VERMICOMPOSTING OF COD (Gadus morhua) OFFAL MIXED WITH SPHAGNUM PEAT

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BY

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ABSTRACT

Vermistabilization is the stabilization of organic wastes using earthworms. Most studies have analysed the use of earthworms in the stabilization of sewage sludge and vegetable wastes, however, there have been no in-depth studies which examine vermicomposting with fish offal.

Sphagnum peat mixed with cod (*Gadus morhua*) offal was vermicomposted for 8 weeks with earthworms (*Eisenia fetida*) following a two week pre-composting period. Vermicomposting samples were compared with the same mixtures in the absence of earthworms. Results showed that the maximum proportion of cod offal that can be used during vermicomposting to ensure a 100% survival rate was 13% (dry wt.). Cod offal of 15% or more (dry wt.) was toxic to the earthworms resulting in death; this was true even when the pre-composting period was extended by more than two weeks. Peat was selected as the bulking agent because peat adsorbs much of the ammonia (NH_3) gas, which is released during the decomposition of the cod offal, as ammonium ion (NH_4^+). As a result of the ammonia gas being adsorbed there is a reduction in the amount of nitrogen that is lost during the decomposition process. Results indicated that, when earthworms were initially added to a compost mixture, the level of ammonium ion should not exceed 1.0 mg/kg to allow for an earthworm survival rate of 100%.

The rate of organic matter stabilization was determined by measuring the reduction in the volatile solids content of the waste. Vermicomposting for 8 weeks produced a material

with a significantly higher percentage of ash compared to composting for a similar period ($P<0.05$). It was concluded that vermicomposting resulted in a more stable material compared to composting. Results also indicated that earthworms increased the proportion of some available nutrients (*i.e.*, phosphorus, potassium, calcium, magnesium) which are needed for good plant growth. Thus, the results showed that vermicomposting is an effective method for stabilizing cod offal.

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ABBREVIATIONS

AAS	=	Atomic Absorption Spectrophotometer
Ca	=	Calcium
$CaCO_3$	=	Calcium Carbonate
CH_3OH	=	Methanol
$CHCl_3$	=	Chloroform
C/N Ratio	=	Carbon/ Nitrogen Ratio
CO_2	=	Carbon Dioxide
H^+	=	Hydrogen Ion
H_2O	=	Water
$H_2PO_4^-$	=	Phosphoric Acid
HPO_4^{2-}	=	Phosphoric Acid
H_2SO_4	=	Sulphuric Acid
K	=	Potassium
KCl	=	Potassium Chloride
Mg	=	Magnesium
N	=	Nitrogen
NaOCl	=	Sodium Hypochlorite
NaOH	=	Sodium Hydroxide
NH_3	=	Ammonia

NH_4^+ = Ammonium Ion

NO_3^- = Nitrate

OH^- = Hydroxide Ion

P = Phosphorus

TKN = Total Kjeldahl Nitrogen

TOC = Total Organic Carbon

Tukey's HSD Test = Tukey's Honest Significant Difference Test

1.0 INTRODUCTION

Various studies have focussed on the use of earthworms in the stabilization of organic residues such as sewage sludge, animal manure, crop residues, and industrial waste (Edwards, 1988; Neuhauser *et al.*, 1988; Albanell *et al.*, 1988; Shanthi *et al.*, 1993; Frederickson *et al.*, 1997; Elvira *et al.*, 1998). Seafood processing also generates a substantial volume of wastes. There is evidence that fish offal has been used as a soil conditioner by traditional composting methods for hundreds of years (Blandford *et al.*, 1916), yet there is very little information available on the use of fish offal as a substrate for vermicomposting. This study examined the feasibility of converting cod offal into a useful fertilizer by vermicomposting.

1.1 Composting

Traditional aerobic composting is the biological decomposition and stabilization of organic substrates, under conditions that allow development of thermophilic temperatures as a result of biologically produced heat, to produce a final product that is stable, free of pathogens and plant seeds, and can be beneficially applied to land (Haug, 1993). Thus, a stable compost product is one in which there is no further decomposition occurring, the temperature is constant and the pile is not longer heating up, and the organic material is completely broken down.

The main products of biological metabolism are carbon dioxide, water, and heat. Microorganisms of importance in composting include bacteria and fungi. The objectives of

composting have traditionally been to biologically convert putrescible organics into a stabilized form and to destroy organisms pathogenic to humans; to retain the nutrient content of the organic waste fraction (nitrogen, phosphorus, potassium and minerals); and to produce a product that can be used to support plant growth and improve soil structure. The Nitrogen-Phosphorus-Potassium (N-P-K) percentages of finished compost are relatively low, however, their benefit lies in the release of nitrogen and phosphorus in the soil at a slow enough rate that plants can use them and they are not lost through leaching (Martin and Gershuny, 1992).

1.1.1 Elements Necessary for Composting

The five elements that are critical to the composting process are: temperature, air, moisture, carbon-nitrogen ratio, and pH levels. Composting can occur at temperatures ranging from 20 °C to 60 °C or higher. Temperature is a major factor in determining the type of microorganisms, the rate of metabolic activities, and the rate of biodegradation of the organic waste (Hobson and Wheatley, 1993).

Air is needed to supply the aerobic microorganisms with oxygen and to keep the compost pile from turning anaerobic. Air movement is also necessary to remove water from wet substrates through drying, and to remove heat generated by organic decomposition which will help to control process temperatures (Haug, 1993).

Moisture is essential to maintain microbial activity. Lack of moisture can impose severe rate limitations on the composting process. Moisture levels must be high enough to assure adequate rates of biological stabilization, yet not so high that free airspace is

eliminated which can reduce the rate of oxygen transfer and in turn the rate of biological activity (Haug, 1993). On the basis of previous research it would seem that the majority of composting processes should operate with a moisture content in the 40-60% range (Hobson and Wheatley, 1993).

The C/N ratio has a fundamental significance because nitrogen is necessary to support cellular synthesis and carbon makes up the largest fraction of organic molecules in the cell (Haug, 1993). Carbon is oxidized (respired) to produce energy and metabolized to synthesize cellular constituents. Nitrogen is an important constituent of protoplasm, proteins, and amino acids. An organism can neither grow nor multiply in the absence of nitrogen in the form that is accessible to it. Although microbes continue to be active without having a nitrogen source, the activity rapidly dwindles as cells age and die (Diaz *et al.*, 1994). During active aerobic metabolism, microbes use about 15 to 30 parts of carbon for each part of nitrogen. Hence, rapid composting is favoured by maintaining a C/N ratio of approximately 30 or less. A higher C/N ratio can slow the compost process, however a C/N ratio that is too low can lead to a loss of nitrogen as ammonium N (Inbar *et al.*, 1991). Mature composts with a high C/N ratio are acceptable as long as further decomposition is slow and the microorganisms do not require additional N from the soil. If microorganisms are still using N from the soil, then the compost is not mature and should not be mixed with regular soil on the ground. The reason for this is that when an immature compost is applied to the ground the microorganisms will continue to use the N that is already present in the soil on the ground and there will actually be a decrease in N for the plants, rather than an increase in N.

However, compost can be used, even if the C/N ratio is high, as long as the product is mature and stable. Gotaas (1956) refers to studies where composting a material with a C/N ratio of 78 produced a biostable product with a C/N ratio of 35.

Very low or very high pH levels can impose rate limitations on microbial activity. The optimum range for most bacteria is between 6.0 and 7.5, whereas the optimum range for fungi is 5.5 to 8.0 (Diaz *et al.*, 1994). Fortunately, composting has a rather unique ability to buffer both high and low pHs back to a neutral range as composting proceeds. This ability to buffer extremes of pH is caused by the fact that both carbon dioxide (a weak acid) and ammonia (a weak base) are released as a result of organic decomposition (Haug, 1993). Carbon dioxide is an end product of all organic decomposition, while ammonia is an end product of protein decomposition. These components will tend to neutralize extremes of low or high pH, therefore, pH adjustment of the starting substrates is usually not required.

1.1.2 Compost Quality

Quality of the final compost product depends on a number of important criteria. These include physical, biological, and chemical criteria such as the presence of physical contaminants (such as glass, metal, or plastic), particle size, organic matter content, pH, moisture content, trace element content, and nutrient content. Criteria can also be established to define compost stability or maturity. Immature composts induce high microbial activity in the soil for some time after their incorporation, causing oxygen deficiency and a variety of indirect toxicity problems to plant roots (Hobson and Wheatley, 1993). For example,

when an immature compost is used as a soil amendment or plant growth medium, it may immobilize nitrogen (N), thereby causing N deficiency in fast growing crops. Stability and maturity usually go together (Golueke, 1977). Specific oxygen consumption rate, absence of phytotoxic compounds, reduction of biodegradable volatile solids across the system, and a return to near ambient temperatures at the end of the process can be used to measure compost stability (Haug, 1993). Compost stabilization can also be determined through measuring ammonia levels. Ammonia is usually present in the early stages of composting as organic nitrogen is decomposed. The ammonia concentration is eventually reduced through volatilization or oxidation to the nitrate form. Thus, the presence of nitrate and absence of ammonia are indicative of a stabilized condition (Haug, 1993).

Compost produced in Canada may be subject to regulation by the federal and the provincial governments. Several provinces have guidelines and standards which determine the suitability of the material for use on a regulated or unregulated basis. The standards in Canada are based on four criteria for product safety and quality: maturity, foreign matter, trace elements, and pathogens (Composting Council of Canada, 1997).

1.2 Vermicomposting

1.2.1 The Basis of Vermicomposting

The principles behind vermicomposting are relatively simple and related to those involved in traditional composting. Certain species of earthworms can consume organic

wastes very rapidly and fragment them into much finer particles by passing them through a gizzard, an organ that all earthworms possess.

The worms derive their nourishment from the microorganisms involved in waste decomposition and from the organic waste that is being decomposed. The worms maintain aerobic conditions in the wastes, ingest solids, convert a portion of the organics to worm biomass and respiration products, and expel partially stabilized matter as discrete particles (castings) (Loehr *et al.*, 1985). The composition of the castings depends upon the original material that the earthworms digest.

The earthworms also promote further microbial activity in the compost so that the faecal material, or "casts" that they produce, are much more fragmented and microbially active than what the earthworms consume (Edwards and Fletcher, 1988). Thus, the worms and the microorganisms act symbiotically to accelerate and enhance the decomposition of the organic matter. The performance of the process is a function of a) the portion of the waste that is biodegradable, b) maintenance of aerobic conditions, and c) avoidance of toxic conditions (Loehr *et al.*, 1985). Overall, the vermicomposting process is a result of the combined action of earthworms and of microflora living in earthworm intestines and in the growth medium (Albanell *et al.*, 1988).

1.2.2 Optimal Conditions for Vermicomposting

The processing of organic materials by earthworms occurs most rapidly at temperatures between 15 °C and 25 °C and at moisture contents of 70 percent to 90 percent

(Edwards, 1995). Outside these limits, earthworm activity and productivity, and thus the rate of waste processing, falls off dramatically. For maximum efficiency, the feedstock should be maintained as close to these environmental limits as possible.

Earthworms also have well defined limits of tolerance to certain chemical conditions. If these limits are exceeded greatly, the worms may move to more suitable zones in the pile, die, or, at best, process the materials very slowly. In particular, earthworms are very sensitive to ammonia and salts and certain other chemicals. For instance, they will die quite quickly if exposed to more than 0.5 mg/g of ammonia and more than 5.0 mg/g of salts (Edwards, 1988). However, salts and ammonia can be washed out of the organics readily or dispersed by pre-composting.

1.2.3 Benefits and Drawbacks of Vermicomposting

Vermicomposting is very beneficial because it can break down organic residuals into a valuable, finely divided plant growth medium with excellent porosity, aeration, and water-holding capacity, rich in available nutrients with superior plant growth characteristics. As well, in the traditional aerobic composting process, the organic materials have to be turned regularly or aerated in some way in order to maintain aerobic conditions. In vermicomposting, the earthworms, which survive only under aerobic conditions, take over both the roles of turning and maintaining the organics in an aerobic condition, thereby lessening the need for expensive engineering.

The major constraint to vermicomposting is that, in contrast to traditional composting

(a thermophilic process), vermicomposting systems must be maintained at temperatures below 35 °C. Exposure of the earthworms to temperatures above this, even for short periods, will kill them (Edwards, 1995). To avoid such overheating requires careful management. Since vermicomposting does not go through a high temperature phase, if materials containing pathogens are used they may need an additional pre-composting phase or sterilization process to ensure that pathogens are killed. There is, however, scientific evidence that human pathogens do not survive the vermicomposting process (Edwards and Bohlen, 1995).

1.3 Benefits of Earthworms

Earthworms are one of the most important and valuable of all the soil organisms because their activities are entirely beneficial to the soil and its crops. The most important function of earthworms (and microorganisms) is to break down plant and animal litter. Soil flora and fauna (including earthworms) break down plant material into a finely-divided organic complex known as "humus" (Mishra and Tiwari, 1993). Earthworms thrive on compost and contribute to its quality through both physical and chemical processes.

The importance of earthworms in affecting soil structure, organic matter processing, and nutrient cycling has long been recognized (Darwin, 1881; Edwards and Lofty, 1977; Lee, 1985; Blair *et al.*, 1995). For example, earthworm activity usually 1) improves soil structure, 2) enhances the number of beneficial microorganisms in the soil, 3) accelerates mineralization of organic matter, and 4) increases the availability of nutrients (Edwards and

Lofty, 1977; Lee, 1985; Martin and Gershuny, 1992).

1.3.1 Eisenia fetida

Many earthworm species are suitable for vermicomposting, but attention has focussed on a few species that are known to prefer habitats with high concentrations of organic wastes and to breed prolifically. The earthworm used most often in vermicompost studies is the lumbricid *Eisenia fetida*, otherwise known by its common name as the tiger or brandling worm (Lee, 1985). This species is favoured because it has a wide temperature tolerance and can live in wastes with a good range of moisture contents. It is a hardy worm, readily handled, and, in mixed cultures, usually becomes dominant (Edwards, 1988). As well, *E. fetida* has a high consumption and a high reproductive rate, for example, each worm is capable of eating its own body weight in 24 hours, and *E. fetida* will reproduce very fast and will mature in approximately 9 weeks (Edwards, 1988).

