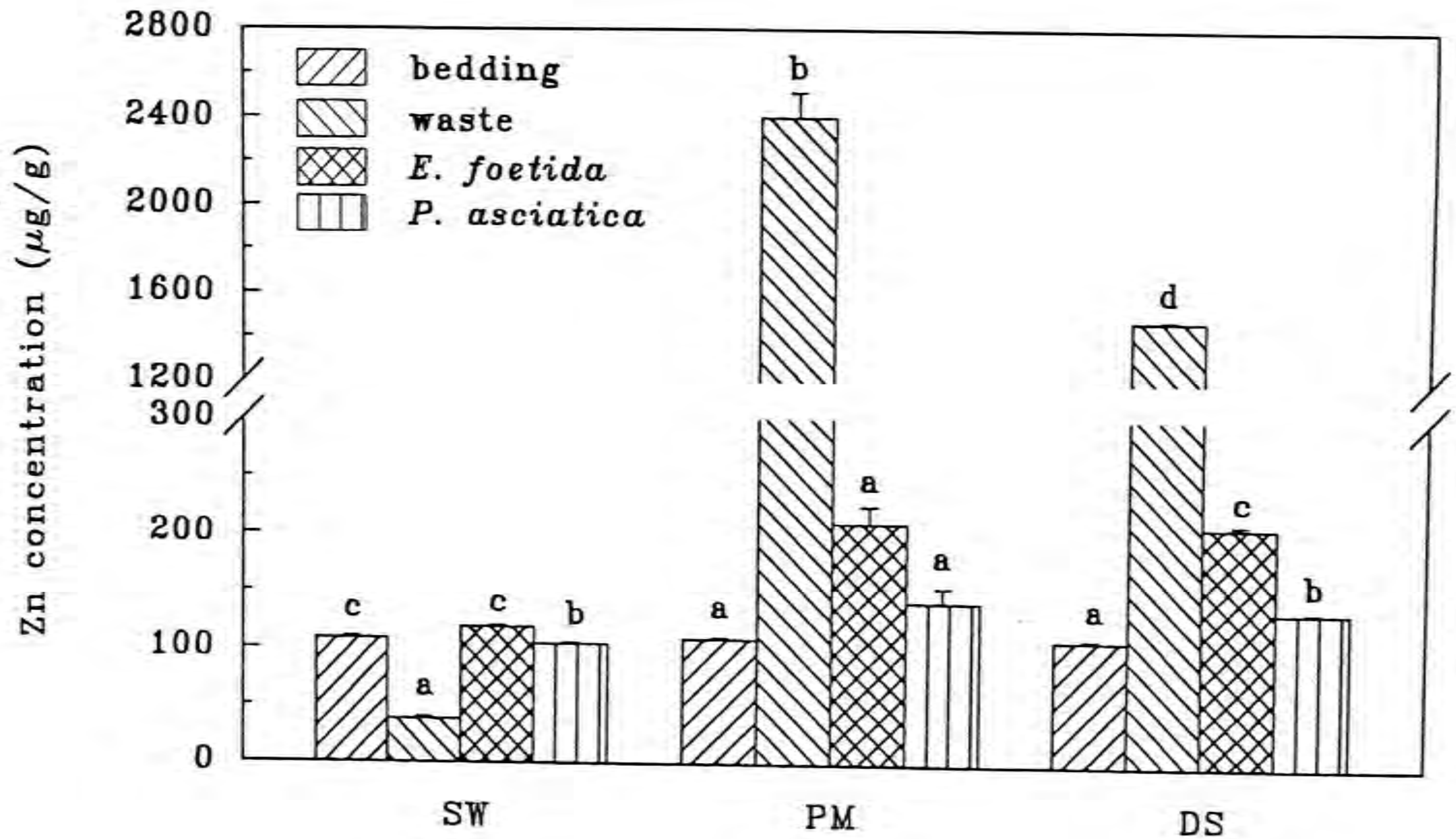


Fig. 3.7 Zn concentrations of bedding material, soybean waste, pig manure, digested sludge and tissue of worms cultured in these wastes (means of four replicates). Error lines denote standard errors. Bars within each waste group having the same letter were not significantly different at 95% according to the Fisher PLSD test. SW, PM and DS are the abbreviations for soybean waste, pig manure and digested sludge respectively.

Table 3.7 Dietary allowances (dry wt, g/day) of worm meals for man.

	Essential element						Toxic element	
	K	Mg	Na	Cr	Cu	Zn	Cd	Pb
RDA/PTI	200 mg/day	350 mg/day	500 mg/day	50 μg/day	1.5 mg/day	15 mg/day	65 μg/day	455 μg/day
E/SW	238.1	159.1	57.5	21.6	96.0	126.7	52.0	133.8
E/PM	235.3	145.8	58.1	2.21	11.5	71.5	17.3	59.7
E/DS	235.3	175.0	60.2	3.62	53.7	72.1	18.2	78.4
P/SW	212.8	194.4	116.3	89.3	38.3	144.6	65.0	364.0
P/PM	190.5	140.0	92.6	13.0	9.88	106.3	18.2	112.3
P/DS	204.1	166.7	92.6	7.00	29.2	116.6	91.5	80.3

RDA is the US recommended dietary allowances of essential elements (Cr, Cu, K, Mg, Na, Zn) for adults (NRC, 1989).

PTI is the provisional tolerate intake for toxic minerals of Cd and Pb (WHO, 1987 and 1989).

E/SW, E/PM, E/DS, P/SW, P/PM and P/DS are abbreviations for worm meals of *E. foetida* from soybean waste (E/SW), pig manure (E/PM), digested sludge (E/DS),and *P. asciatica* from soybean waste (P/SW), pig manure (P/PM), digested sludge (P/DS), respectively.

Organic wastes from agricultural or municipal origin were contaminated with heavy metals to various extent. Earthworms fed with heavily contaminated wastes take up and even accumulate metals in the wastes such that worm meals derived from these organic wastes are not safe for human consumption directly in most cases.

3.4 Conclusions

Both *E. foetida* and *P. asciatica* were very suitable species that could be exploited in vermiculture due to their high growth rate and excellent reproduction potential, as well as their ability in converting organic wastes into worm biomass. Meanwhile, they were rich in protein with high proportion of amino acids, which were comparable with fish meal, except that methionine, lysine and valine were found deficient in some worm meals. The presence of heavy metals in worm meals was the major barrier in the utilization of worm meal as animal feed. To safeguard the contamination of the consumers along the food chain, worm meals with elevated metal contents should be restricted from utilization as feed. A feeding trial should be conducted to evaluate the quality of worm meal as a feed and to determine the extent of transfer of metals from the earthworm tissue to their consumers.

Chapter 4. WORM MEAL AS PROTEIN SOURCE FOR FISH DIET

4.1 Introduction

A wide spectrum of organic wastes have been commonly used as one of the elements in integrated aquaculture. However, some fishes, especially those of highly valued carnivores, cannot thrive at high density in waste-fed ponds either because the natural feed which they require is insufficient or the water quality is too poor in terms of dissolved oxygen content, levels of toxic substances and pathogen counts (Little and Muir, 1987). Thus, artificial, completed diets which are formulated with balanced nutrients are required by these fishes.

The protein component of a fish diet is the single most expensive composition in formulated feed when the conventional protein source of fish meal is used. For this reason, if less expensive and readily available protein sources are available, they are more desirable with respect to production cost. Unconventional protein sources such as rapeseed meal (Davies *et al.*, 1990), cotton seed (El-Sayed, 1990), cassava leaf (Ng and Wee, 1989), soybean meal (Shiau *et al.*, 1989), biogas slurry (Edwards *et al.*, 1988), sewage sludge (Wong and Chiu, 1993) and dried poultry waste (Yousif and Alhadhrami, 1993) have been tested for their potential for the replacement of fish meal. Results often showed that these material can be used only to a limited extent, because high rate inclusion usually caused a repression in growth. Their economic value as protein source is mostly limited either by inadequate nutritional profile or the presence of various antinutritional factors, e.g. methionine deficiency in soybean meal (Shiau *et al.*, 1989), glucosinolates in rapeseed meal (Davies *et al.*, 1990), gossypol in cotton seed (El-Sayed, 1990) and heavy metals in sewage sludge (Wong and Chiu, 1993).

Protein, which is the material for building up organism and producing enzymes, is a very important constituent of animal feedstuff. Amino acids, as the basic component of the protein, are important for the quality of protein, and partially determine its value as a feed ingredient. A number of amino acids cannot be synthesized by organism directly, and therefore they must be supplied in diets and are referred to as essential amino acids. Amino acids, only when supplied in an optimal level, can benefit the animal in growth and development. A balanced amino acid profile, which means that every individual amino acid is not in excess or deficient, is a premise of a good quality protein.

Earthworms, with a high tissue protein content (54-64%) may provide an alternative protein source for fish and livestock (Huang, 1982). Feasibility of replacing fish meal by worm meal or using earthworm as direct feed for fishes has been demonstrated in a few studies. Worm species used include *E. foetida*, *Allolobophora* spp. and *Lumbricus terrestis* for rainbow trout (Tacon *et al.*, 1983); *Eudrilus eugenige* for juvenile trout (Hilton, 1983); *Dendrodrilus subrubicundus* for trout (Stafford and Tacon, 1984); and three *Enchytraeus* spp. for *Brachydanio rerio* (an ornamental fish), *Rutilus rutilus* (a fodder fish) and

Perca fluviatilis (a carnivorous fish) (Bouguenec, 1992). Growth responses varied, depending on the species of earthworm and fish. Only frozen *A. longa* and *L. terrestis* were found comparable to the commercial diet for trout. Unfortunately, they are not generally considered to be good candidates for vermiculture due to their relatively low growth and reproductive rate (Tacon *et al.*, 1983). Most of the other earthworm species reduced fish growth at high rates of inclusion in the fish diets (Bouguenec, 1992; Hilton, 1983; Stafford and Tacon, 1984; Tacon *et al.*, 1983).