1.3.2 Basic Environmental Requirements of Earthworms

1.3.2.1 Food Supplies

Earthworms can use a wide variety of organic materials for food, and even in adverse conditions extract sufficient nourishment from soil to survive. However, earthworms feed mainly on dead and decaying plant (and animal) remains and on free-living soil microflora and fauna (Lee, 1985). Although the bulk of food ingested is dead plant tissue, living microorganisms, fungi, microfauna, and mesofauna and their dead tissues are also ingested,

and there is evidence that they are an important part of the diet. As they eat, however, earthworms also ingest large amounts of soil, sand, and tiny pebbles. It has been estimated that an earthworm ingests and discards its own weight in food and soil every day. Earthworms literally eat their way through the soil, obtaining nourishment from the organic matter contained in it, and bring their wastes to the surface. It is in this way that they turn over the soil.

1.3.2.2 Adequate Moisture

Perhaps the most important requirement of earthworms is adequate moisture. Water constitutes 75 - 90% of the body weight of earthworms, so prevention of water loss is important for earthworm survival (Edwards and Lofty, 1977). In a study by Edwards (1988), the optimum moisture conditions for *E. fetida* was 80 - 90% with a limit of 60 - 90%.

1.3.2.3 Suitable Temperature

The activity, metabolism, growth, respiration, and reproduction of earthworms are all greatly influenced by temperature. The temperature limit for survival of earthworms varies between species (Lee, 1985). It was determined that the optimum temperature for *E. fetida* was 25 °C and it had a tolerance of temperatures from 0 °C - 35 °C (Edwards, 1988).

1.3.2.4 Suitable pH

Earthworms are sensitive to pH, which limits the distribution, numbers, and species

of earthworms that live in any particular soil. *E. fetida* are relatively more tolerant than other species with regard to pH, but when given a choice in pH gradient, they move towards the more acid material, with a pH preference of 5.0 (Edwards, 1997). Edwards (1988) concluded that the optimal conditions for *E. fetida* was a pH of > 5 and < 9.

1.3.3 Physical Effects on Soils

Earthworms are probably the most important soil-inhabiting invertebrates in terms of their influence on soil formation and maintenance of fertility in soil ecosystems. Earthworms turn over tremendous quantities of soil on an annual basis and can induce major structural changes in soil which have important consequences for both water and nutrient fluxes. The final physical structure of the plant growth medium or vermicompost is usually a finely-divided peat-like material with excellent structure, porosity, aeration, drainage, and moisture-holding capacity. Some of the earthworm activities includes breaking down plant remains into smaller pieces which provides a greater surface area for further microbial activity. As well, earthworms ingest and turn over substantial amounts of organic waste (Lee, 1985).

Two of the most important structural features of soils that are influenced by earthworms are soil aggregation and macroporosity. Aggregates are mineral granules joined together in such a manner that they can resist wetting, erosion or compaction and remain loose when the soil is either dry or wet. A soil that is rich in aggregates remains well-aerated and drained, so that the formation of aggregates is of prime importance to fertility. The most

direct way that earthworms contribute to the stability of soil aggregates is through the production of casts (Edwards *et al.*, 1995). Most workers have agreed that earthworm casts contain more water-stable aggregates than the surrounding soil (Edwards and Lofty, 1977). However, freshly-deposited casts appear less stable than other soil aggregates and stability increases with time after deposition (Shipitalo and Protz, 1987). As earthworm casts age, various physical, chemical, and biological processes influence their stabilization, maximum stability being attained 15 days after excretion (Tomlin *et al.*, 1995). Thus, stabilized casts contribute significantly to the improved aggregation of soil.

Earthworms also influence the porosity of soils. Soils with earthworms drain from four to ten times faster than soils without earthworms (Edwards and Lofty, 1977). Thus, earthworms influence the drainage of water from soil and the moisture-holding capacity of soil, both of which are important factors for growing crops (Edwards *et al.*, 1995).

1.3.4 Earthworms and Microorganisms

There are many reports of an increase in the numbers of microorganisms in the earthworm gut, or in cast material, relative to the surrounding soil. The role of these microorganisms is unclear but it seems likely that they form an essential part of the earthworm diet, enabling the animal to grow. *E. fetida* does not gain weight when feeding on mineral soil or cellulose, but does when feeding on microorganisms. Bacteria and protozoa are preferred to fungi, and protozoa may be essential for earthworm development (Wood, 1989).

It is generally agreed that the earthworm gut contains essentially the same kinds of organisms as are present in the soil in which the worms are living. Microbial numbers are generally higher in casts than in the surrounding soil and may remain so for some weeks. For example, according to Edwards and Lofty (1977) earthworms increased the number of microorganisms in soil as much as five times (Satchell, 1983). This is partly explained by the fact that earthworm casts, rich in ammonia and partially-digested organic matter, provide a good environment for microbial growth (Wood, 1989). Thus, the worms and the microorganisms act symbiotically to accelerate and enhance the decomposition of organic matter.

1.3.5 Stabilization of Organic Matter

Soil organic matter is, by definition, the organic fraction derived from living organisms. It includes living organisms, and partly-decomposed and decomposed plant and animal residue. The decomposed organic fraction is usually called "humus" (Tan, 1996). Thus, the quicker that organic matter is decomposed then the faster humus will be formed.

Neuhauser *et al.* (1988) demonstrated that vermicomposting sewage sludge could accelerate organic matter stabilization compared to incubating sludge without earthworms. Earthworms can accelerate the stabilization of many organic materials and produce a compost with superior plant-growth enhancement properties (Harris *et al.*, 1991). Thus, it is well established that earthworms can increase the rate of volatile solids destruction when present. The usual effect of earthworms is to accelerate the breakdown of coarse organic

matter and contribute directly and indirectly to increased soil respiration, processes leading to loss of C from the system (Blair *et al.*, 1995). The more rapid degradation of the organic matter is probably due to increased aeration and other factors brought about by the earthworms (Edwards, 1997). Stabilization is influenced by the amount and nature of organic matter incorporated, cast age, and moisture status. Casts also contain enzymes such as proteases, amylases, lipase, cellulase, and chitinase, which continue to disintegrate organic matter even after they have been excreted (Edwards and Lofty, 1977). Decomposition of organic matter is much faster and more intensive in the casts than in the soil.

1.3.6 Nutrient Transformations

Nutrients are also an important aspect of the final compost product. Nitrogen, phosphorus, and potassium are the elements which are most likely to be lower than required for optimum plant growth. Thus, commercial fertilizer is defined by the concentration of N-P-K. Calcium and magnesium are considered secondary fertilizer elements because they are often naturally present in soil in adequate concentrations (Winegardner, 1996).

The soil mixing process carried out by earthworms involves chemical changes that release potential nutrients from 'bound', and unavailable, states into forms that can be assimilated by plants. During the vermicomposting process, the important plant nutrients in the organic material - particularly nitrogen, phosphorus, potassium, and calcium - are released and converted through microbial action into forms that are much more soluble and available to plants than those in the parent compounds (Edwards, 1995). It is accepted that

energy and nutrients obtained by plants are usually tied up in plant residue at the end of the growing season (Berry, 1994). Mineralization, the release of inorganic nutrients during the decomposition of organic materials, must occur before this material can be returned to the ecosystem. Earthworms may participate in these processes directly and indirectly.

Earthworms cannot increase the total amount of nutrients in the soil but can make them more available (Barley and Jennings, 1959; Sharpley and Syers, 1977) and they may increase the rate of nutrient cycling, thereby increasing the quantity available at any one time (Syers and Springett, 1984). Thus, availability of nutrients (*i.e.*, N, P, K, Ca, Mg) in earthworm casts is generally higher than in bulk soils, and many studies have indicated that earthworm casts are important microsites for some specific nutrient transformations (Edwards and Lofty, 1977).

1.3.6.1 Nitrogen

Nitrogen exists in soils in two major forms: (1) organic N and (2) inorganic N. Plants satisfy their nitrogen requirements from the inorganic fraction. The organic fraction serves as a reserve of nitrogen in plant nutrition, and will be released only after decomposition and mineralization of organic matter. Most of the nitrogen in soils (more than 90%) is in organic form, and only a small portion is present in inorganic form (Baruah and Barthakur, 1997). Under normal conditions, only 0.5-2.5% of the total N is converted into forms accessible to the plant (Baruah and Barthakur, 1997). Inorganic nitrogen, mainly nitrates and ammonium, are the available nitrogen forms that are used by plants (Tan, 1996).

Nitrogen is a vital component of all protein, essential for the formation of new plant protoplasm. Without sufficient nitrogen, a plant is stunted and turns pale green or yellow, starting with the lower leaves. The stems of members of the grass family, such as corn, will be slender, and the whole plant will lack vigour (Martin and Gershuny, 1992).

Earthworms can significantly affect N transformations in casts. An example of a direct effect of earthworms is the mineralization of nitrogen. Nitrogen is commonly bound in organic complexes and, as such, is not readily available to plants. Passage through the earthworm gut apparently converts this bound nitrogen into more readily 'available' forms, such as ammonia, nitrites, and nitrates (Wallwork, 1983). Thus, earthworms are capable of N mineralization, which is the release of inorganic nutrients during the decomposition of organic materials. However, there is much debate over the topic of earthworms and the extent to which they enhance N fixation, which is the ability to reduce nitrogen to ammonia gas, and the potential significance of this phenomenon (Blair *et al.*, 1995).

Thus, the concentration of inorganic nitrogen in fresh earthworm casts is usually much greater than in surrounding soil, with ammonium usually being the dominant form of inorganic nitrogen in casts (Scheu, 1987; Lavelle *et al.*, 1992). The increase in inorganic nitrogen in earthworm casts is due to excretory products and mucus from the earthworm, as well as from increased rates of mineralization of organic nitrogen by microorganisms in the casts (Parkin and Berry, 1994). The increase in inorganic N is also a result of excretion of ammonia into soil as it passes through the gut (Blair *et al.*, 1995). The rate of nitrification (conversion from ammonia N to nitrate) in casts can be high and some authors have noted

a simultaneous increase in nitrate and decrease in ammonium as casts age (Parle, 1963; Syers *et al.*, 1979).

Parle (1963) observed that freshly-deposited casts were high in ammonium, but with time the ammonium decreased with a concomitant increase in nitrate, indicating high nitrification. Similar findings were reported by Syers *et al.* (1979), who investigated the mineral-N dynamics of earthworm casts incubated for 12 days and found that 87% of the ammonium initially present in casts was lost, but that increases in the nitrate pool did not match losses in ammonium. It was suggested that the resulting N deficit was due to a combination of immobilization and denitrification (Parkin and Berry, 1994). Moreover, Barley and Jennings (1959) observed that a proportion (6%) of the non-plant available N ingested by the lumbricid *Allolobophora caliginosa* (Savigny) was excreted in forms readily available to plants. The above observations suggest that the major participation of earthworms in the N cycle lies, directly or indirectly, in their ability to increase the rate of mineralization of organic N and to increase the amount of available N in vermicomposts (Syers *et al.*, 1979).

1.3.6.2 Phosphorus

Phosphorus is an essential plant nutrient, and is taken up by plants in the form of inorganic ions: $H_2PO_4^-$, and HPO_4^{2-} (orthophosphates) (Hesse, 1971). Phosphorus is necessary for photosynthesis, for energy transfers within plants, and for good flower and fruit growth. Unlike nitrogen, phosphorus has more to do with plant maturation than with plant

growth. Deficiencies of phosphorus are characterized by stunted early growth, poor root development, and most notably by reddish or purple colouration on the undersides of leaves. Because seed production is influenced by phosphorus, seed abnormalities may also indicate a lack of this element. Adding phosphate to your compost also prevents nitrogen loss through ammonia volatilization (Martin and Gershuny, 1992).

Phosphorus availability in casts is often significantly greater than in bulk soils (Sharpley and Syers, 1977; Tiwari *et al.*, 1989; Krishnamoorthy, 1990). Increased phosphorus availability in fresh earthworm casts has been attributed to an increase in phosphatase activity in egested material (Satchell and Martin, 1984), although it remains unclear to what extent the increased phosphatase activity is due directly to earthworm-derived enzymes, as opposed to increased microbial activity (Park *et al.*, 1992).

1.3.6.3 Potassium

Potassium, K, is an essential element for plant growth. In the fertilizers industry this element is called potash. Together with nitrogen and phosphorus, it is one of the major fertilizer elements (Tan, 1996). Potassium is used by plants in many life processes, including the manufacture and movement of sugars, and cell division. It is necessary for root development and helps plants to retain water (Martin and Gershuny, 1992). Potassium in soil is often bound up with silicates. Symptoms of deficiency appear in older leaves first and take the form of yellowing at the edges. Later, leaf edges turn brown and may crinkle or curl. Other potassium deficiency effects are: poor keeping quality of fruit, increase in disease

susceptibility, increase in incidence of low temperature damage, and retardation of maturity (Hesse, 1971).

Amounts of available potassium have also been reported to be elevated in earthworm casts, relative to bulk soils (Tiwari *et al.*, 1989). Basker *et al.* (1992) found that earthworm activity increased the proportion of total soil potassium in exchangeable form, presumably by shifting the equilibrium between exchangeable and non-exchangeable forms in the soil.

1.3.6.4 Calcium

Calcium, Ca, is a very important cation in soils. In addition to increasing pH of acid soils, it is believed to have a beneficial effect in development of soil structure. It is also an essential macronutrient for plant growth (Tan, 1996). In plants, calcium is essential for the growth of meristems and root tips and tends to accumulate in leaves as calcium pectate. At least 30% of the adsorption complex of a soil must be saturated with calcium for the average crop to obtain sufficient amounts. A deficiency of calcium stunts plant roots and gives recognizable leaf symptoms (Hesse, 1971). A lack of calcium appears to affect the stems and roots of growing plants. Plants deficient in calcium are retarded in growth and develop thick woody stems; seedlings will have stubby roots with brownish discolouration.