Earthworm species of *Eisenia foetida* and *Pheretima asciatica* have been cultured successfully in the laboratory in a number of agro-industrial wastes, particularly soybean waste, pig manure and digested sludge (Chapters 2 and 3). Worm meals of *Eisenia foetida* and *Pheretima asciatica* produced from agro-industrial wastes were sufficient in most of the essential amino acids, which are comparable to fish meal (Table 3.6), except that methionine and valine in both worm meals and lysine in worm meal of *P. asciatica* were found significantly lower than that in fish meal. Some other amino acids, e.g. histidine, in most of the worm meals were found to be significantly higher than that in fish meal. The present study was carried out to evaluate the use of worm meal of *E. foetida* and *P. asciatica* from soybean waste, pig manure and digested sludge as the sole protein source in the diets for the omnivorous tilapia. The response of the fish to the worm meals and the uptake of the heavy metals from the worm meal by the fish were determined so as to see if the worm meals could substitute the conventional protein source in fish diets.

4.2 Materials and Methods

4.2.1 Preparation of experimental diets

Freeze-dried whole tissues of *E. foetida* and *P. asciatica* was obtained from the second earthworm culture trial (Chapter 3). The worm meal was finely ground by a micro-hammer mill into powder before being made into fish diets. For comparison, brown fish meal was used as the single protein source in the control diet. It contained 65.2% crude protein, 10.5% crude fat, and its amino acid profile was listed in Table 3.6. The fish meal contained 19.4 μg/g Cu, 1.80 μg/g Ni, 1.62 μg/g Pb and 92.3 μg/g Zn.

Experimental diets were formulated by computer basically according to the protein and lipid requirements of tilapia stated by De Silva *et al.* (1989) and Hanley (1991) in the levels of 38% and 10% respectively. Vitamin mix, mineral mix and CMC as binder were supplemented in levels of 1%, 3% and 1% respectively, and starch was used to balance the diet formula to 100% (Table 4.1).

The composition of the experimental diets is shown in Table 4.2. Control, E/SW, E/PM, E/DS, P/SW, P/PM and P/DS were the diets which contained the sole dietary protein of fish meal (Control), worm meal of *E. foetida* from soybean waste (E/SW), pig manure (E/PM), digested sludge

Table 4.1 Basic composition (%) of conventional diets for tilapia

Crude protein	38.0
Crude fat	11.0
Mineral mix	3.00
Vitamin mix	1.00
CMC	1.00
Starch and others	46.0

CMC: carboxymethylcellulose.

Table 4.2 Composition (%) of diets used for the feeding trial with tilapia.

	Control	E/SW	E/PM	E/DS	P/SW	P/PM	P/DS
Fish meal	58.3	0.00	0.00	0.00	0.00	0.00	0.00
Worm meal	0.00	57.7	58.1	56.1	57.5	56.2	55.6
Corn oil	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Lard	0.00	1.99	1.87	2.46	0.86	2.57	2.89
Starch	31.7	30.3	30.0	31.4	31.6	31.2	31.5
Mineral mix	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Vitamin mix	1.00	1.00	1.00	1.00	1.00	1.00	1.00
CMC	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Total	100	100	100	100	100	100	100

Control, E/SW, E/PM, E/DS, P/SW, P/PM and P/DS were the fish diets which contained dietary protein of fish meal (Control), worm meal of *E. foetida* from soybean waste (E/SW), pig manure (E/PM), digested sludge (E/DS), and worm meal of *P. asciatica* from soybean waste (P/SW), pig manure (P/PM) and digested sludge (P/DS) respectively.

Mineral mix (per 100 g of mix)(Ng and Wee, 1989; Soliman *et al.*, 1994): $Ca(HPO_4)_2.2H_2O$, 72.78 g; $MgSO_4.7H_2O$, 12.75 g; NaCl, 6.0 g; KCl, 5.0 g; $FeSO_4.7H_2O$, 2.5 g; $ZnSO_4.7H_2O$, 0.5 g; $MnSO_4.H_2O$, 0.25 g; $CuSO_4.5H_2O$, 0.08; $CoSO_4.7H_2O$, 0.05 g; $Ca(IO_3).6H_2O$, 0.03 g; $CrCl_3.6H_2O$, 0.01 g.

Vitamin mix (per 100 g of mix) (Ng and Wee, 1989): thiamine (B1), 250 mg; riboflavin (B2), 250 mg; pyridoxine (B6), 200 mg; pantothenic acid, 500 mg; inositol, 10 g; biotin, 30 mg; folic acid, 75 mg; para-aminobenzoic, 250 mg; choline, 20 g; niacin, 1 g; cyanocobalamine (B12), 0.5 mg; retinol palmitate (A), 10,000 i.u.; α-tocopherol acetate (E), 2.01 g; ascorbic acid (C), 5.0g; menadione (K), 200 mg; cholecalciferol (D3), 50,000 i.u..

CMC: carboxymethylcellulose as binder.

(E/DS), and worm meal of *P. asciatica* from soybean waste (P/SW), pig manure (P/PM) and digested sludge (P/DS), respectively. All ingredients in the diets were mixed well by adding 60 g distilled water to 100 g dry matter. The moistened diets were packed by cling wrap and keep in 4°C refrigerator before used.

4.2.2 Feeding experiment

Fry fish of tilapia (*Oreochromis mossambicus*) were stocked in a 30 l glass tank with water filtering system, and the water was aerated with air stones. The fish were acclimated for 10 days before the start of the experiment during which the fish were fed with micro-algae and daphnids.

Fish of similar size were collected from the stock and starved for one day prior to the feeding trial. Fifteen fry tilapia, average approximately 43 mg body weight were released into each aquarium. The feeding trial was conducted in 28 x 18 x 28 cm^3 aquaria with 12 l capacity (Plate 4.1). The aquaria were arranged in randomized blocks and were kept in an air-conditioned room with illumination set at 12-12 light-dark cycle. There were seven experimental diets (including the control diet). Each treatment was replicated four times. The water in the aquaria was aerated and filtered with sponge filters to remove suspended particles. The water was replenished daily to compensate evaporation, and was changed every two days. The feeding lasted for a period of six weeks, during which the fish were fed with the experimental diets at the rate of about 10% fish

Plate 4.1 Setup for the fish feeding trial.

wet weight in two equal feedings per day. Every week the fish were weighed and mortality recorded. The fish were weighed after 12 h of the last feeding, sacrificed, dried in an oven at 65°C until the weights were constant, and ground into powder before subjected to chemical analysis.

4.2.3 Chemical analysis

The whole fish was determined for their total N content (semi-micro Kjeldahl digestion followed by salicylate nitroprusside method using a QuickChem AE Lachat Automated Ion Analyzer), crude protein (total N x 6.25), crude fat content (diethylether extraction for 24 h, and evaporation of the solvent before weighing), ash (550°C for 2 h) and heavy metal contents (70% nitric acid digestion followed by a Hitachi Polarized Zeeman Atomic Absorption Spectrophotometer) (Allen *et al.*, 1974).

4.2.4 Statistical analysis

The data were subjected to analysis of variance and significant differences between the means were calculated according to the Fisher PLSD test. The regression analysis using the F-test at 90-95% interval was performed where necessary.

4.3 Results and Discussion

4.3.1 Growth response

4.3.1.1 Growth

Fig. 4.1 shows the changes in fresh weight of fish fed with the seven diets during the experimental period. Fish from the control group which were fed with the diet containing brown fish meal as its sole protein source gained the greatest weight (Table 4.3), followed by P/SW, P/DS, P/PM, E/SW, E/DS and E/PM. Significant difference ($P<0.05$) was found between the control and those fed with worm meals, indicating that worm meal from various wastes used in this experiment were not as good as fish meal in supporting fish growth. Since all the diets were formulated to contain the same level of crude protein, protein content was not the cause of growth depression.

High levels of inclusion of worm meal in the diets usually resulted in reduced feed intake and feed utilization efficiency, and a consequent reduction in the growth of fish have been reported by Stafford and Tacon (1984). They attributed unpalatability, nutritional deficiency and the presence of antinutritional factors in the diets as the main reasons of growth repression. Similarly, the same reasons may have led to poorer growth response in the present study when compared with conventional fish meal.

The coelomic fluid may be responsible for the unpalatable nature of *E. foetida*. Tacon *et al.* (1983) suggested that enhanced palatability of *E. foetida* can be achieved by the removal of the coelomic fluid by chemical irritation with water saturated with ether, formalin or salt, or by blanching with hot water. However, there is no experimental evidence or solid data to support this.