Earthworm activity may significantly affect calcium availability in soils. Many species of earthworms possess calciferous glands or esophageal regions which are involved in production of $CaCO_3$ spherules. Spiers *et al.* (1986) reported that earthworms can convert calcium oxalate crystals on ingested fungal hyphae to calcium bicarbonate, which then is

egested in cast material. This temporarily increases calcium availability in the fresh casts, and increases pH which could affect concentrations of other soluble nutrients available for plant uptake (Blair *et al.*, 1995).

1.3.6.5 Magnesium

Magnesium, Mg, is one of the essential macronutrients for plant growth. It is an essential constituent of chlorophyll, vital in photosynthesis (Tan, 1996). Magnesium is also involved in enzyme reactions. The element affects the translocation of phosphorus and has been reported to increase sugars, vitamins, starches, and inulin in root crops (Hesse, 1971). It also functions as a carrier for phosphorus, and the two deficiencies often go together. Insufficient magnesium is manifested as discolouration in the tissue between veins, which may cause leaves to look streaked. In some plants, leaves develop a reddish or purplish colouration, and the leaf margins turn brown or yellow while the veins remain green (Martin and Gershuny, 1992). Earthworm activity may also significantly affect magnesium availability in soils.

1.4 Vermicomposting of Fish Offal

There are only a few literature references concerning the changes in chemical and biochemical parameters during vermicomposting. Of those studies that do exist none of them examine the vermicomposting of fish offal. However, fish offal is a very useful substrate

because it contains valuable nutrients such as nitrogen and phosphorus (Mathur *et al.*, 1986).

1.4.1 Problems with Vermicomposting Fish Offal

It is likely that there is not much information on the vermicomposting of fish offal because of the problems that may be encountered. For example, all earthworms have difficulty degrading protein matter in meat and fish offal due to toxic effects from ammonia, produced from decomposition of protein, and from inorganic salts (Edwards, 1988). As discussed earlier, both ammonia and inorganic salts have very sharp cutoff points between toxic and nontoxic effects, *i.e.* > 0.5 mg/g of ammonia and > 5.0 mg/g salts (Edwards, 1988). However, wastes that have too much ammonia become acceptable after this is removed by a period of composting because the ammonia is released as a gas, also excessive ammonia and salts can be washed out of the waste. Thus, if toxicity problems can be reduced, then vermicomposting of fish offal may be a very beneficial process and result in a useful soil conditioner.

1.4.2 Peat as a Bulking Agent

When vermicomposting fish offal it is necessary to use generous amounts of bulky, high-carbon materials such as shredded brush, straw, sawdust, or peat to balance the high nitrogen and moisture of the fish, to increase aeration, and to discourage packing down which may lead to anaerobic composting (Martin and Gershuny, 1992).

Peat was chosen in this study as the best bulking agent to examine vermicomposting

of fish offal because it has good odour control and has excellent water absorption capacities, which renders it very suitable for fish offal which has a high moisture content. The high air:water ratio in most friable peats, wide C:N ratio, and large capacities for retaining water, heat, and odours should also help when composting fish offal with peat (Mathur *et al.*, 1986).

It was decided that *sphagnum* peat could meet these requirements well because it is a fibrous peat and as a result there are still microorganisms present as there is still decomposition occurring. In addition, peat was chosen because peats have high market acceptability as soil conditioners although by themselves they are generally poor sources of fertilizer elements. One potential constraint could be the occurrence of biostatic phenolics in some peats. These inhibitors, however, are largely deactivated by calcium and proteins (Mathur, 1991; Mathur, 1998).

Peat is also best for composting fish offal in terms of nitrogen conservation (Liao *et al.*, 1995). For plant use nitrogen needs to be in nitrate or ammonium forms. Peat adsorbs ammonia, a major gaseous product of protein decomposition, as ammonium ion by the moist, acidic peat fibres which reduces the loss of nitrogen (Mathur *et al.*, 1986). It has been found that the final product obtained from peat mixed with fish offal through traditional composting methods is of high quality with an earthy odour and good concentrations of organic and inorganic nutrients (Martin and Patel, 1991; Martin, 1998). Peat is low in nutrients; however, these will be added through the fish offal. Consequently, fish offal mixed with peat may produce an excellent finished product when vermicomposted.

1.5. Peat

Peat accumulates typically at about 3 cm per 100 years in a bog whose vegetation is well-established, and where hydrological conditions are favourable. Chemically, peats are largely organic material; *i.e.*, peat which has been dried and then burned, leaves little ash. The amount varies with type of peat, but ash content of 2-10% can be regarded as typical. The greater the decomposition, the greater the ash content. Thus, the amount of ash in lower levels of peat is greater than that in upper levels (Fuchsman, 1980).

The degree to which plants have undergone "humification", *i.e.*, have lost their original character and become an organic soil, is of considerable importance in the preliminary assessment of the possible uses of the peat. Peat with some fibre is acceptable for composting, however peat in which almost all the fibres are broken down is useful as a fuel. The measurement of the degree of decomposition of peat is inexact. The more widely accepted measurements consider that decomposition corresponds to replacement of the fibrous structure characteristic of plant tissue by exceedingly fine particles of no regular structure. A system, used in field testing, consists simply of squeezing a fresh sample of wet peat in the hand and assessing how much water drips from the hand and also whether or not the peat is more like mud that squeezes through the fingers, which is quite subjective and difficult to use in a quantitative sense (Fuchsman, 1980).

The Post system distinguished ten grades of "humification". Horticultural peat is made up of light brown, slightly humified (H-1 to H-2) *Sphagnum* and an underlying, darker

layer of some humification (H-3 to H-5) representing a minerotrophic-ombrotrophic transition and consisting of sedges in mixture with *Sphagnum*. Below that (H-5 to H-10) is a dark brown, stringy deposit suitable for fuel and consisting of more than 50 percent by volume of sedges (Crum, 1988).

1.6 Availability of Materials

The materials used in this study are of major importance in the Newfoundland area due to their availability. For example, Canada has one of the largest fisheries of all countries in the world which produces a large amount of fish offal. Traditionally, fishery wastes have been disposed of by returning them to the sea, however, this practice is being discouraged due to environmental problems. Fish wastes include whole waste fish, offal that contains viscera, and fish scrap that is the residue of filleting. The scraps (racks) contain skin, heads, fins, tails, and backbones (Mathur, 1991; Mathur, 1998). The fish frames and guts are the most important source of wastes (Martin and Patel, 1991; Martin, 1998).

Northern regions such as Newfoundland, which possess a limited amount of good soil, can benefit from the production of a nutrient-rich product such as compost or vermicompost for soil enhancement. As for bulking agents to be employed for composting, Newfoundland possesses peat resources as well as a forestry industry that produces waste organic materials in the form of sawdust, bark, and wood chips (Martin *et al.*, 1993). In particular, peatland makes up 17% of Newfoundland & Labrador (Keys, 1992).

2.0 OBJECTIVES

The goal of this study is to turn cod offal into a useful material which will improve soil structure and fertility, and also provide a good plant growth medium. It must first be determined whether or not vermicomposting is a viable option to dispose of cod offal. To examine this aim further, this study has three main objectives:

1) to determine the appropriate percentage of peat mixed with cod offal for the vermicomposting process;
2) to determine the maximum proportion of ammonium (mg/kg) at the initial point of vermicomposting in which *Eisenia fetida* can have a 100% survival rate; and
3) to determine the chemical changes during vermicomposting of cod offal, in comparison to controls without worms.

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Earthworms (Eisenia fetida)

The earthworm, *Eisenia fetida*, was used for all vermicomposting experiments. The earthworms were purchased from Trouter's Special Worm Farm in Bay Bulls, Nfld. Adult worms were used and the average weight of 30 worms was between 3.0 - 4.0 grams.

3.1.2 Sphagnum Peat Moss

Sphagnum peat was used as the bulking agent. This peat was collected near Bay Bulls Big Pond just outside of St. John's, Nfld. Samples were collected from the upper layers of the peatland at a depth of about 30 cm. The humification rate ranged from 2 - 5 according to the Post scale, which means that there was some fibrous materials remaining in the peat (Fuchsman, 1980). The peat was used approximately 1-2 months after it was collected. The peat was stored in plastic bags to maintain moisture content. The physical and chemical characteristics of the peat are listed in Table 1.

3.1.3 Cod (Gadus morhua) Offal

The fish offal was cod *(Gadus morhua)* racks which consisted of the skin, spine, fins, tails, and cheeks. All pieces were ground up (< $1cm^2$) and then frozen. The cod offal was

thawed before use in the experiment. The cod offal was obtained from Skipper's Fish Market in Mount Pearl, Newfoundland. The physical and chemical characteristics of the cod offal are listed in Table 1.

3.1.4 Containers

The plastic containers were 0.5 litres (tube-style). The dimensions were 2.5 inches high with a circumference of 5 inches. Holes in the lids allowed aeration.

3.2 Experimental Design

The experiments were carried out in the presence and absence of the earthworm, *Eisenia fetida*, and continued for 10 weeks (including 2 weeks of pre-composting). The samples were kept at room temperature (25 ± 2 °C) and the humidity in the room was between 55 ± 10%. The moisture content inside the containers was maintained at approximately 80%. To maintain the moisture content the containers were weighed every week and the amount of weight loss in grams was the amount of distilled water added in mL.

Processing of the feedstocks prior to the first stage of composting is termed "pre" or front-end processing (Haug, 1993). The pre-composting involved traditional composting methods without the addition of earthworms. Frederickson *et al.* (1997) demonstrated that to ensure the vermicomposting system operates at maximum efficiency, in terms of worm growth and reproduction, pre-composting should be kept to a minimum, consistent with effective sanitization of the waste. Wastes, such as animal manures and fish offal, can

Table 1. Composition of raw materials used in the vermicomposting process.*

	Cod Offal	Sphagnum Peat
Moisture	77.76% ± 1.34%	83.39% ± 1.62%
pH	7.48 ± 0.17	4.17 ± 1.05
Ash	20.42% ± 2.50%	1.35% ± 0.01%
Total Kjeldahl Nitrogen (TKN)	11.67% ± 1.33%	0.76% ± 0.00%
Total Organic Carbon (TOC)	44.21% ± 1.39%	54.80% ± 0.00%
C:N Ratio	3.82 ± 0.34	72.99 ± 2.59
Exchangeable Ammonium (NH_4^+)	n.d.[1]	2.80 μg/kg ± 0.30
$P_{Available}$	6.11%[2]	14 mg/kg ± 3
$K_{Available}$	0.37%[2]	31.21 mg/kg ± 5.07
$Ca_{Available}$	10.19%[2]	232.92 mg/kg ± 40.65
$Mg_{Available}$	0.27%[2]	169.17 mg/kg ± 20.97
Total Lipids	2.98% ± 0.31%	n.d.[1]

* All results are in dry weight, except for moisture content.
[1] Not Determined
[2] (Mathur et al., 1986)

require ageing to reduce produced ammonia content before they are acceptable to worms (Edwards, 1988). Thus, there was an initial pre-composting period of 2 weeks, after which 30 adult *E. fetida* were added to the vermicompost containers.

Initially, the cod offal was completely surrounded by peat. The containers were mixed after the first and second week during pre-composting. A pre-composting period of two weeks was selected because by the time the worms were to be added the samples were almost homogenous and the cod offal was almost completely broken down (by visual inspection). There was manual mixing every week to allow for further aeration. The composition of the mixtures used in the study is indicated in Table 2. In each container there was always a combined total weight of peat and cod offal of 30 g (dry wt.)

A vermicomposting period of 8 weeks was selected because at this point almost all the material in the containers had been digested by the earthworms into castings. Analysis was conducted on control samples (without earthworms) at the initial pre-composting period, after the initial two weeks (at week 0) when the worms were added, and then after 2, 4, 6, and 8 weeks of vermicomposting. Vermicomposting samples were analyzed after the worms had been added for 2, 4, 6, and 8 weeks. Three replicates of each mixture were studied for each analysis period (3 without worms and 3 with worms). Samples were sacrificed for each analysis period. This means that there were 36 containers established for each of the mixtures. Chemical analysis was conducted for each separate container (results are the average of three containers).

Table 2. Initial composition of vermicompost mixtures.

Compost Mixture	Composition[1]
1	100% Peat
2	7% Cod Offal, 93% Peat
3	9% Cod Offal, 91% Peat
4	11% Cod Offal, 89% Peat
5	13% Cod Offal, 87% Peat
6	15% Cod Offal, 85% Peat
7	17% Cod Offal, 83% Peat
8	19% Cod Offal, 81% Peat
9	21% Cod Offal, 79% Peat

[1] All mixtures had a total dry weight of 30g.

For the mixtures in which there was 100% survival of the earthworms at week 0, the earthworms remained in the containers and digested the material until the containers were sacrificed at 2 week intervals for a total of 8 weeks of vermicomposting. However, for the mixtures in which there was less than 100% survival at week 0, there were further studies to determine whether or not there would be a better survival rate if the pre-composting period was extended longer than 2 weeks. Earthworms were then added after 4, 6, 8, and 10 weeks of pre-composting and the survival rate was recorded. Chemical analysis was conducted every two weeks on all samples with 100% peat, as well as all samples with the highest % of cod offal in which there was 100% survival of the earthworms at week 0. For the other percentages of cod offal in which there was 100% survival, chemical analysis was only completed on samples for the final week of vermicomposting (week 8). In the mixtures in which there was a full 8 weeks of vermicomposting, the earthworms started to show signs of malnourishment after 6 weeks because no further cod offal was added; however, all of the worms survived for the completion of the study.