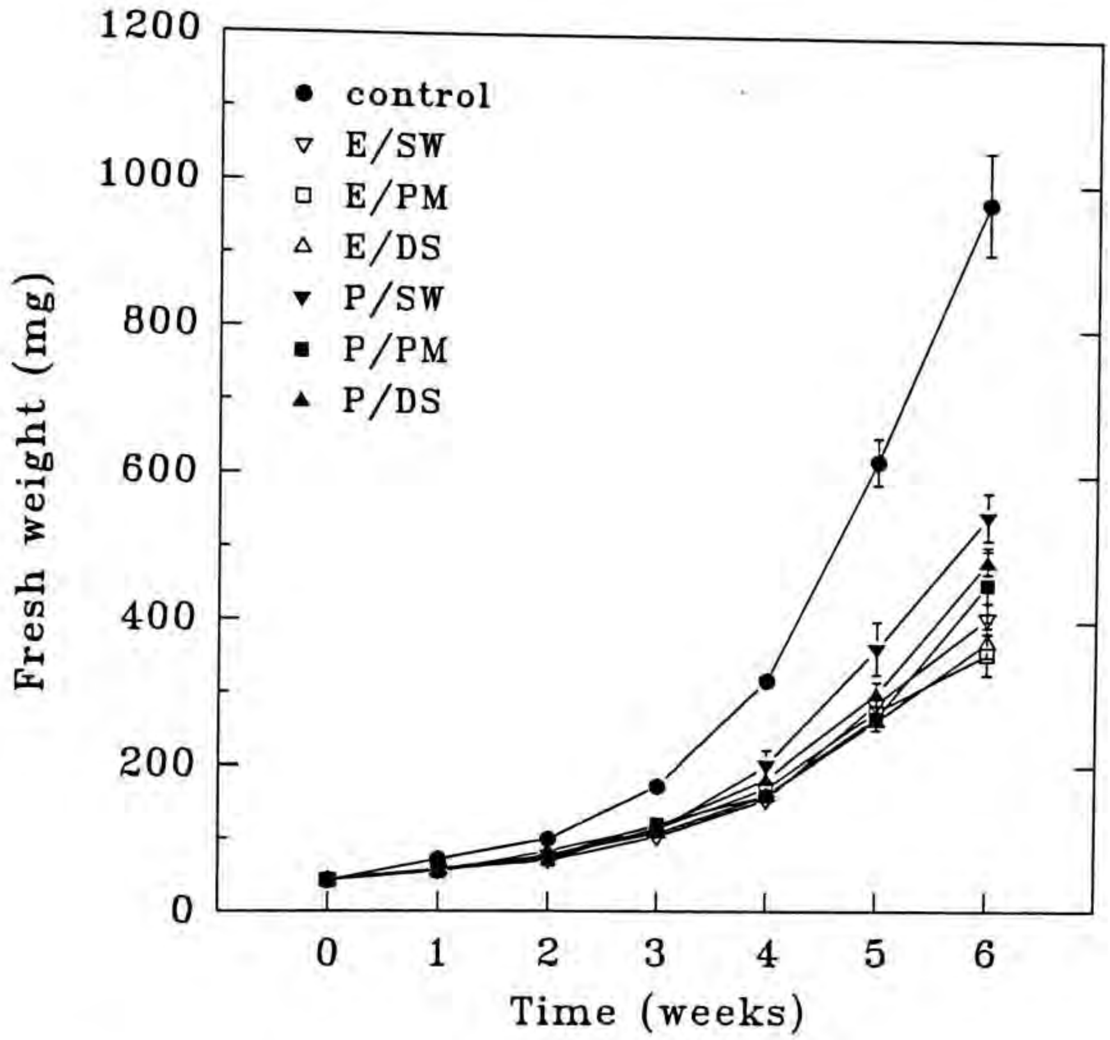


Fig. 4.1 Change in fresh weight in tilapia fed with diets containing various protein sources over 6 weeks (means of four replicates). Error lines denote standard errors. Control, E/SW, E/PM, E/DS, P/SW, P/PM and P/DS contained dietary protein from fish meal (control), worm meal of *E. foetida* from soybean waste (E/SW), pig manure (P/PM), digested sludge (E/DS), and worm meal of *P. asciatica* from soybean waste (P/SW), pig manure (P/PM), digested sludge (P/DS) respectively.

Table 4.3 Effect of diets containing various protein sources on fish growth and mortality (means of four replicates)

	Control	E/SW	E/PM	E/DS	P/SW	P/PM	P/DS
Initial weight (mg)	42.7[a] (1.75)	43.4[a] (1.66)	43.3[a] (1.57)	43.4[a] (1.38)	43.0[a] (1.79)	43.1[a] (2.44)	42.9[a] (1.63)
Final weight (mg)	975[c] (70.4)	405[ab] (21.6)	356[a] (29.6)	372[ab] (21.0)	545[b] (33.1)	452[ab] (51.4)	482[b] (15.6)
Growth rate (%)	2190[c] (155)	835[a] (40.7)	728[a] (83.3)	761[a] (63.2)	1180[b] (115)	979[ab] (181)	1030[ab] (78.5)
Mortarlity (%)	6.25[a] (2.08)	8.00[a] (4.00)	14.6[a] (2.09)	12.5[a] (2.42)	6.25[a] (6.25)	14.6[a] (4.00)	4.17[a] (2.41)

Control, E/SW, E/PM, E/DS, P/SW, P/PM and P/DS were the fish diets which contained dietary protein of fish meal (Control), worm meal of *E. foetida* from soybean waste (E/SW), pig manure (E/PM), digested sludge (E/DS), and worm meal of *P. asciatica* from soybean waste (P/SW), pig manure (P/PM) and digested sludge (P/DS) respectively.

Figures within a row followed by the same letter within a row were not significantly different at 95% according to the Fisher PLSD test.

Figures within brackets are standard errors.

It was evident that deficiencies of methionine, valine and lysine occurred in some or all of the worm meals of *E. foetida* and *P. asciatica*, and differences in these amino acids among fish meal and worm meal were generally small although some were significant ($P<0.05$). These may account partially for the poorer fish growth, since it was found that supplementary methionine and lysine in the diet for Nile tilapia (*Tilapia nilotica*) improved growth performance and feed utilization (El-Dahhar and El-Shazly, 1993). Thus, supplement of the deficient amino acids can increase the nutritional value of worm meal.

The antinutritional factors such as heavy metals or toxic organics present in the worm meals may be derived from deposition in worm tissue or contamination from waste substrate on body surface. Soaking in solution before the earthworms are processed may reduce the contaminants both inside and outside the earthworm bodies and help to get rid of the antinutritional factors from the worm meals (Tacon *et al.*, 1983).

Compared with worm meal of *E. foetida*, worm meal of *P. asciatica* supported better weight gain, although no significant difference ($P>0.05$) was found among the treatments of E/SW, E/PM, E/DS, P/PM and P/DS. It seems likely that the growth response associated with the diets was not only related to the earthworm species, but also the organic wastes on which the earthworms fed. Generally speaking, worm meal of *P. asciatica* gave better fish growth than that of *E. foetida*; and worm meal from soybean waste supported the greatest growth, followed by digested sludge and pig manure. Variation of growth rates of fish from worm meals of *E. foetida* and *P. asciatica* may be related to their different palatability, while the differences in fish growth among various worm meals from the different organic wastes were likely to be associated with the heavy metal contents of the worm meals.

4.3.1.2 Mortality

Mortality, though low, was noted in almost all of the treatments, especially at the beginning of the experiment. No significant difference ($P>0.05$) was found among treatments (Table 4.3). It is not sure whether toxic substances such as heavy metals in the diets was the cause of fish kill, since mortality was not statistically different among treatments and was not correlated with the metal content in the diets. It was accountable for fry fish that competition for food resulted in the death of the smaller ones.

4.3.2 Tissue chemical composition

4.3.2.1 Nutritional contents

The body composition of fish fed with various diets is shown in Table 4.4. Fish fed with P/PM had the highest protein content in fish body, which was significantly higher ($P<0.05$) than those fed with other worm meals. The highest crude fat content was found in fish from P/SW, while the lowest was from E/PM and P/PM. Fish from E/PM and P/PM possessed the greatest ash content, while the lowest was found in fish from P/SW. It is of interest that the

Table 4.4 The effect of diets containing various protein sources on the nutritional composition of fish tissue (%) (means of four replicates)

	Control	E/SW	E/PM	E/DS	P/SW	P/PM	P/DS
Crude protein	73.4^{a} (2.04)	72.5^{a} (1.48)	72.4^{a} (0.89)	72.1^{a} (0.62)	72.9^{a} (1.02)	79.8^{b} (1.00)	74.7^{a} (1.57)
Crude fat	13.4^{ab} (0.55)	12.9^{ab} (0.85)	12.0^{a} (0.67)	14.8^{ab} (1.53)	18.0^{c} (1.34)	12.1^{a} (1.18)	15.5^{bc} (1.14)
Ash	12.8^{bc} (0.43)	13.1^{bc} (0.61)	13.7^{c} (0.37)	11.6^{ab} (0.22)	10.8^{a} (0.88)	14.0^{c} (0.53)	12.4^{abc} (0.80)

Control, E/SW, E/PM, E/DS, P/SW, P/PM and P/DS were the fish diets which contained dietary protein from fish meal (Control), worm meal of *E. foetida* from soybean waste (E/SW), pig manure (E/PM), digested sludge (E/DS), and worm meal of *P. asciatica* from soybean waste (P/SW), pig manure (P/PM), digested sludge (P/DS) respectively.

Figures within a row followed by the same letter were not significantly different at 95% according to the Fisher PLSD test.

Figures within brackets are standard errors.

higher crude fat content, the lower was the ash content in fish body. Similar inverse relationship of crude fat and ash content has been reported by Mazid *et al.* (1979). However, they attributed this relationship to the difference in dietary protein levels in the diets, which was not the case in the present study.

4.3.2.2 Heavy metal contents

The concentrations of four heavy metals measured in the fish carcass are shown in Table 4.5. Deposit of Cu in fish carcass was found to be positively related to the Cu levels in the worm meals (Chapter 3 and Table 4.5). For Cd, Pb and Zn, no obvious relationship between the metal concentrations of the dietary proteins and the fish carcass was observed.

The calculated maximum allowance of the fish from worm meals for human consumption according to the US recommended dietary allowance (NRC, 1989) and the provisional tolerable intakes (WHO, 1987 and 1989) was limited by Pb contents to very low levels of 8.8, 9.1, 14.8, 16.4, 18.8 and 5.39 g in a dry weight basis for fish from the diets of E/SW, E/PM, E/DS, P/SW, P/PM and P/DS respectively. However, sources of Pb in the diets were not clear. If Pb content in fish diets came mainly from the worm meals, tilapia may be a species that tended to accumulate Pb in their bodies. Contents of Cd, Cu and Zn in fish tissue were not limitations for the consumption of fish from worm meals by human. The results of the metal contents in fish were derived from the whole fish analysis, which cannot specify the exact location of metal deposit in fish

Table 4.5 Heavy metal contents (μg/g) in tissues of tilapia fed with diets containing various protein sources (means of four replicates)

	Control	E/SW	E/PM	E/DS	P/SW	P/PM	P/DS
Cd	0.00^{a}	0.00^{a}	0.47^{bc} (0.16)	0.21^{ab} (0.15)	0.63^{c} (0.00)	1.10^{d} (0.16)	0.63^{c} (0.00)
Cu	8.60^{a} (0.16)	16.1^{b} (0.30)	36.6^{d} (2.53)	17.7^{bc} (0.39)	20.9^{c} (0.83)	33.0^{d} (3.35)	21.3^{c} (0.63)
Pb	17.5^{a} (7.37)	51.6^{ab} (17.5)	50.0^{ab} (25.2)	30.8^{a} (6.32)	27.7^{a} (5.91)	24.2^{a} (12.2)	84.4^{b} (18.1)
Zn	136^{a} (3.10)	181^{bc} (3.51)	189^{c} (2.03)	184^{c} (4.64)	171^{b} (6.52)	176^{bc} (6.22)	186^{c} (2.95)

Control, E/SW, E/PM, E/DS, P/SW, P/PM and P/DS were the fish diets which contained dietary protein of fish meal (Control), worm meal of *E. foetida* from soybean waste (E/SW), pig manure (E/PM), digested sludge (E/DS), and worm meal of *P. asciatica* from soybean waste (P/SW), pig manure (P/PM) and digested sludge (P/DS) respectively.

Figures within a row followed by the same letter were not significantly different at 95% according to the Fisher PLSD test.

Figures within brackets are standard errors.

tissue. From the results of Stafford and Tacon (1984), most trace elements were accumulated in tissues of liver, kidney, fin, bone and intestine rather than muscle, suggesting that fish from worm meals, although high in heavy metals, can be safely consumed by humans if the bone and viscera are removed and the metals in the worm meals do not elevated to alarming levels.

Fish growth was not affected by the uptake of Cd, Cu and Pb in fish tissue (Table 4.6); only Zn in fish tissue was found to be negatively related ($R^2 = 0.95$, $P < 0.001$) with fish growth (Fig. 4.2). Although protein source from worm meals supported poorer fish growth and resulted in higher heavy metal contents in fish carcass when compared with fish meal, to what extent the heavy metals in the worm meals are responsible for the growth repression is not known.

4.4 Conclusions

Both the worm meals of *E. foetida* and *P. asciatica* from soybean waste, pig manure and digested sludge as sole protein source in fish diet resulted in poorer growth when compared with conventional fish meal. Palatability and antinutritional factors in the worm meals which may originate from the waste substrates for the earthworms may be responsible for the growth repression. Growth response associated with the diets was not only related to the earthworm species, but the organic wastes on which the earthworms fed. Worm meals of *P. asciatica* supported better weight gain when compared with those of *E. foetida*;

Table 4.6 Relationship of fish growth rates and concentrations of various metals in fish carcass at the end of the experiment.

	Equation	R^2	P
Growth rate vs Cd	y=-394x+1270	0.10	0.50
Growth rate vs Cu	y=-32.4x+1810	0.39	0.14
Growth rate vs Pb	y=-9.54x+1490	0.19	0.33
Growth rate vs Zn	y=-27.3x+5880	0.95	0.0002

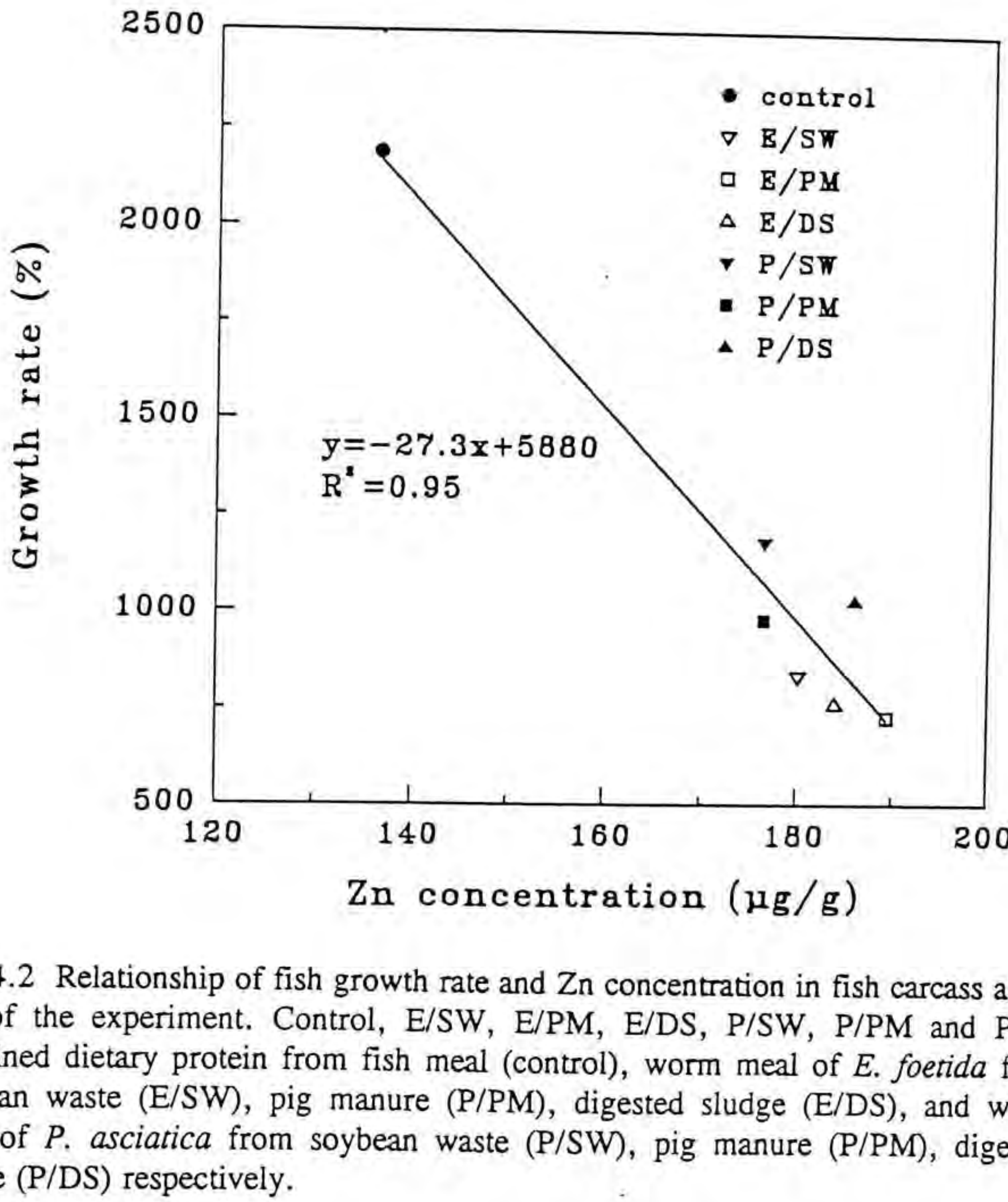


Fig. 4.2 Relationship of fish growth rate and Zn concentration in fish carcass at the end of the experiment. Control, E/SW, E/PM, E/DS, P/SW, P/PM and P/DS contained dietary protein from fish meal (control), worm meal of *E. foetida* from soybean waste (E/SW), pig manure (P/PM), digested sludge (E/DS), and worm meal of *P. asciatica* from soybean waste (P/SW), pig manure (P/PM), digested sludge (P/DS) respectively.

however, among the worm meal treatments, only the weight gain of fish from P/SW was found to be significantly higher ($P<0.05$) than those from other worm meals. Worm meals from soybean waste were the most promising one which supported better growth in terms of weight gain than those from pig manure and digested sludge. Worm meal as protein source elevated the heavy metal contents in the diets which resulted in higher levels of heavy metal deposition in fish carcass when compared with the control. However, toxic effect of heavy metals was only found in Zn which was highly correlated with the decline in fish growth.

Tilapia contained higher concentration of crude protein than those of worm meals of *E. foetida* and *P. asciatica*. Through feeding the diets containing worm meals, protein was concentrated in fish tissues at higher levels. Meanwhile, some heavy metals were found to deposit in worm tissues. To reduce the risk of heavy metal contamination, the use of worm meals should be restricted to these from innocuous wastes and earthworms cultured should be subject to process such as depuration prior to the preparation of fish diets.

CHAPTER 5. WORM-WORKED BEDDING AS POTTING MEDIA FOR PLANT GROWTH

5.1 Introduction

Earthworms have long been recognized for their abilities to improve soil quality in terms of structure and fertility. Some physical and biochemical processes are involved. Physical decomposition is accomplished by the activities of burrowing and feeding, which results in breaking down larger particles into smaller ones; while biochemical decomposition involves the action of some digestive enzymes in earthworm intestine, which results in the degradation of complex molecules into simpler ones (Wallwork, 1983). Earthworm participates in soil-forming process through their influence on soil pH, as agents of physical decomposition, by promoting humus formation, by improving soil texture and by enriching the soil with N and P (Syers and Springett, 1983; Wallwork, 1983). The products of decomposition are the humus-rich organic matter which are essential for plant growth.

Moreover, earthworms are found to reduce population of pathogens such as *Escherichia coli* and *Salmonella typhimurium* (Carmody, 1979). However, whether they kill these pathogens directly by biochemical methods or indirectly by improving aeration is uncertain. To exploit their contributions to soil, earthworms have been used to restore industrial wasteland and improve the quality of agricultural land (Curry and Cotton, 1983; Syers and Springett, 1983; Vimmerstedt, 1983).

Effects of earthworms on soil mostly appeared on soil physical and chemical characteristics. Most of the physical effects are related to the changes in pore size distribution and aggregate stability to applied stress (Syers and Springett, 1983). The larger pores created by burrowing enhance water infiltration, aeration and root penetration, while the medium pores created by casting increase water-holding capacity. All these characteristics are important to the normal functioning of the root system. However, these benefits to soil physical properties are available only in the presence of earthworms.

Compared with physical characteristics, effects of earthworms on soil chemical properties appear to be longer lasting and remain effective after the removal of earthworms. Earthworms chemically influence nutrient contents in soil (Syers and Springett, 1983). They not only increase the total amount of nutrients in soil, but also make them more available to plant uptake (Sharpley and Syers, 1977). Earthworms derive most of the nutrients from soil organic matter which includes plant detritus, animal residues and soil micro-organisms. These organic matters are partially assimilated into earthworm tissues, while the metabolic by-products are released into the soil as earthworm casts. Worm casts contain more available nutrients to plants than the soil matrix does (Chan and Griffiths, 1988; Parle, 1963). Chan and Griffiths (1988) showed that humus content increased by 40-60% in worm cast in a 10-day earthworm feeding trial.

Up to half of the N excreted by earthworms was in protein form, other was released as ammonia and urea (Needham 1957). Similarly, P availability was found to increase by the effect of earthworms. Earthworms which are fed on organic matter increase mineralization rates, at least for N and P (Syers and Springett, 1983). Through physical and chemical processes, earthworms can improve soil physical structure and enrich soil nutrient content.

Organic wastes are potential soil additive for improving both fertility and tillage. Application of organic wastes to soil increases soil aggregation, porosity, water holding capacity & hydraulic conductivity and decreases buck density which cannot be achieved directly by using chemical fertilizers. Worm-worked bedding which is a by-product of vermiculture is organic in nature and rich in microflora. Spent bedding from vermiculture can be an valuable source of soil amendment for agriculture and horticulture. However, the use of metal-rich wastes for culturing earthworms may result in the contamination of the bedding with heavy metals, and this limits its value as a medium for plant growth.

A greenhouse pot experiment was conducted to assess the agronomic value of used bedding materials from vermiculture as potting media for plant growth. *Brassica chinensis* (Pak choi), a common Chinese vegetable, was selected for the study. Particular attention was paid to heavy metal accumulation in edible tissues to assess the risk of the transfer of heavy metals along the food chain.