For chemical analysis, the worms were removed from the vermicomposting samples. Then, all the soils were emptied and spread to air dry on flat trays. In order to compare soils it is essential to standardize by air-drying even though the results may be different from those which would be obtained in the field (Hesse, 1971). Analysis of undried samples is not recommended because of the effect of moisture content on soil to extractant ratio and the need for determination of moisture content (Bates, 1993). After air-drying, the soil was homogenized by mechanical grinding.

These experiments were conducted in small containers under controlled laboratory conditions and the results may not be exactly the same as those that would occur under field conditions. However, the experiments did evaluate fundamental factors affecting the activity of earthworms and the results should be indicative of the impact of field conditions on the vermistabilization process.

3.3 Analytical Methods

3.3.1 pH

The degree of acidity or alkalinity in soils is determined by the resting hydrogen ion (H^+) concentration in the soil solution after a known amount of water to soil is added. An acid soil has more H^+ than OH^- ions, whereas a basic or alkaline soil contains more OH^- than H^+ ions. To characterize these conditions the term, soil pH, is used (Tan, 1996). The pH value of a soil is a measure only of intensity of acidity and not of the amount of acid present. When measuring soil pH in water, the main concern is that an increase in the amount of water added will cause an increase in pH; it is therefore important to keep the ratio constant and as low as possible. The soil pH tends to be higher when measured at wider (higher) soil:water ratios (Jackson, 1958).

The pH of the compost samples was measured with an Accumet 950 pH Meter and electrode (AOAC Method 973.04, 1990). Approximately 3.00 grams of air-dried soil was put into a 125 mL conical flask. Then, 50.0 mL of distilled water was added and the contents

were stirred occasionally. After 1 hour a pH reading was taken. For pH of the cod offal, 20.00 grams of cod offal was mixed with 40.0 mL of distilled water in a blender for one minute. The contents were poured into a 125 mL conical flask, after which a pH reading was taken.

3.3.2 Moisture

Moisture content for compost samples and cod offal was determined by drying at 105°C for 24 hours (AOAC Method 967.03, 1990). The following equation was used to calculate moisture:

$$\% \text{ Moisture} = (W_1 - W_2 / W_1 - W_0) \times 100$$

W_0 = Weight of Empty Dish
W_1 = Weight of Dish plus Wet Sample
W_2 = Weight of Dish plus Dry Sample

3.3.3 Ash

Ash content was used to determine volatile solids content by heating samples at 550°C for 24 hours (AOAC Method 967.04, 1990). The following equation was used to calculate ash:

$$\% \text{ Ash} = (W_1 - W_0 / W_2 - W_0) \times 100$$

W_0 = Weigh of empty crucible & cover after drying to constant weight
W_1 = Weight of crucible, cover, & ash after drying
W_2 = Weight of crucible, cover, & sample before ashing

3.3.4 Total Organic Carbon (TOC)

Total Organic Carbon (TOC) was calculated by the following equation (Golueke, 1977):

$$\%TOC = (100 - \% \text{ ash residue})/ 1.8$$

3.3.5 Total Kjeldahl Nitrogen (TKN)

Total N was analysed by the Kjeldahl method using a Buchi 426 Digestion Unit and a Buchi 315 Distillation Unit. The method involved digestion, distillation, and titration (AOAC 955.04, 1990).

For the digestion phase, approximately 0.20 grams of sample was weighed onto nitrogen-free paper and placed in a Kjeldahl digestion tube (actual weight recorded to 4 decimal places). Two Kjeltabs were added to each tube. 20.0 mL of concentrated H_2SO_4 was added to the digestion tubes. The samples were digested for approximately 70 minutes. The digestion continued until the sample turned colourless or clear. About 60.0 mL of distilled water was added to the cooled sample.

Then, 50.0 mL of 4% boric acid (40.00 grams of boric acid crystals in 1.0 L distilled water) was measured into six 200 mL conical flasks. 0.50 mL N-point indicator was added to each flask. The Kjeldahl tube was placed on the distillation unit and the conical flask containing the boric acid was placed on the swivel shelf. After the tube was connected to the Distillation Unit, approximately 100.0 mL of 40% NaOH solution was added. The distillation was continued until a total of 150.0 mL distillate (NH_3/H_2O) was collected

(totalling 200 mL of solution in the conical flask).

The last step involved the titration of the distilled NH_3 against a standard solution of 0.1 *N* H_2SO_4 until the green turned back to the original pink. The values obtained for the titration were used to calculate the % nitrogen values. The calculation used was:

$$\% \text{ Nitrogen} = \frac{(\text{volume titrated (mL)} - \text{blank (mL)}) * (\text{Normality of } H_2SO_4 \text{ (mol/L)}) * 14.0067}{(\text{weight of sample (g)}) * (1000 \text{ (mL/L)})} * 100$$

3.3.6 C:N Ratio

C/N ratio was calculated using the values for Total Organic Carbon (TOC) and Total Kjeldahl Nitrogen (TKN) analyses.

3.3.7 Exchangeable Ammonium (NH_4^+)

The quantity of ammonium in a soil at any one time is dependent upon prevailing conditions of moisture, temperature, degree of aeration, pH, and microbial activity. It follows, therefore, that a random measurement of ammonium in a soil is almost meaningless and such a determination should always be made as part of a well-planned experiment which takes into account all the variables (Hesse, 1971).

As stated earlier, the soil samples were air-dried and ground up to provide a homogenous sample. However, many workers have found that drying of soil samples or storage of samples after drying leads to a marked increase in their content of exchangeable ammonium (Bremner, 1965). For most test procedures and fertilizer recommendations, air-

dried samples at low temperatures (e.g. room temperature) in an NH_4^+-free environment are used (Maynard and Kalra, 1993). Thus, in this study analysis was done as soon as possible after drying so that there was as little change as possible in the ammonium levels.

Exchangeable ammonium (NH_4^+) was determined by a colorimetric method (Tan, 1996). As a general extractant of ammonium from soil, 2 *M* neutral potassium chloride solution (KCl) was finally decided upon by Bremner (1965) as being the most suitable. The following reagents were prepared and used when analysing ammonium (NH_4^+) concentrations (Tan, 1996).

Reagents:

1) KCl solution, 2*M*: Dissolve 149.00 grams KCl in 800.0 mL distilled water in a 1 L volumetric flask, and dilute to the mark with distilled water.

2) Sodium salicylate-Sodium nitroprusside solution: Dissolve 300.00 mg of Na-nitroprusside [$Na_2Fe(CN)_5NO^{\cdot}2H_2O$] and 150.00 g Na-salicylate ($NaC_7H_5O_3$) in 600.0 mL water. Store in dark-coloured bottle in a refrigerator.

3) Sodium hypochlorite (NaOCl, or Clorox): Measure 6.00 mL Clorox, containing 5.25% Cl, in a 100 mL volumetric flask, and dilute to the mark with distilled water. This reagent must be prepared daily, immediately before use to obtain optimum results because the NaOCl concentration in this reagent decreases on standing.

4) Buffer Solution: Dissolve 50.00 g of Na-K-tartrate ($NaKC_4H_4O_6 \cdot H_2O$) and 26.80 g disodium phosphate (Na_2HPO_4) in 600.0 mL distilled water in a 1 L volumetric flask. Add 54.00 g NaOH, and allow this to dissolve by constant stirring, before the volume is made up to 1.0 L with distilled water.

5) Standard NH_4^+ solution: Dissolve 412.50 g of ammonium sulfate [$(NH_4)_2SO_4$] in 1.0 L of distilled water. This solution contains 100 μg of NH_4^+/mL. Pipet 5.00 mL of this solution into a 250 mL volumetric flask, and dilute to the mark with distilled water. The resulting solution, containing 2 μg NH_4^+ per mL, is used for making a standard (calibration) curve.

Extraction of Exchangeable NH_4^+:

To extract the ammonium from the soil 1.00 g of soil was put into a 125 mL conical flask. Then, 10.00 mL of the 2.0 *M* KCl solution was added to the conical flask (10:1 KCl:soil). After a stopper was put on the flasks, the samples were shaken on a mechanical shaker for 60 minutes at 200 rpm. The samples were filtered through Whatman No. 2 filter paper into glass vials. Ammonium KCl extracts were analysed as soon as possible (< 24 hours).

Procedure:

Using a pipet, 1.00 mL of extracted sample was added to 5.50 mL buffer solution,

4.00 mL Na-salicylate-nitroprusside solution, and 2.00 mL hypochlorite solution and mixed. The samples stood for 45 minutes at 25°C for complete colour development. The absorbency of the coloured solution was measured at 650 nm on a spectrophotometer. A standard curve was prepared with 4 or 5 different known amounts of NH_4^+ using the same procedure as above. However, 1.00 mL of KCl solution was used rather than 1.00 mL of extracted sample.

Some samples had to be diluted 10 times and 200 times so that they could be read on the spectrophotometer. For a dilution of 10 times, 1.00 mL of the extracted solution was added to a 10 mL volumetric flask and diluted to the mark with KCl solution. Then, 1.00 mL of the diluted solution was added to the colorimetric tube and the other solutions were added, as above. For a 200 times dilution, 0.05 mL of the extracted solution was added to a 10 mL volumetric flask with a micropipette (Pipetman P1000 (200μL - 1000μL). The volume was diluted to the mark with KCl solution, then 1.00 mL of the diluted solution was added to the colorimetric tube and the other solutions were added as above.

3.3.8 Available Phosphorus, Potassium, Calcium, Magnesium

Phosphorus, potassium, calcium, and magnesium were determined with a Mehlich III Extraction Solution (Mehlich, 1984). Mehlich III Extraction Solution was developed by Mehlich (1984) as a multielement soil extraction. In the Mehlich III procedure, phosphorus is extracted by reaction with acetic acid and fluoride compounds. Exchangeable potassium, calcium, and magnesium are extracted by the action of ammonium nitrate and nitric acid

(Tran and Simard, 1993). To analyse phosphorus, potassium, calcium, and magnesium using the Mehlich III Method the following reagents were used:

Reagents:

1) Mehlich III Extracting Solution (0.2*M* CH_3COOH + 0.25*M* NH_4NO_3 + 0.015*M* NH_4F + 0.013*M* HNO_3 + 0.001*M* ethylene diamine tetraacetic acid (EDTA))
2) Activated Carbon Powder
3) 2% Strontium Solution: Measure 15.21 g of strontium chloride into 250.0 mL Mehlich III extracting solution
4) Aqueous Standards: Ca^{2+} (40 mg/kg - 200 mg/kg), Mg^{2+} (10 mg/kg - 50 mg/kg), K^{+} (5 mg/kg - 40 mg/kg) (All with 2% strontium solution in the standards)

Extraction:

Research on soil testing for plant-available nutrients (phosphorus, potassium, calcium, magnesium) should use exactly the same procedures as are used in the routine soil test laboratory. Thus, samples are measured instead of weighed (Bates, 1993). For the extraction, 2.5 cm^3 of air-dried soil was measured using a calibrated scoop and put into a 125 mL conical flask. The heaping scoopful of soil was tapped with a plastic rod, after which the rod was used to scrape the excess soil off the scoop to provide a consistent volume (Bates, 1993).

Then, 25.0 mL of Mehlich III Extraction Solution was added (soil/solution ratio

1:10). The flasks were shaken at 200 oscillations per minute for 15 minutes. The extract was filtered through a medium porosity paper (Whatman No. 2 filter paper or equivalent) into glass vials. Analyses were made as soon as possible (< 24 hours) (Tran and Simard, 1993). A colourless filtrate was necessary for phosphorus, so 1 cm^3 of activated carbon was added to each funnel when filtering.

A 4:1 ratio was used of extracted solution to 2% strontium solution (e.g. 8.00 mL extracted solution and 2.00 mL 2% strontium solution). The 2% strontium solution helped to avoid spectral interference in the readings between potassium, calcium, and magnesium. Some samples had to be diluted 5 or 10 times. Thus, the extracted solution would be diluted, and then 8.00 mL of the diluted solution would be added to 2.00 mL of 2% strontium solution. The same 4:1 ratio was used in the standard solutions.

Procedure to Analyse Extracts:

Filtrates for phosphorus were analysed using an Auto Analyser. Analysis was conducted at the Soil Plant and Seed Lab of the Newfoundland Department of Forest Resources and Agri-Foods. The most commonly used automated analysis system is the Technicon AutoAnalyser. In a typical automated system, a sampler automatically introduces sample into the flow stream. A proportioning pump and manifold moves the sample and reagent streams into the system. Samples and reagents are then mixed in a mixing cell, allowing a chemical reaction to proceed at constant temperature. The chemical reaction results in the formation of a coloured complex which absorbs light of specific wavelengths.

The coloured solution is then pumped in an air-segmented stream through a colorimeter where absorbency is measured. The absorbency reading is proportional to the concentration of the ion. For phosphate, absorbency readings are taken at a 712 nm wavelength using a spectrophotometer (Schoenau and Karamanos, 1993).

Potassium, calcium, and magnesium were analysed with an Atomic Absorption Spectrophotometer. This analysis was also completed at the Soil Plant and Seed Lab. The most frequently encountered interference effects in AAS are chemical effects which alter, and usually reduce, the extent to which atom formation occurs in the flame. These effects were ameliorated by the use of strontium chloride (Ure, 1983). Thus, 2% strontium chloride was used in the samples for AAS analysis to decrease any interference between readings of the potassium, calcium, and magnesium levels.

3.3.9 Seed Germination

Perhaps the best indicator of whether a compost is biomature is the absence of bioinhibitory aliphatic acids and phenolics which can be determined by seed germination tests (Mathur, 1991; Mathur, 1998). To determine if there were any plant inhibitors in the final product a test on seed germination was completed on compost and vermicompost samples for the final week (week 8) of the experiment. If there are no plant inhibitors the seeds placed on a filter paper soaking in a water extract of the compost in a Petri dish should germinate to the same percentage level as those placed on the paper soaked in distilled water (Mathur *et al.*, 1986). To test seed germination 10.00 grams of air-dried soil was put into a

200 mL conical flask. Then, 100.0 mL of distilled water was added (10 parts distilled water to 1 part air-dried soil) and the samples were mechanically shaken for 24 hours. The samples were filtered using a Buchner funnel with the aid of suction. The extract for the control and vermicomposting samples at week 8 were used to moisten Whatman No. 1 filter paper in petri dishes on which there were 20 seeds of radish (*Raphanus sativus* cv. Sparkler) (Mathur *et al.*, 1986). The seeds were certified and had a guaranteed germination percentage of 99%. The petri dishes were kept at 25 °C with constant light at all times for a total of 3 days. After 3 days the germination rates were determined. Germination rates for the control soil and the vermicompost-soil mix were obtained by dividing the total amount of seeds that had emerged by the total amount of seeds planted.