5.2 Materials and Methods

5.2.1 Preparation of potting media

Six spent bedding materials that had been used for the culture of *Eisenia foetida* and *Pheretima asciatica* on soybean waste, pig manure or digested sludge were mixed with a sandy loam soil (red-yellow podzol of 62% sand, 23% silt and 15% clay; pH, 4.9; organic C, 0.12%) in a ratio of 2:3 (v/v). At this ratio, the soil mixes had C/N ratios between 11 and 15 which was similar to that of nomal soil (about 10) (Allen *et al.*, 1974). Raw bedding, i.e. horse manure compost which had not been used for vermiculture, was also mixed with the sandy soil in the same ratio for comparison. The abbreviations of HMC, E/SW, E/PM, E/DS, P/SW, P/PM and P/DS are employed to stand for the mix of sand and horse manure compost (HMC), and the mixes of sand and spent bedding of *E. foetida* form soybean waste (E/SW), pig manure (E/PM), digested sludge (E/DS), and *P. asciatica* from soybean waste (P/SW), pig manure (P/PM), digested sludge (P/DS) respectively.

5.2.2 Chemical analysis of potting media

The potting media were analyzed for their pH (pH meter, sample:distilled H_2O = 1:10 (w:v)), electrical conductivity (conductivity meter, sample:distilled H_2O = 1:10 (w:v)), organic carbon (Walkley and Black, 1934), total nitrogen (semi-micro Kjeldahl digestion followed by salicylate nitroprusside method using a Lachat QuickChem AE Automated Ion Analyzer), extractable NH_4^+ (2M KCl extraction followed by salicylate nitroprusside method using a Lachat QuickChem AE Automated Ion Analyzer), total phosphorus and extractable phosphorus (semi-micro Kjeldahl digestion and 0.5 M pH 8.5 $NaHCO_3$ extraction respectively, followed by molybdenum blue method using a Lachat QuickChem AE Automated Ion Analyzer), total macronutrients (K, Na and Mg) and total heavy metals (Cu, Zn, Pb and Ni) by 70% nitric acid digestion followed by determination using a Hitachi Atomic Absorption Spectrophotometer (Allen *et al.*, 1974), and extractable macronutrients and heavy metals by 1 M neutral ammonium acetate extraction followed by determination using a Hitachi Atomic Absorption Spectrophotometer (Allen *et al.*, 1974).

5.2.3 Plant growth experiment

The plant growth experiment was carried out in a greenhouse using 20 cm diameter plastic pots arranged in completely randomized blocks (Plate 5.1). Five seeds of *Brassica chinensis* were sown to each pot containing the soil mixes. After ten days, the pots were thinned to result in two seedlings per pot. Half of the pots were left untreated, whilst the remainder were fertilized with commercial NPK fertilizer (15 N : 15 P :15 K) applied by surface broadcast at a rate equivalent to 50 kg N/ha. Each treatment was replicated four times. The plants were watered daily. The plants were harvested after 50 days. They were

Plate 5.1 Potting trial with *Brassica chinensis* in a greenhouse.

rinsed with distilled water and dried at 70° C in an oven for three days. The dry weight of the aerial part of the plants was determined.

5.2.4 Tissue analysis

The heavy metal contents of the shoot and root were determined by 70% nitric acid digestion followed by determination using a Hitachi Atomic Absorption Spectrophotometer (Allen *et al.*, 1974). The total nitrogen and phosphorus of the shoot portion were determined by semi-micro Kjeldahl digestion followed by salicylate nitroprusside method and molydenum blue method respectively using a Lachat QuickChem AE Automated Ion Analyzer.

5.2.5 Statistical analysis

The data were subjected to analysis of variance and significant differences between the means were calculated according to the Fisher PLSD test. The regression analysis using the F-test at 90-95% interval was performed where necessary.

5.3 Results and Discussion

5.3.1. Chemical properties of potting media

Chemical properties of potting mixes without additional fertilizer are shown in Table 5.1. The chemical nature of the soil mixes were that of the sandy soil and the spent bedding which had been used for vermiculture on

Table 5.1. Chemical properties of potting media which were mixture of horse manure compost or spent bedding (which had been used for the culture of earthworms on an organic waste) and a sandy soil at the ratio of 2:3 (v/v) (means of three replicates)

	HMC	E/SW	E/PM	E/DS	P/SW	P/PM	P/DS
pH	5.7 (0.07)	6.0 (0.00)	6.7 (0.03)	5.3 (0.07)	5.9 (0.03)	6.8 (0.07)	5.3 (0.07)
Conductivity ($\mu S/cm \times 10^2$)	1.79 (0.07)	2.48 (0.02)	3.19 (0.08)	1.74 (0.06)	2.87 (0.03)	3.43 (0.21)	2.37 (0.07)
Organic C (%)	5.82 (0.21)	4.97 (0.05)	5.68 (0.18)	5.08 (0.06)	5.39 (0.04)	5.77 (0.04)	5.38 (0.02)
Total N (%)	0.37 (0.02)	0.41 (0.01)	0.49 (0.01)	0.47 (0.02)	0.45 (0.00)	0.43 (0.01)	0.48 (0.02)
Extractable NH_4^+-N ($\mu g/g$)	28.7 (1.44)	37.5 (1.83)	24.9 (3.44)	44.9 (3.44)	18.7 (0.63)	21.2 (0.79)	15.2 (1.04)
C/N ratio	15.7	12.1	12.1	10.8	12.0	13.4	11.2
Total P (%)	0.069 (0.01)	0.12 (0.00)	0.15 (0.01)	0.12 (0.01)	0.11 (0.00)	0.20 (0.01)	0.12 (0.01)
Extractable P ($\mu g/g$)	559 (45.7)	518 (72.5)	1350 (132)	569 (30.1)	782 (56.5)	1360 (78.0)	1050 (169)
Total K (%)	0.09 (0.00)	0.10 (0.00)	0.13 (0.01)	0.06 (0.01)	0.12 (0.01)	0.17 (0.01)	0.08 (0.07)
Extractable K ($\mu g/g$)	685 (6.33)	843 (12.2)	1060 (15.9)	388 (21.9)	797 (11.6)	1060 (4.00)	525 (20.9)
Total Na (%)	0.08 (0.00)	0.08 (0.00)	0.09 (0.00)	0.07 (0.00)	0.09 (0.00)	0.09 (0.00)	0.08 (0.00)
Extractable Na ($\mu g/g$)	311 (5.69)	290 (1.73)	312 (1.86)	246 (2.89)	301 (0.33)	340 (6.89)	306 (2.85)
Total Mg (%)	0.08 (0.00)	0.10 (0.00)	0.11 (0.00)	0.07 (0.00)	0.10 (0.00)	0.14 (0.00)	0.09 (0.00)
Extractable Mg ($\mu g/g$)	417 (12.0)	393 (0.00)	691 (31.4)	381 (42.9)	536 (20.5)	762 (12)	500 (0.00)

HMC, E/SW, E/PM, E/DS, P/SW, P/PM, P/DS stand for the potting media which were mixture of sand and horse manure compost (HMC), mixture of sand and used-bedding of *E. foetida* from soybean waste (E/SW), pig manure (E/PM), or digested sludge (E/DS) and mixture of sand and used bedding of *P. asciatica* from soybean waste (P/SW), pig manure (P/DS), or digseted sludge (P/DS) respectively.

Figures within brackets are standard errors.

different wastes. The properties of the latter were the results of the physical and physiological activities of the earthworms feeding on a particular waste as well as the substances leached out and residues left from the organic waste added for vermiculture. Their properties largely depended on the organic waste added for growing worms, and to a much lesser extent on the species of earthworm cultured.

The potting mixes were slightly to moderately acidic. Potting media which contained bedding with worms fed on digested sludge (i.e. E/DS and P/DS) were the most acidic (pH=5.3), whilst those with bedding with worms fed on pig manure were only slightly acidic (pH 6.7-6.8). Electrical conductivity, which reflects the soluble salt concentration in soil, was the highest in E/PM and P/PM and lowest in HMC. Contents of organic C and total N in all soil mixes varied slightly, ranging from 4.97-5.82% and 0.37-0.49% respectively. Among the soil mixes, those with spent bedding of *E. foetida* contained higher concentrations of extractable NH_4^+ than those of *P. asciatica*, while that containing HMC had a concentration in between. Total P was the highest in P/PM and E/PM, and the lowest in HMC. Extractable P was the highest in pig manure-sand mixtures. Similarly, levels of K and Mg was the highest in spent beddings of manure, while those of Na were in a narrow range.

Much attention has been paid to heavy metal contamination in soils or soil amendments (Steffen, 1979). Although some trace metals such as Co, Cu, Fe, Mn, Mo, Ni and Zn are required for plant metabolism (Davidescu and

Davidescu, 1982), excessively high contents of these metals in soil will inhibit plant growth, and may be accumulated in plant tissue which may be taken up by their consumers. Moreover, non-essential heavy metals such as Cd and Pb are commonly found in organic wastes. With the application of these wastes in vermiculture, worm-worked beddings are unavoidably contaminated with heavy metals. This may reduce the agronomic value of spent bedding as a medium for plant growth.

Heavy metals of Cd, Cr, Cu, Ni, Pb and Zn in the various potting mixes were determined (Table 5.2). Cd and Cr were undetectable in all soil mixes, while Cu, Ni, Pb and Zn were found at various levels. Cu content was found to be exceptionally high in E/PM and P/PM, which is due to the fact that Cu is added to feed to promote pig fattening. Ni and Pb concentrations varied slightly among treatments, and they probably came from the sand used. Zn content was found to be slightly higher in E/PM and P/PM, which was derived from feed additive. When the metal contents in the potting mixes were compared to those of uncontaminated soil (Cd, <1-2; Cu, 2-60; Ni, 2-150; Pb, 10-150 and Zn, 25-200) (Thornton, 1981), E/PM and P/PM were heavily contaminated with Cu, and Pb was slightly excessive in almost all the soil mixes used.