3.3.10 Total Lipids

The lipid content was calculated for the cod offal using the Bligh and Dyer method (Bligh and Dyer, 1959). In a fumehood, 50.00 g of homogenized cod offal was put into a blender with 100.0 mL methanol (CH_3OH) and 50.0 mL of chloroform ($CHCl_3$). The sample was blended for 2 minutes and then an additional 50.0 mL of chloroform ($CHCl_3$) was added and blended for 30 seconds. Finally, 50.0 mL of distilled water was added and blended for 30 seconds. The sample was filtered through a Buchner funnel using Whatman filter paper No. 4 with the aid of suction. The filtrate was poured into a 500 mL separatory funnel. After separation and clarification (overnight), the chloroform layer (lower layer) was poured into a 100 mL graduated cylinder and the volume was recorded. Three aluminum weighing

dishes were pre-weighed. 10.00 mL of the chloroform layer was pipetted into the pre-weighed aluminum dishes and dried in an oven for 1 hour. The dried samples were weighed and all values obtained were used to calculate % total lipids. The following equation was used:

$$F = [(W_2 - W_0)(V_1)(100)]/(V_2)(W_3)$$

F	=	fat content in sample (%)
V_1	=	total volume of lower chloroform layer in graduated cylinder (mL)
V_2	=	volume of chloroform extract aliquot removed to the preweighed aluminum weighing dish (mL)
W_0	=	weight of empty aluminum weighing dish (g)
W_2	=	weight of aluminum weighing dish with dried lipid residue (g)
W_3	=	weight of tissue sample blended (g)

3.4 Statistical Analysis

One set of chemical analyses was conducted for each separate container. Results were calculated using the average of the 3 containers that were sacrificed for each mixture for each separate analysis period (with and without worms). Comparisons between the means were made using Tukey's HSD (Honest Significant Difference) Test (Sprinthall, 1987).

4.0 RESULTS AND DISCUSSION

4.1 Appropriate % of Cod Offal for Vermicomposting

Results indicated that as the percentage of cod offal increased in the compost mixtures, there was a corresponding decrease in the survival of *E. fetida*. As shown in Table 3, the highest amount of cod offal in which there was 100% survival of *E. fetida* for the full 8 week vermicomposting period was 13% cod offal (dry wt.). When earthworms were added to the compost mixtures at week 0 there was 100% survival in the containers with 7%, 9%, 11% and 13% cod offal. Thus, the earthworms remained in these containers until they were sacrificed at 2 week intervals, for a total of 8 weeks of vermicomposting. At 15, 17, 19, and 21% cod offal the earthworms died within a 48 hour period. Many earthworms ejected a yellow coelomic fluid through the dorsal pores, which is a response to chemical irritation or a sign of stress (Edwards and Lofty, 1977). There were further studies for the mixtures in which there was less than 100% survival at week 0 to see if a longer pre-composting period would affect the survival rate of the worms. Thus, for 15%, 17%, 19%, and 21% cod offal, earthworms were also added at weeks 2, 4, 6, and 8, which means that the earthworms were introduced after 4, 6, 8, and 10 weeks of pre-composting. Results showed that there was some survival at 15 and 17% cod offal. However, at all time intervals during the study, even after 10 weeks of pre-composting, there was 0% survival of *E. fetida* at 19 and 21% cod offal. Results showed that the highest percentage of cod offal which resulted in 100%

Table 3. Survival Rate of *Eisenia fetida* in vermicompost mixtures containing cod (*Gadus morhua*) offal mixed with peat.

	Week 0[a]	Week 2[a]	Week 4[a]	Week 6[a]	Week 8[a]
7% Cod Offal, 93% Peat	100%[b]	n.a.[c]	n.a.[c]	n.a.[c]	n.a.[c]
9% Cod Offal, 91% Peat	100%[b]	n.a.[c]	n.a.[c]	n.a.[c]	n.a.[c]
11% Cod Offal, 89% Peat	100%[b]	n.a.[c]	n.a.[c]	n.a.[c]	n.a.[c]
13% Cod Offal, 87% Peat	100%[b]	n.a.[c]	n.a.[c]	n.a.[c]	n.a.[c]
15% Cod Offal, 85% Peat	0%[b]	0%[b]	0%, 0%, 80%[d]	80%, 100%, 100%[d]	80%, 100%, 100%[d]
17% Cod Offal, 83% Peat	0%[b]	0%[b]	0%, 0%, 20%[d]	0%, 40%, 80%[d]	20%, 100%, 100%[d]
19% Cod Offal, 81% Peat	0%[b]	0%[b]	0%[b]	0%[b]	0%[b]
21% Cod Offal, 79% Peat	0%[b]	0%[b]	0%[b]	0%[b]	0%[b]

[a] There was a pre-composting period of two weeks prior to adding the worms at Week 0. This meant that at week 2 the earthworms were introduced after 4 weeks of pre-composting, at week 4 there was 6 weeks of pre-composting, at week 6 there was 8 weeks of pre-composting, and at week 8 the earthworms were introduced after 10 weeks of pre-composting.

[b] Mean of three determinations.

[c] Not Applicable - Since there was a survival rate of 100% at week 0 there were no further studies to determine if a longer pre-composting period would increase the survival rate. All of the worms in the containers from 7% to 13% cod offal survived for the full 8 weeks of vermicomposting.

[d] Unaveraged data for three separate containers.

survival of the earthworms was 13% (dry wt.), regardless of the length of time of the pre-composting period.

4.2 pH

The pH levels ranged from very acidic in the pure peat to almost neutral in the mixtures with cod offal. The pH levels were higher in the mixtures with the cod offal because the $CaCO_3$ in the cod bones raised the pH. Previous studies have confirmed that the pH values of worm castings will tend towards neutrality (Albanell *et al.*, 1988; Buchanan *et al.*, 1988). This is possibly due to the fact that as soil or organic matter is passed through an earthworm's digestive system, it is broken up and neutralized by secretions of calcium carbonate from calciferous glands near the worm's gizzard (Lee, 1985).

The results in Table 4 show that most of the time there is no significant difference between samples in the presence and the absence of *E. fetida*. However, there is a trend that the pH levels are higher in the samples with worms, however, by the end of the eight week period the pH levels in the vermicomposting samples are significantly lower than in the control samples (Figures 1 - 5). The lower pH recorded in the final week may have been due to the production of CO_2 and organic acids by microbial activity during the process of bioconversion (Haimi and Huhta, 1987). An increase in pH may be an important factor in nitrogen retention, as this element is lost as volatile ammonia at lower pH values (Hartenstein and Hartenstein, 1981; Elvira *et al.*, 1998).

Table 4. Changes in pH during composting of cod (*Gadus morhua*) offal mixed with *Sphagnum* peat in the presence and absence of *Eisenia fetida* over a 10 week period (including 2 weeks of pre-composting).*

Time (Weeks)	Before Pre-Composting	0**	2		4		6		8	
	No Worms	No Worms	No Worms	With Worms	No Worms	With Worms	No Worms	With Worms	No Worms	With Worms
100% Peat	4.17 ± 0.05	4.14 ± 0.04	4.07 ± 0.02^{a}	4.37 ± 0.05^{b}	4.10 ± 0.01^{a}	4.45 ± 0.03^{b}	4.18 ± 0.07^{a}	4.72 ± 0.05^{b}	4.02 ± 0.02^{a}	4.68 ± 0.01^{b}
7% Cod Offal, 93% Peat	4.10 ± 0.02	5.56 ± 0.09	5.59 ± 0.06^{a}	5.69 ± 0.07^{a}	5.74 ± 0.06^{a}	5.88 ± 0.10^{a}	5.88 ± 0.06^{a}	5.76 ± 0.04^{a}	5.65 ± 0.07^{a}	5.62 ± 0.23^{a}
9% Cod Offal, 91% Peat	4.17 ± 0.20	5.79 ± 0.12	5.75 ± 0.15^{a}	5.75 ± 0.02^{a}	5.80 ± 0.04^{a}	5.94 ± 0.09^{a}	6.07 ± 0.16^{a}	5.99 ± 0.07^{a}	5.78 ± 0.09^{a}	5.59 ± 0.10^{a}
11% Cod Offal, 89% Peat	4.40 ± 0.39	5.89 ± 0.03	6.10 ± 0.12^{a}	6.07 ± 0.17^{a}	6.15 ± 0.21^{a}	6.33 ± 0.16^{a}	6.19 ± 0.03^{a}	5.90 ± 0.23^{a}	5.97 ± 0.04^{a}	5.58 ± 0.15^{b}
13% Cod Offal, 87% Peat	4.21 ± 0.01	6.38 ± 0.23	6.35 ± 0.01^{a}	6.35 ± 0.16^{a}	6.65 ± 0.13^{a}	6.54 ± 0.10^{a}	6.53 ± 0.07^{a}	5.86 ± 0.39^{a}	6.44 ± 0.05^{a}	4.91 ± 0.22^{b}

* All results are expressed in dry weight. Mean values of three determinations ± standard deviations.

** Week 0 occurs after two weeks of pre-composting and is also time period at which the earthworms were added to the containers for vermicomposting.

^{a,a} Values in the same row for the same week with the same superscript are not statistically different ($P > 0.05$).

^{a,b} Values in the same row for the same week with a different superscript are statistically different ($P > 0.05$).

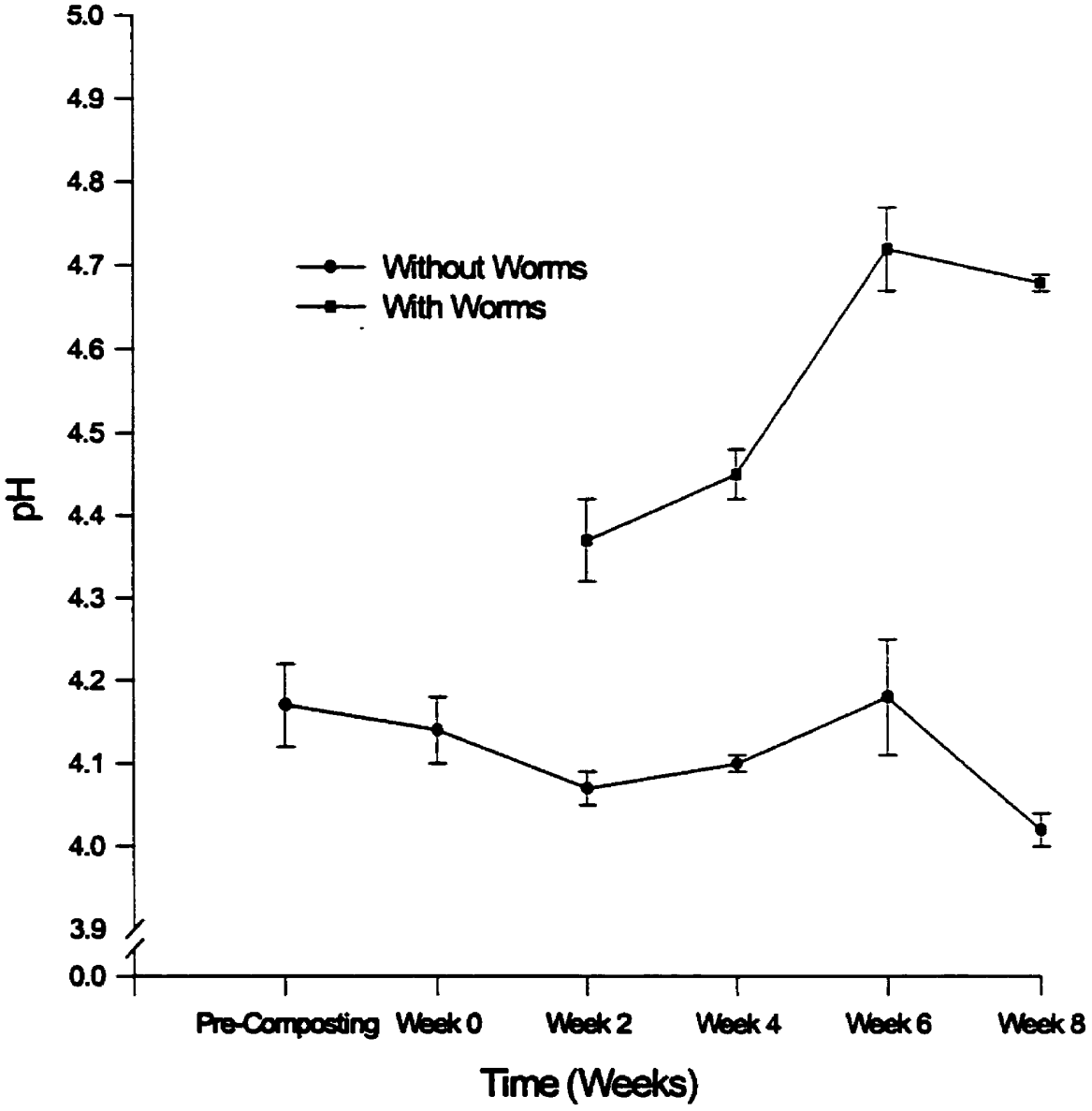

Figure 1. Changes in pH during composting of samples with 100% *Sphagnum* peat in the presence and absence of *Eisenia fetida* over a 10 week period (including 2 weeks of pre-composting prior to adding the earthworms) (dry wt).