Judging from the chemical composition of the potting mixes, it seems that worm-worked bedding was inferior to NPK fertilizers. However, a complete assessment of plant growth media can never be satisfied by conducting

Table 5.2 Total heavy metal contents ($\mu g/g$) of potting media which were mixture of horse manure compost or spent bedding (which had been used for the culture of earthworms on an organic waste) and a sandy soil at the rate of 2:3 (v/v) (means of three replicates)

	HMC	E/SW	E/PM	E/DS	P/SW	P/PM	P/DS
Cd	ND	ND	ND	ND	ND	ND	ND
Cr	ND	ND	ND	ND	ND	ND	ND
Cu	8.10 (0.17)	7.38 (0.17)	132 (4.38)	13.1 (0.17)	9.53 (0.17)	141 (1.78)	16.0 (0.17)
Ni	5.36 (0.36)	5.00 (0.41)	8.33 (1.19)	3.09 (0.48)	5.24 (0.63)	7.14 (0.72)	5.72 (0.72)
Pb	167 (2.18)	165 (9.13)	150 (1.35)	169 (1.91)	154 (2.23)	140 (6.83)	162 (5.56)
Zn	40.5 (0.61)	32.6 (0.74)	76.9 (1.18)	58.3 (4.39)	36.4 (0.29)	82.9 (0.29)	67.9 (0.29)

HMC, E/SW, E/PM, E/DS, P/SW, P/PM, P/DS stand for the mixture of sand and horse manure compost (HMC), mixture of sand and used-bedding of *E. foetida* from soybean waste (E/SW), pig manure (E/PM), digested sludge (E/DS) and mixture of sand and used-bedding of *P. asciatica* from soybean waste (P/SW), pig manure (P/PM), digested sludge (P/DS) respectively.

Figures within brackets are standard errors.

ND means not detectable.

chemical analysis alone, and should be complemented by planting trials to evaluate their edaphic quality for plant growth.

5.3.2 Plant growth status

As observed, plants without fertilizer grew well in E/SW, E/DS, P/SW and P/DS in the first 30 days, and were even better than in HMC. Vegetable in HMC grew faster in later stage and ultimately had a greater weight than those in spent beddings. Chlorosis was noted after 30 days and continued until harvest in all spent beddings (Plate 5.2). Dry weight of shoot from HMC was significantly higher ($P < 0.05$) than those from other treatments, except that from E/SW which was comparable to that from the control (Fig. 5.1). Nutrient deficiencies, especially that of N may occur in plants from potting mixes with worm-worked beddings as demonstrated by the lower N contents in shoot (Fig. 5.2).

With the application of NPK fertilizer, the soil mixes supported greater yields of *Brassica chinensis* than those without fertilizer (Plate 5.2). For soil mixes with additional fertilizer, a similar pattern of plant yields was obtained. HMC gave the highest yield, followed by the spent beddings containing soybean waste, digested sludge and pig manure. Application of NPK fertilizer in a rate of 50 kg N/ha resulted in greater shoot yields, as well as higher contents of N and P in shoot (Figs. 5.2 and 5.3).

It was found that shoot yield from unfertilized treatments were highly correlated ($P < 0.05$) with extractable Mg and total Zn content of the soil mixes,

Plate 5.2 Plant growth on various potting mixes with or without additional NPK fertilizer.

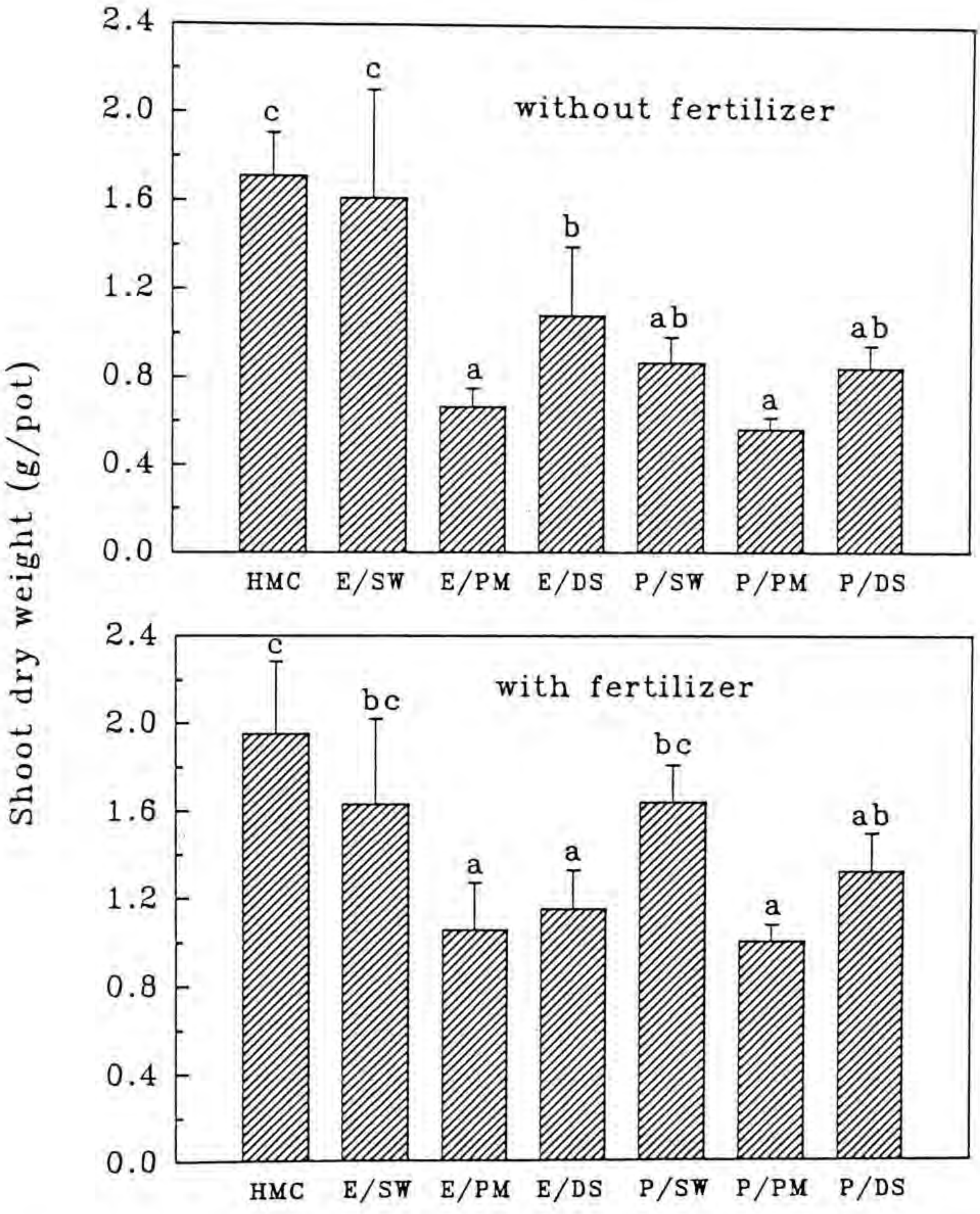


Fig. 5.1 Dry weight of shoot of *Brassica chinensis* grown on different worm-worked bedding and sand mixture with or without additional fertilizer (means of four replicates). Error lines denote standard errors. Bars having the same letters were not significantly different at 95% according to the Fisher PLSD test.

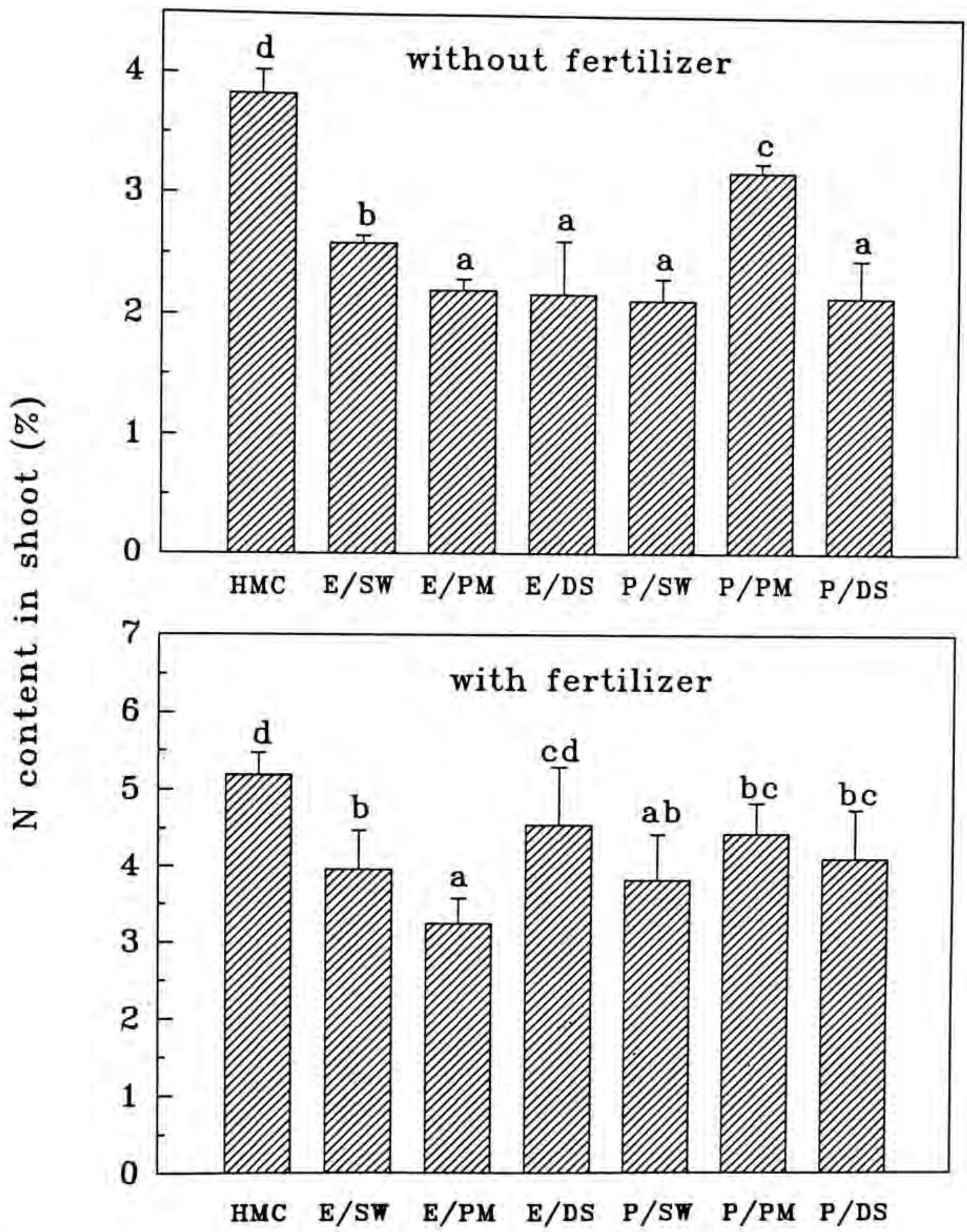


Fig. 5.2 Nitrogen contents in shoot of *Brassica chinensis* grown on different worm-worked bedding and sand mixture with or without additional fertilizer (means of four replicates). Error lines denote standard errors. Bars having the same letters were not significantly different at 95% according to the Fisher PLSD test.