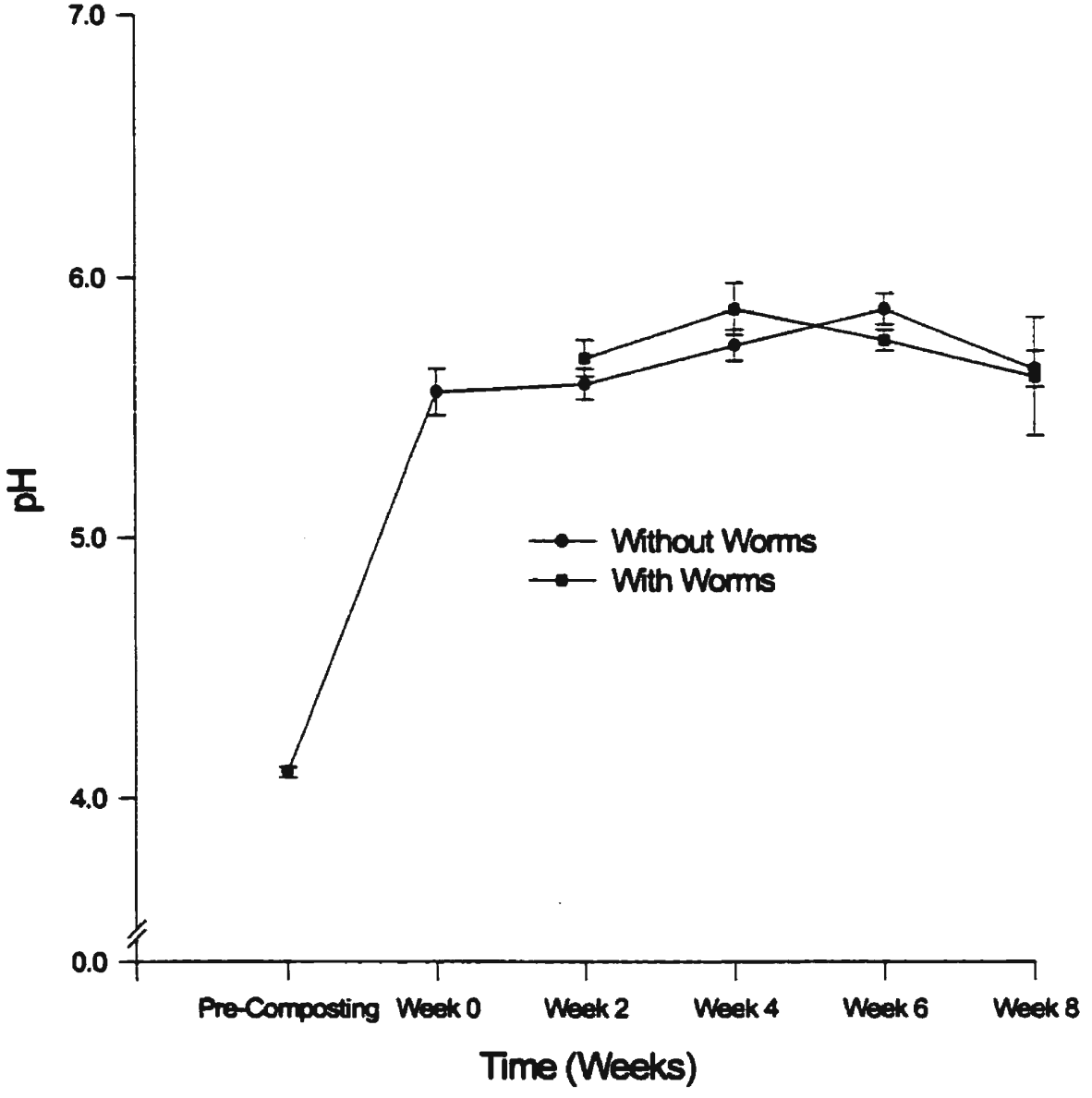

Figure 2. Changes in pH during composting of samples with 7% cod (*Gadus morhua*) offal and 93% *Sphagnum* peat in the presence and absence of *Eisenia fetida* over a 10 week period (including 2 weeks of pre-composting prior to adding the earthworms) (dry wt.).

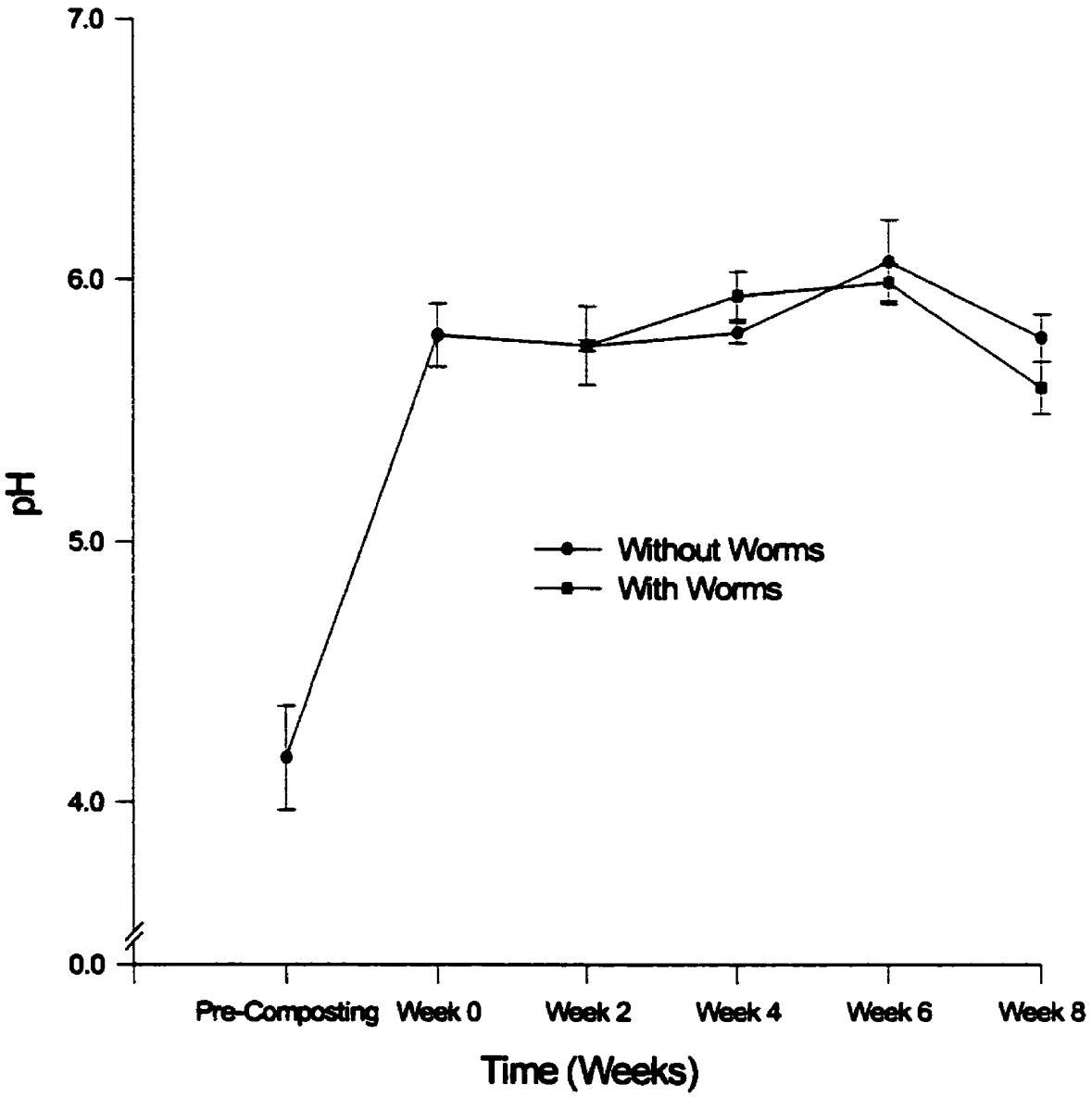

Figure 3. Changes in pH during composting of samples with 9% cod (*Gadus morhua*) offal and 91% *Sphagnum* peat in the presence and absence of *Eisenia fetida* over a 10 week period (including 2 weeks of pre-composting prior to adding the earthworms) (dry wt.).

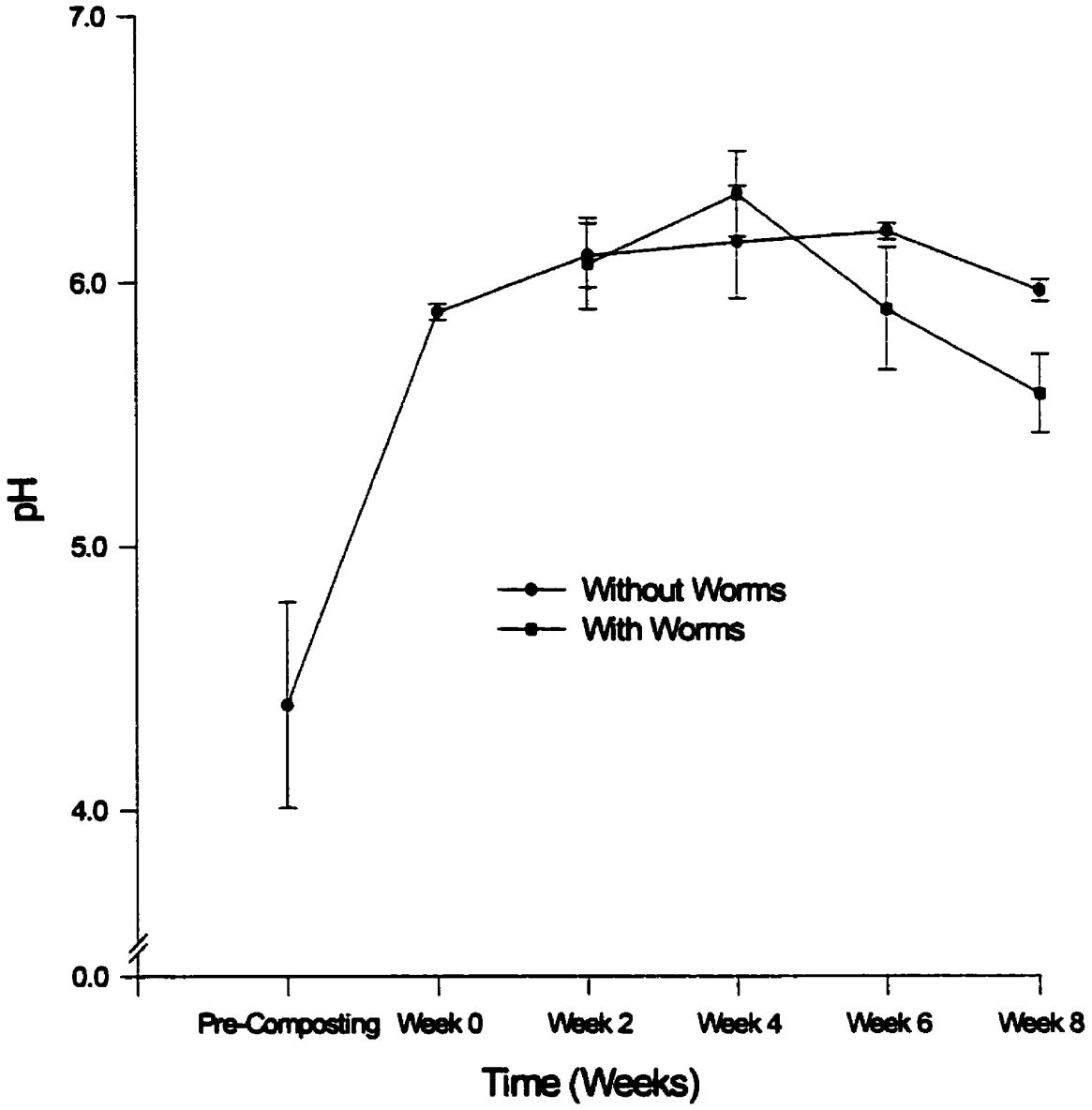

Figure 4. Changes in pH during composting of samples with 11% cod (*Gadus morhua*) offal and 89% *Sphagnum* peat in the presence and absence of *Eisenia fetida* over a 10 week period (including 2 weeks of pre-composting prior to adding the earthworms) (dry wt.).