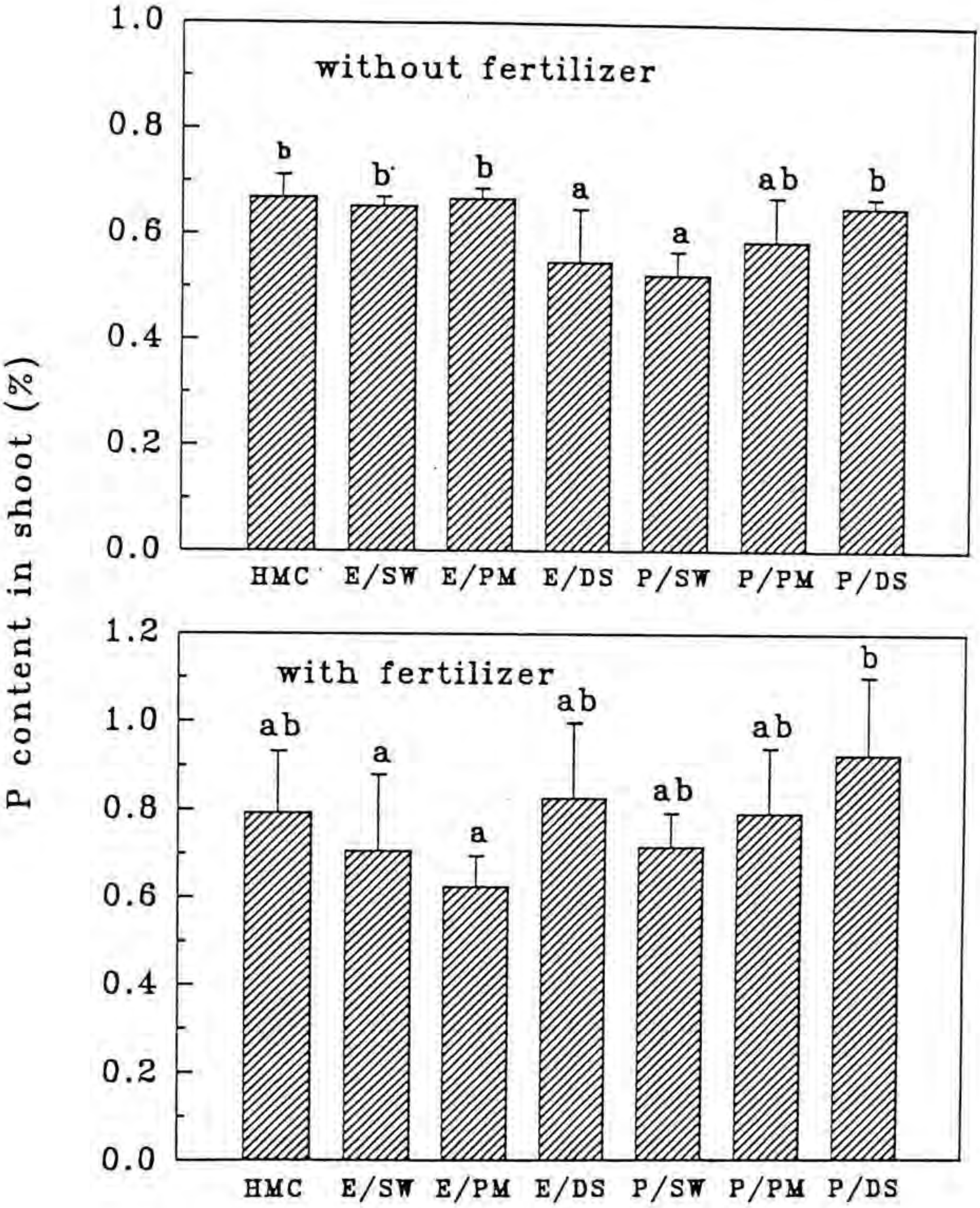


Fig. 5.3 Phosphorus contents in shoot of *Brassica chinensis* grown on different worm-worked bedding and sand mixture with or without additional fertilizer (means of four replicates). Error lines denote standard errors. Bars having the same letters were not significantly different at 95% according to the Fisher PLSD test.

and to those of conductivity and total Pb content to lesser extents ($P < 0.1$) (Table 5.3); while the shoot yield from fertilized treatments was found significantly correlated to total Zn content of the soil mixes, and to that of total Cu content to a lesser extent. Very poor growth was observed in soil mixes containing E/PM and P/PM in both unfertilized and fertilized treatments. This was probably attributed to the high salt content and extremely high contents of Cu and Zn in E/PM and P/PM (Tables 5.1 and 5.2). Salt damage may be a reason for the reduced yield in *B. chinensis* as shoot dry weight tended to decrease with increasing conductivity (Hirai *et al.*, 1986). The range exhibited by the various soil mixes with spent beddings all indicated that salinity may be a problem in the utilization of spent beddings for growing plants.

When compared with the soil mix of HMC, the most potential factors which caused growth depression in soil mixes containing spent beddings were high conductivity and heavy metal contents. The application of additional fertilizer obviously improved plant yields, indicating that nutrient deficiency was a limiting factor in unfertilized treatments and potential damage by salinity and phytotoxicity caused by high heavy metal contents may be reduced by soil enrichment with some of the macronutrients.

5.3.3 Heavy metal contents in plant tissue

Of the four heavy metal studied in the potting media (Table 5.2), only Cu, Pb and Zn were taken up by plant tissues. High Cu concentrations were

Table 5.3 Relationship of shoot dry weight and various chemical parameters of the soil mixes.

	± fertilizer	Equation	R^2	P
Conductivity	- fertilizer	y=-0.005x+2.27	0.48	0.09
	+ fertilizer	y=-0.003x+2.11	0.26	0.24
Extractable Mg	- fertilizer	y=-0.002x+2.30	0.62	0.04
	+ fertilizer	y=-0.002x+2.18	0.40	0.13
Total Cu	- fertilizer	y=-0.005x+1.27	0.43	0.11
	+ fertilizer	y=-0.004x+1.59	0.53	0.06
Total Pb	- fertilizer	y=0.031x-3.85	0.54	0.06
	+ fertilizer	y=0.081x-1.48	0.31	0.20
Total Zn	- fertilizer	y=-0.018x+2.05	0.63	0.03
	+ fertilizer	y=-0.015x+2.26	0.77	0.009

observed in plant tissues from E/PM and P/PM which were heavily contaminated with Cu. According to Lepp (1981), Cu had a low shoot/root ratio when compared with other metals in cotton, corn and sunflower. This was also true for *Brassica chinensis* (Table 5.4). Irrespective of the Cu content in the various potting media, shoot/root ratios were lower than one in all treatments, indicating that the Cu absorbed by roots remained mostly in root rather than moving to the aerial parts.

Pb contents in most of the potting media were slightly higher than that in normal soils (Table 5.2). Regardless of the high Pb content in soil, Pb was not elevated in the plant tissues to serious levels, especially for those in shoots (Table 5.5). Pb shoot/root ratios of all treatments were lower than one, indicating that Pb tended to accumulate mainly in root rather than shoot. Pb uptake was affected by pH, organic matter, and phosphorus (Koeppe, 1981). For the present study, no obvious pattern of Pb content in plant tissues as related to that in soil was observed. Moreover, application of NPK fertilizer did not make very much difference in Pb uptake.

Compared with the concentrations of 25-200 μg/g in non-contaminated soils (Table 5.2), Zn in tissue from the various potting media were in the average levels (Table 5.6). Tissues from mixes with spent beddings from pig manure and digested sludge were loaded with relatively higher concentrations of Zn when compared with the control (HMC) due to the high Zn content in beddings containing pig manure and digested sludge. *B. chinensis* was likely to

Table 5.4 Cu concentrations (μg/g) in potting mixes and shoot & root of *Brassica chinensis* grown on the different mixes with or without additional fertilizer (means of four replicates).

		HMC	E/SW	E/PM	E/DS	P/SW	P/PM	P/DS
potting mix		8.10^{Aa} (0.17)	7.38^{Aa} (0.17)	132^{Cc} (4.38)	13.1^{Aab} (0.17)	9.53^{Aa} (0.17)	141^{Bd} (1.78)	16.0^{Ab} (0.17)
- fertilizer	shoot	25.0^{Bb} (1.40)	19.5^{Ba} (0.34)	33.5^{Ac} (1.17)	20.5^{Bab} (0.84)	22.0^{Bab} (0.18)	58.1^{Ad} (3.97)	21.7^{ABab} (0.61)
	root	37.7^{Cc} (0.30)	27.7^{Ca} (1.06)	49.2^{Bd} (2.46)	27.8^{Ca} (2.70)	29.0^{Ca} (1.24)	60.8^{Ae} (3.68)	36.3^{Cb} (5.03)
+ fertilizer	shoot	23.2^{Ba} (0.62)	20.0^{Ba} (0.50)	45.4^{Bb} (2.52)	23.9^{BCa} (0.62)	21.1^{Ba} (0.36)	51.3^{Ac} (3.69)	21.3^{ABa} (0.74)
	root	44.0^{Cb} (4.49)	27.2^{Ca} (1.21)	50.4^{Bb} (3.07)	24.6^{BCa} (1.29)	23.0^{Ba} (0.71)	61.0^{Ac} (4.70)	23.7^{Ba} (1.47)

HMC, E/SW, E/PM, E/DS, P/SW, P/PM, P/DS stand for the potting mixes of sandy soil and horse manure compost (HMC), or spent bedding of *E. foetida* from soybean waste (E/SW), pig manure (E/PM), or digested sludge (E/DS), or spent bedding of *P. asciatica* from soybean waste (P/SW), pig manure (P/PM), or digested sludge (P/DS) respectively.