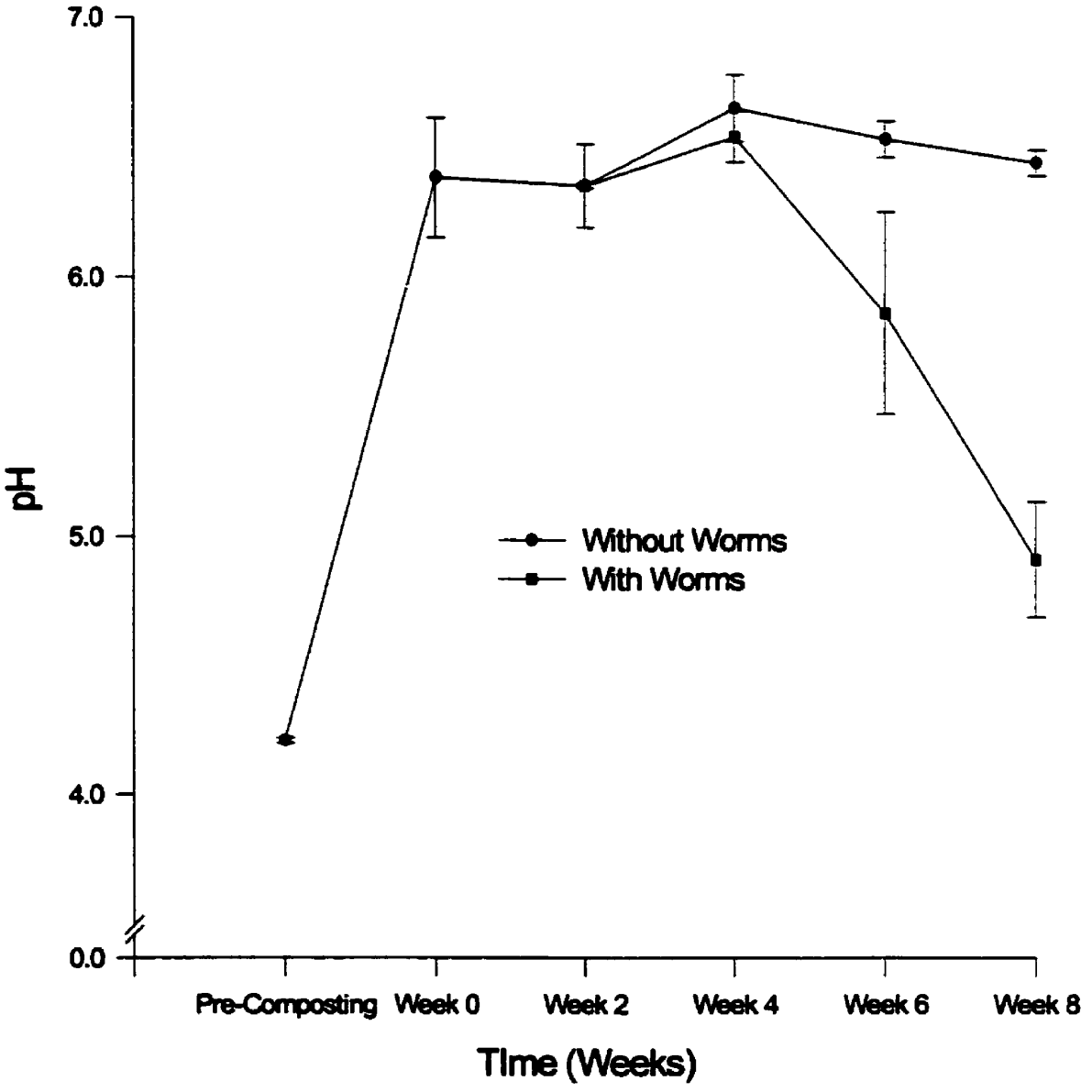

Figure 5. Changes in pH during composting of samples with 13% cod (*Gadus morhua*) offal and 87% *Sphagnum* peat in the presence and absence of *Eisenia fetida* over a 10 week period (including 2 weeks of pre-composting prior to adding the earthworms) (dry wt.).

4.3 Stabilization of Organic Matter - Ash and Total Organic Carbon (TOC)

This study is similar to other work which concludes that vermicomposting can accelerate organic matter stabilization compared to traditional composting (Hartenstein and Hartenstein, 1981; Neuhauser *et al.*, 1988; Frederickson *et al.*, 1997). The rate of organic matter stabilization was determined by measuring the reduction of combustible carbon content of the compost by measuring percentage ash. A higher percentage of ash indicates that the material is broken down and more stabilized.

Results indicated that there was a significant difference in the percentage of ash in samples in the presence and the absence of E. *fetida* (Table 5). For samples without worms, the percentage of ash remained constant for the entire composting period. However, during vermicomposting the percentage of ash continued to increase until the earthworms had digested all the material at week 6. After week 6 there was less of an increase in the percentage of ash because most of the material was already digested (Figure 6). The most change in percentage of ash occurred during the first 2 weeks of vermicomposting. Thus, vermicomposting for 8 weeks produced a material with a significantly higher percentage of ash compared to the control without worms (Figure 7).

The results for Total Organic Carbon (TOC) also showed that vermicomposting increased the stabilization of organic matter. The percentage of total organic carbon was significantly less in mixtures which had worms which means that earthworms increased the rate at which organic matter was stabilized (Table 6). The amount of TOC remained constant

in samples without worms, however, TOC constantly decreased over time in samples with worms (Figures 8 and 9).

The fact that organic matter is transformed differently in compost and vermicompost can be partly explained by the mutualistic relationship between soil microflora, ingested microorganisms, and intestinal mucus (Vinceslas-Akpa and Loquet, 1997). Organic matter that passes through the earthworm gut and is egested in their casts is broken down into much finer particles, so that a greater surface area of the organic matter is exposed to microbial decomposition. Thus, vermicomposting results in a more stable material and earthworms can speed up the stabilization or maturation of organic matter (Albanell *et al.*, 1988).

Table 5. Changes in Ash (%) during composting of cod (*Gadus morhua*) offal mixed with *Sphagnum* peat in the presence and absence of *Eisenia fetida* over a 10 week period (including 2 weeks of pre-composting).*

Time (Weeks)	Pre-Composting	0**	2		4		6		8	
	No Worms	No Worms	No Worms	With Worms	No Worms	With Worms	No Worms	With Worms	No Worms	With Worms
100% Peat	1.35% ± 0.00%	1.51% ± 0.00%	1.51% ± 0.00%^{a}	2.81% ± 0.01%^{b}	1.53% ± 0.00%^{a}	3.93% ± 0.01%^{b}	1.47% ± 0.01%^{a}	4.76% ± 0.01%^{b}	1.55% ± 0.00%^{a}	4.63% ± 0.01%^{b}
7% Cod Offal, 93% Peat	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.78% ± 0.00%^{a}	4.94% ± 0.01%^{b}
9% Cod Offal, 91% Peat	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.91% ± 0.00%^{a}	5.43% ± 0.01%^{b}
11% Cod Offal, 89% Peat	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	3.07% ± 0.01%^{a}	5.39% ± 0.00%^{b}
13% Cod Offal, 87% Peat	3.92% ± 0.01%	3.97% ± 0.00%	4.42% ± 0.00%^{a}	6.38% ± 0.01%^{b}	3.92% ± 0.00%^{a}	6.86% ± 0.01%^{b}	4.60% ± 0.01%^{a}	6.51% ± 0.00%^{b}	4.35% ± 0.00%^{a}	6.39% ± 0.01%^{b}

* All results are expressed in dry weight. Mean values of three determinations ± standard deviations.

** Week 0 occurs after two weeks of pre-composting and is also time period at which the earthworms were added to the containers for vermicomposting.

^{a,a} Values in the same row for the same week with the same superscript are not statistically different ($P > 0.05$).

^{a,b} Values in the same row for the same week with a different superscript are statistically different ($P > 0.05$).

n.d. Not Determined

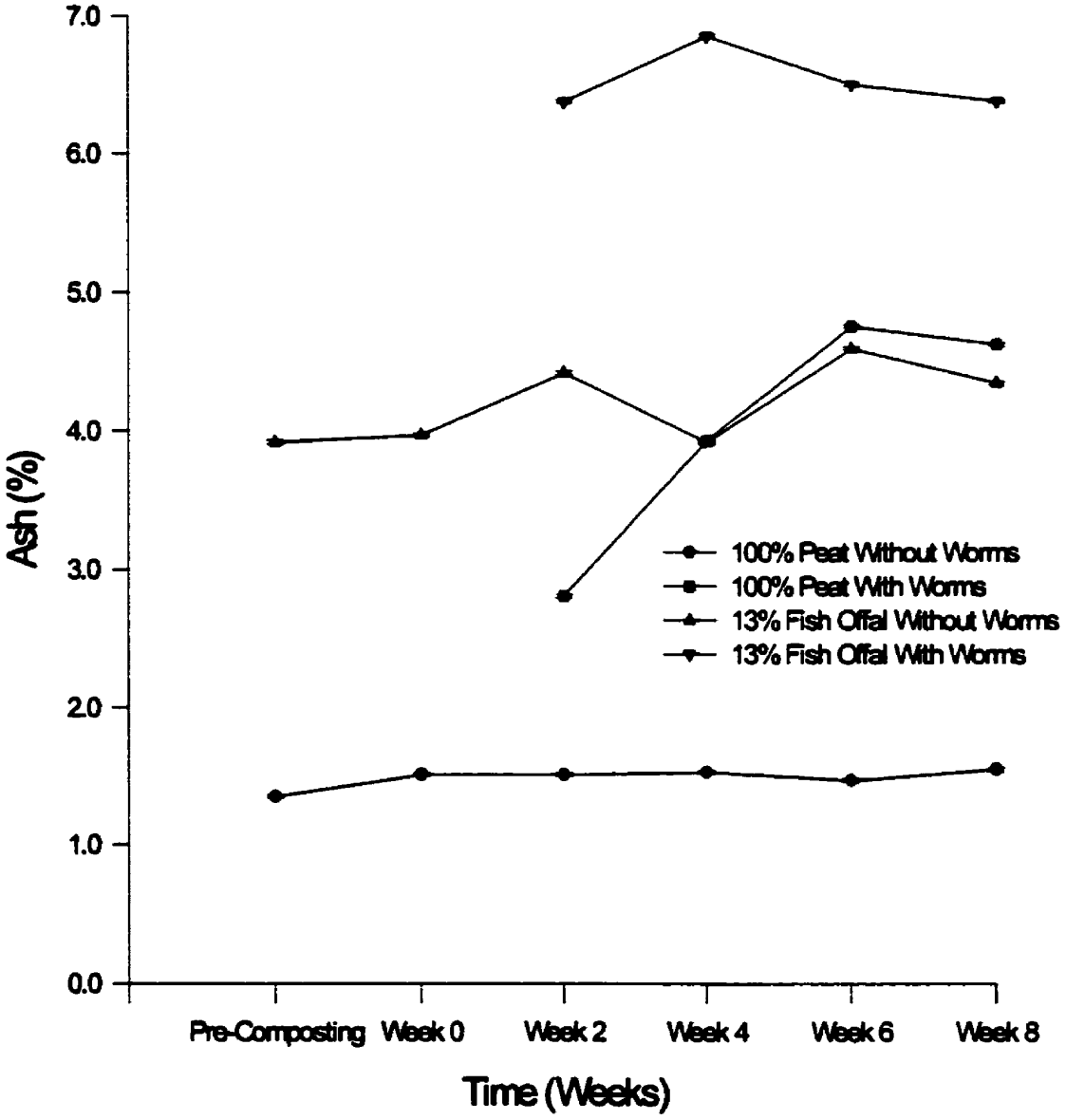

Figure 6. Changes in Ash (%) during composting in the presence and absence of *Eisenia fetida* for samples with 100% *Sphagnum* peat and for samples with 13% cod (*Gadus morhua*) offal and 87% *Sphagnum* peat over a 10 week period (including 2 weeks of pre-composting prior to adding the earthworms) (dry wt.).

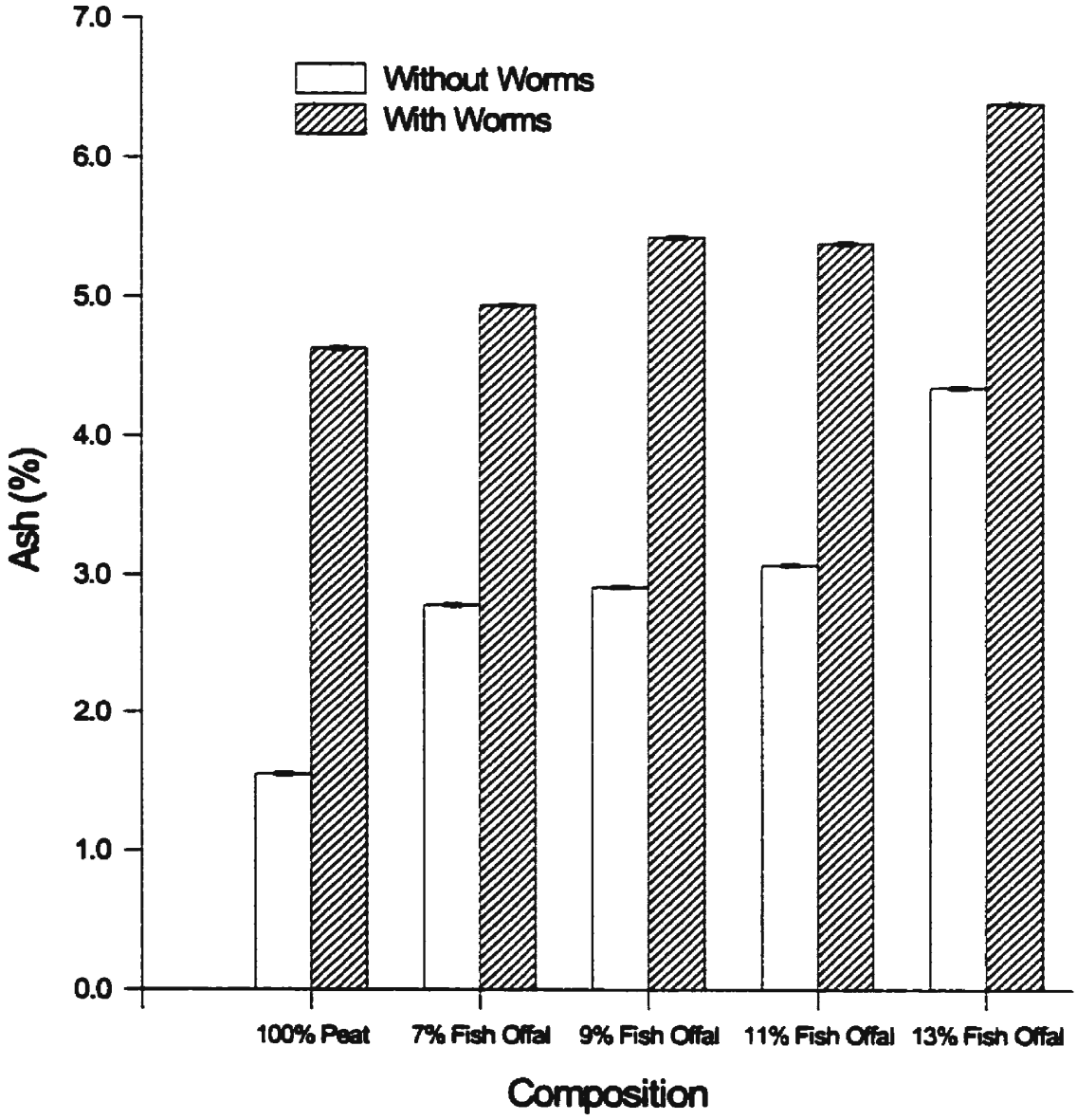

Figure 7. Changes in Ash (%) during composting in the presence and absence of *Eisenia fetida* for samples with 0%, 7%, 9%, 11%, and 13% cod (*Gadus morhua*) offal mixed with *Sphagnum* peat at the 8th week of vermicomposting (there was 2 weeks of pre-composting prior to adding the earthworms) (dry wt.).