Figures within a column followed by the same letter in upper case were not significantly different at 95% according to the Fisher PLSD test. Figures within a row followed by the same letter in lower case were not significantly different at 95% according to the Fisher PLSD test.

Figures within brackets are standard errors.

Table 5.5 Pb concentrations (μg/g) in potting mixes and shoot & root of *Brassica chinensis* grown on the different mixes with or without additional fertilizer (means of four replicates).

		HMC	E/SW	E/PM	E/DS	P/SW	P/PM	P/DS
potting mix		167^{Dcd}	165^{Ccd}	150^{Dab}	169^{Dd}	154^{Cabc}	140^{Ca}	162^{Dbcd}
		(2.18)	(9.13)	(1.35)	(1.91)	(2.23)	(6.83)	(5.56)
- fertilizer	shoot	8.75^{Aa}	9.28^{Aab}	9.47^{Aab}	10.7^{Ab}	10.7^{Ab}	8.75^{Aa}	9.52^{Aab}
		(0.18)	(0.87)	(0.45)	(0.88)	(0.58)	(0.18)	(0.45)
	root	33.9^{Bab}	39.8^{Babc}	32.7^{Ca}	45.0^{Cc}	32.3^{Ba}	35.2^{Bab}	42.1^{Cbc}
		(2.21)	(2.96)	(0.47)	(3.07)	(1.21)	(4.59)	(4.40)
+ fertilizer	shoot	9.65^{Aab}	10.7^{Abc}	9.29^{Aa}	11.6^{Ac}	9.65^{Aab}	9.47^{Aab}	9.76^{Aab}
		(0.36)	(0.58)	(0.00)	(0.79)	(0.36)	(0.45)	(0.34)
	root	44.2^{Cd}	33.0^{Bbc}	25.0^{Ba}	37.3^{Bc}	30.0^{Bab}	28.9^{Bab}	27.2^{Bab}
		(3.38)	(1.28)	(2.39)	(1.50)	(1.70)	(0.89)	(2.22)

HMC, E/SW, E/PM, E/DS, P/SW, P/PM, P/DS stand for the potting mixes of sandy soil and horse manure compost (HMC), or spent bedding of *E. foetida* from soybean waste (E/SW), pig manure (E/PM), or digested sludge (E/DS), or spent bedding of *P. asciatica* from soybean waste (P/SW), pig manure (P/PM), or digested sludge (P/DS) respectively.

Figures within a column followed by the same letter in upper case were not significantly different at 95% according to the Fisher PLSD test. Figures within a row followed by the same letter in lower case were not significantly different at 95% according to the Fisher PLSD test.

Figures within brackets are standard errors.

Table 5.6 Zn concentrations (μg/g) in potting mixes and shoot & root of *Brassica chinensis* grown on the different mixes with or without additional fertilizer (means of four replicates).

		HMC	E/SW	E/PM	E/DS	P/SW	P/PM	P/DS
potting mix		40.5^{Ab} (0.61)	32.6^{Aa} (0.74)	76.9^{Ae} (1.18)	58.3^{Ac} (4.39)	36.4^{Aab} (0.29)	82.9^{Af} (0.29)	67.9^{Ad} (0.29)
- fertilizer	shoot	172^{Bbc} (27.9)	103^{Ba} (10.2)	199^{Bc} (15.2)	193^{Bc} (32.00)	121^{BCab} (7.00)	101^{Aa} (5.42)	207^{Cc} (26.72)
	root	239^{Cc} (14.1)	127^{Ba} (14.5)	178^{Bb} (14.3)	166^{Bb} (6.50)	153^{Cab} (8.41)	157^{Bab} (17.2)	158^{Bab} (9.89)
+ fertilizer	shoot	230^{Cbc} (18.5)	113^{Bab} (7.50)	256^{Ccd} (18.8)	278^{Cd} (9.14)	109^{Ba} (5.91)	237^{Ccd} (20.1)	242^{Ccd} (14.7)
	root	172^{Bb} (18.8)	156^{Cab} (7.40)	219^{BCc} (13.7)	178^{Bb} (6.32)	130^{BCa} (21.63)	238^{Cc} (5.44)	156^{Bab} (7.27)

HMC, E/SW, E/PM, E/DS, P/SW, P/PM, P/DS stand for the potting mixes of sandy soil and horse manure compost (HMC), or spent bedding of *E. foetida* from soybean waste (E/SW), pig manure (E/PM), or digested sludge (E/DS), or spent bedding of *P. asciatica* from soybean waste (P/SW), pig manure (P/PM), or digested sludge (P/DS) respectively.

Figures within a column followed by the same letter in upper case were not significantly different at 95% according to the Fisher PLSD test. Figures within a row followed by the same letter in lower case were not significantly different at 95% according to the Fisher PLSD test.

Figures within brackets are standard errors.

take up and even accumulate Zn in plant tissues which had the plant/soil ratios higher than 1.0. In the present study, shoot/root ratios were around 1.0, and might be influenced by pH, soil Zn levels or other soil properties.

5.4 Conclusions

Potting mixes containing spent beddings from various sources supported poorer growth of *B. chinensis* when compared with that containing unused bedding of horse manure compost. Exception was the soil mixes with spent beddings containing soybean waste which was the best among the various wastes and was comparable to the control in terms of shoot yield.

Potting mixes containing spent beddings from pig manure contained elevated concentrations of Cu & Zn and other soluble salts, and were not suitable for growing *Brassica chinensis* which had poor yield and high tissue metal contents.

Addition of NPK fertilizer improved plant growth in all treatments to various extent. Heavy metal accumulation was not raised to an elevated levels in plant tissues, except those of Cu and Zn from soil mixes of E/PM and P/PM. Worm-worked bedding should be regarded as a soil conditioner rather than a fertilizer due to high organic matter content and low N level. Its value was affected by the organic wastes added for vermiculture and its application rate should be adjusted depending on their chemical properties, and that of the soil to be mixed.

Chapter 6. GENERAL CONCLUSIONS

Recycling of agro-industrial wastes not only minimize the quantity of wastes for disposal but also conserve our finite resources. The bioconversion of wastes into invertebrate biomass provides a protein source which can be used as animal feedstuff and the residual medium can be utilized for agricultural purpose. Earthworms, especially the epiges, which live on plant litter, animal manure and other decaying matter, demonstrate a great ability to convert a wide spectrum of organic wastes into earthworm biomass with a high protein content. This combined with their rapid growth and high reproduction potential makes them the suitable candidate which can be exploited in protein production from organic wastes.

The culture trials with *Eisenia foetida* and *Pheretima asciatica* demonstrated that both earthworm species were suitable candidates for vermiculture because of their high growth rates and reproductive potential. However, not all the waste materials were readily utilized by earthworms for maximum growth. Large particle size, poor digestibility and unfavourable nutritional quality might be the main constraints. To increase the utilization efficiency, the nutrient contents of the substrates can be adjusted by mixing various wastes materials with complementary chemical properties, while the large particle size can be reduced by mechanical or biochemical means.

It is undoubtedly that the protein contents of the organic wastes are concentrated to very high levels in earthworm tissues. The amino acid profiles of the worm meals are excellent which are comparable to that of fish meal. Vermiculture is a very efficient system for the production of animal protein from organic wastes which may be otherwise low in protein content or not of food grade for fish or livestock.

However, heavy metal problems are generally encountered in vermiculture system which reduce the value of worm meals as animal feedstuffs. Applying heavy metal contaminated organic wastes, such as pig manure and digested sludge, to vermiculture may result in biomagnification along the food chain, i.e. organic waste→earthworm→fish (Table 6.1), as noted in most of the cases, e.g. Cd, Cu, Pb and Zn.

Although serious accumulation was not observed in the present study probably due to the duration of the experiment conducted, heavy metal contamination of worm meals is still the major barrier in the utilization of worm meals from wastes. To safeguard the contamination of the consumers along the food chain, wastes used for vermiculture should be limited to the uncontaminated ones, and worm meals with elevated metal contents should be restricted from utilization as feed.

Palatability is another problem in utilizing worm meals as animal feedstuffs. Worm meals, especially those from species which secret too much coelomic fluid, are not palatable to fish. These earthworms can be fed to other

Table 6.1 Transfer of heavy metals (μg/g) in the food chain (organic wastes → earthworm→ tilapia).

		E. foetida	*P. asciatica*
Cd	soybean waste	0.00 → 1.25 → 0.00	0.00 → 0.00 → 0.63
	pig manure	8.04 → 3.22 → 0.47	8.04 → 1.79 → 1.10
	digested sludge	2.14 → 3.57 → 0.21	2.14 → 0.71 → 0.63
Cr	soybean waste	0.80 → 2.32 → 0.00	0.00 → 1.24 → 0.00
	pig manure	12.8 → 2.21 → 0.00	12.8 → 3.84 → 0.00
	digested sludge	49.3 → 3.62 → 0.00	49.3 → 7.00 → 0.00
Cu	soybean waste	13.2 → 15.6 → 16.1	13.2 → 39.2 → 20.9
	pig manure	2060 → 131 → 36.6	2060 → 152 → 33.0
	digested sludge	235 → 27.9 → 17.7	235 → 51.5 → 21.3
Pb	soybean waste	0.00 → 3.40 → 51.6	0.00 → 1.25 → 27.7
	pig manure	85.7 → 7.62 → 50.0	85.7 → 4.05 → 24.2
	digested sludge	137 → 5.80 → 30.8	137 → 5.54 → 84.4
Zn	soybean waste	38.2 → 118 → 181	38.2 → 104 → 171
	pig manure	2400 → 210 → 189	2400 → 141 → 176
	digested sludge	1480 → 208 → 184	1480 → 134 → 186

animals rather than fish, e.g. frogs, chickens and pigs (Edwards *et al.*, 1985).

Profitability of vermiculture depends on the market of the products from this system, including the worm meals and spent beddings. Assuming that dried earthworms can substitute for the existing protein sources in animal feed rations, their value per tonne is likely to be from £200 to £400; and the value of the spent beddings is to be between £20 and £40 per tonne (Fieldson,1985). Removal of the earthworms from beddings is the most complicated and costly operation. Therefore, vermiculture systems of centralized operations where wastes are available and transportation cost is minimized would have more advantages than the alternative small farm based operations (Fieldson, 1985).

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