Table 6. Changes in Total Organic Carbon (TOC, %) during composting of cod (*Gadus morhua*) offal mixed with *Sphagnum* peat in the presence and absence of *Eisenia fetida* over a 10 week period (including 2 weeks of pre-composting).*

Time (Weeks)	Pre-Composting	0**	2		4		6		8	
	No Worms	No Worms	No Worms	With Worms	No Worms	With Worms	No Worms	With Worms	No Worms	With Worms
100% Peat	54.80% ± 0.00%	54.72% ± 0.00%	54.72% ± 0.00%^{a}	54.00% ± 0.01%^{b}	54.71% ± 0.00%^{a}	53.37% ± 0.01%^{b}	54.74% ± 0.00%^{a}	52.91% ± 0.01%^{b}	54.69% ± 0.00%^{a}	52.99% ± 0.01%^{b}
7% Cod Offal, 93% Peat	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	54.01% ± 0.00%^{a}	52.81% ± 0.01%^{b}
9% Cod Offal, 91% Peat	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	53.94% ± 0.00%^{a}	52.54% ± 0.01%^{b}
11% Cod Offal, 89% Peat	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	53.85% ± 0.01%^{a}	52.56% ± 0.00%^{b}
13% Cod Offal, 87% Peat	53.38% ± 0.01%	53.35% ± 0.00%	53.10% ± 0.00%^{a}	52.50% ± 0.01%^{b}	53.38% ± 0.00%^{a}	52.01% ± 0.01%^{b}	53.00% ± 0.01%^{a}	52.01% ± 0.00%^{b}	53.14% ± 0.00%^{a}	51.47% ± 0.01%^{b}

* All results are expressed in dry weight. Mean values of three determinations ± standard deviations.

** Week 0 occurs after two weeks of pre-composting and is also time period at which the earthworms were added to the containers for vermicomposting.

^{a,a} Values in the same row for the same week with the same superscript are not statistically different ($P > 0.05$).

^{a,b} Values in the same row for the same week with a different superscript are statistically different ($P > 0.05$).

n.d. Not Determined

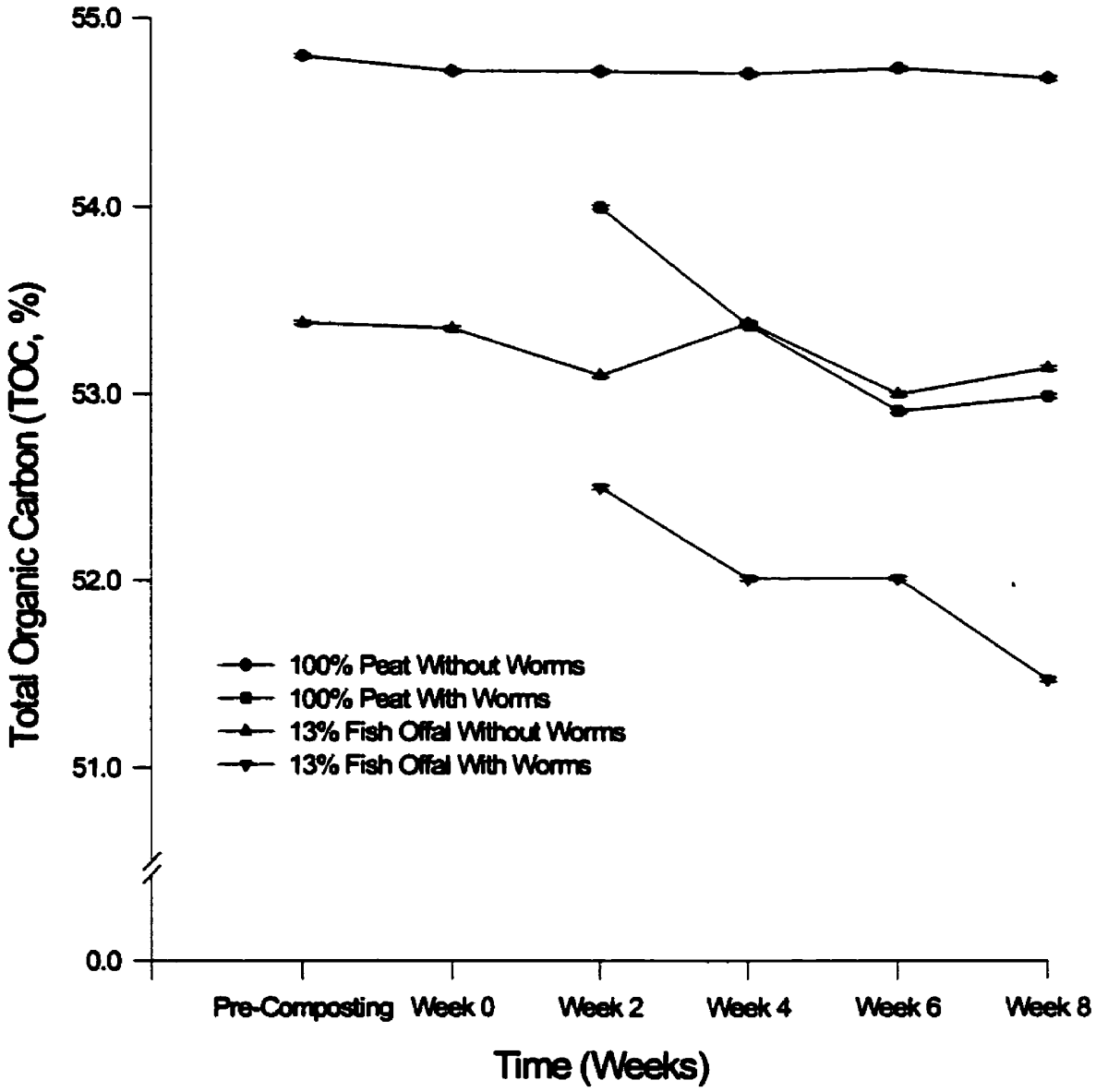

Figure 8. Changes in Total Organic Carbon (TOC, %) during composting in the presence and absence of *Eisenia fetida* for samples with 100% *Sphagnum* peat and for samples with 13% cod (*Gadus morhua*) offal and 87% *Sphagnum* peat over a 10 week period (including 2 weeks of pre-composting prior to adding the earthworms) (dry wt.).

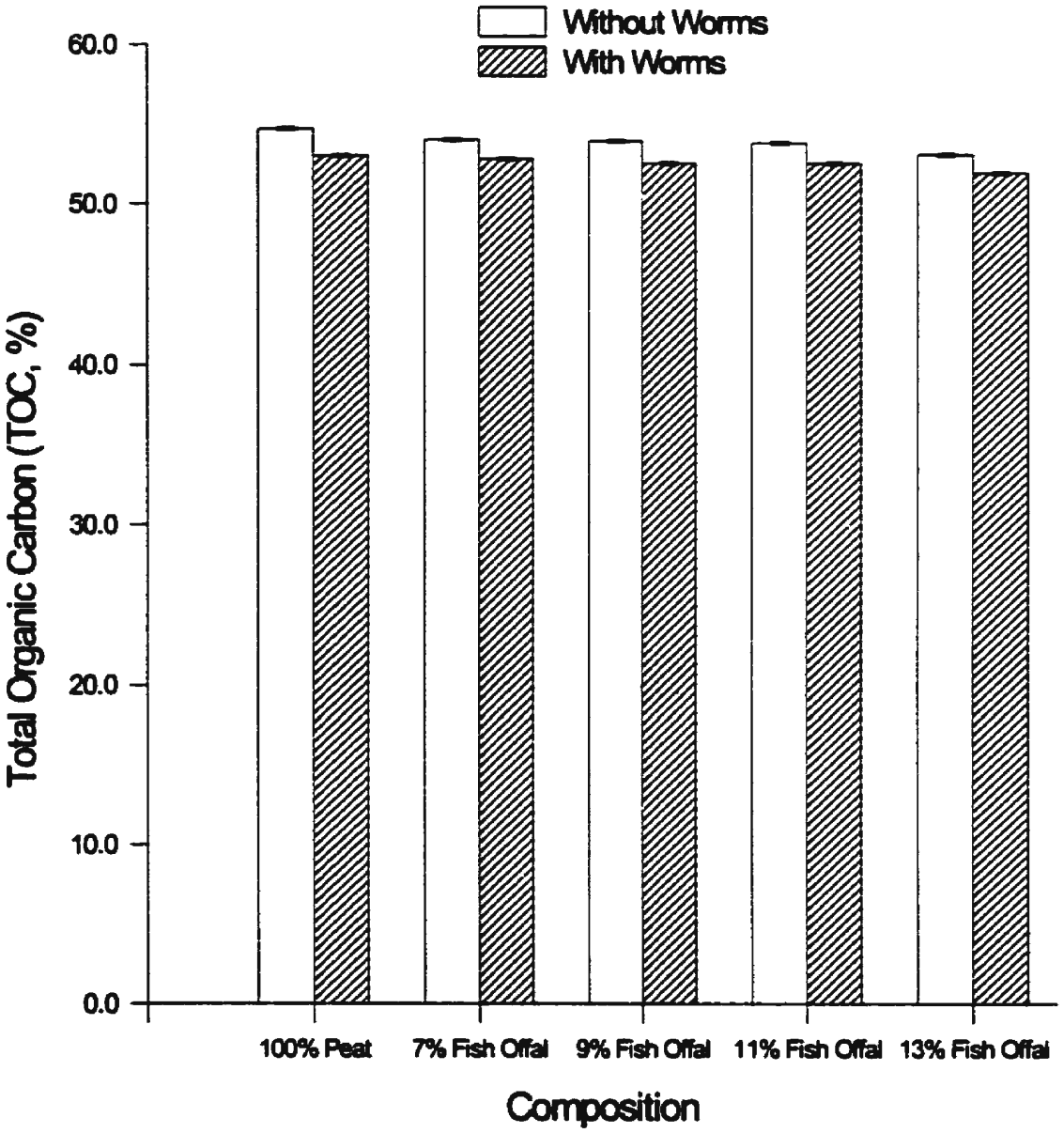

Figure 9. Changes in Total Organic Carbon (TOC, %) during composting in the presence and absence of *Eisenia fetida* for samples with 0%, 7%, 9%, 11%, and 13% cod (*Gadus morhua*) offal mixed with *Sphagnum* peat at the 8th week of vermicomposting (there was 2 weeks of pre-composting prior to adding the earthworms) (dry wt.).

4.4 Total Kjeldahl Nitrogen (TKN)

Studies have shown that nitrogen mineralization was greater in the presence of earthworms, and this mineral nitrogen was retained in the nitrate form (Elvira *et al.*, 1998). Thus, there is an increased availability of N in earthworm castings compared to the non-ingested soil, which has been reported by several workers (Mulongoy, 1986; Scheu, 1987; Tiwari *et al.*, 1989; Hulugalle and Ezumah, 1991) and has been attributed to the higher microbial populations and enzyme activities in the casts. However, when analysing total nitrogen Nye (1955) concluded that when earthworms are kept in a controlled system and all of the nitrogen in the system is accounted for, there is no increase in total nitrogen in the system (Lee, 1985).

The results of this study for Total Kjeldahl Nitrogen (TKN) supports the fact that earthworms do not significantly increase the total amount of nitrogen through vermicomposting (Table 7). Total nitrogen content remains constant throughout the composting period for samples of peat only and for 13% cod offal both in the presence and the absence of earthworms (Figure 10). However, the results in week 8 appear to show a slight increase in the total nitrogen for vermicomposting samples in comparison to the controls, however there is not a significant difference (Figure 11).

Table 7. Changes in Total Kjeldahl Nitrogen (TKN, %) during composting of cod (*Gadus morhua*) offal mixed with *Sphagnum* peat in the presence and absence of *Eisenia fetida* over a 10 week period (including 2 weeks of pre-composting).*

Time (Weeks)	Pre-Composting	0**	2		4		6		8	
	No Worms	No Worms	No Worms	With Worms	No Worms	With Worms	No Worms	With Worms	No Worms	With Worms
100% Peat	0.76% ± 0.00%	0.80% ± 0.00%	0.79% ± 0.00%[a]	0.79% ± 0.00%[a]	0.76% ± 0.01%[a]	0.78% ± 0.01%[a]	0.83% ± 0.01%[a]	0.86% ± 0.01%[b]	0.79% ± 0.01%[a]	0.83% ± 0.02%[a]
7% Cod Offal, 93% Peat	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.39% ± 0.02%[a]	1.43% ± 0.01%[a]
9% Cod Offal, 91% Peat	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.54% ± 0.01%[a]	1.65% ± 0.00%[b]
11% Cod Offal, 89% Peat	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.65% ± 0.01%[a]	1.76% ± 0.01%[b]
13% Cod Offal, 87% Peat	1.76% ± 0.01%	2.05% ± 0.00%	1.98% ± 0.01%[a]	1.99% ± 0.01%[a]	2.05% ± 0.01%[a]	2.06% ± 0.01%[a]	1.91% ± 0.02%[a]	1.97% ± 0.01%[a]	1.93% ± 0.01%[a]	1.99% ± 0.03%[a]

* All results are expressed in dry weight. Mean values of three determinations ± standard deviations.

** Week 0 occurs after two weeks of pre-composting and is also time period at which the earthworms were added to the containers for vermicomposting.

[a,a] Values in the same row for the same week with the same superscript are not statistically different ($P > 0.05$).

[a,b] Values in the same row for the same week with a different superscript are statistically different ($P > 0.05$).

n.d. Not Determined

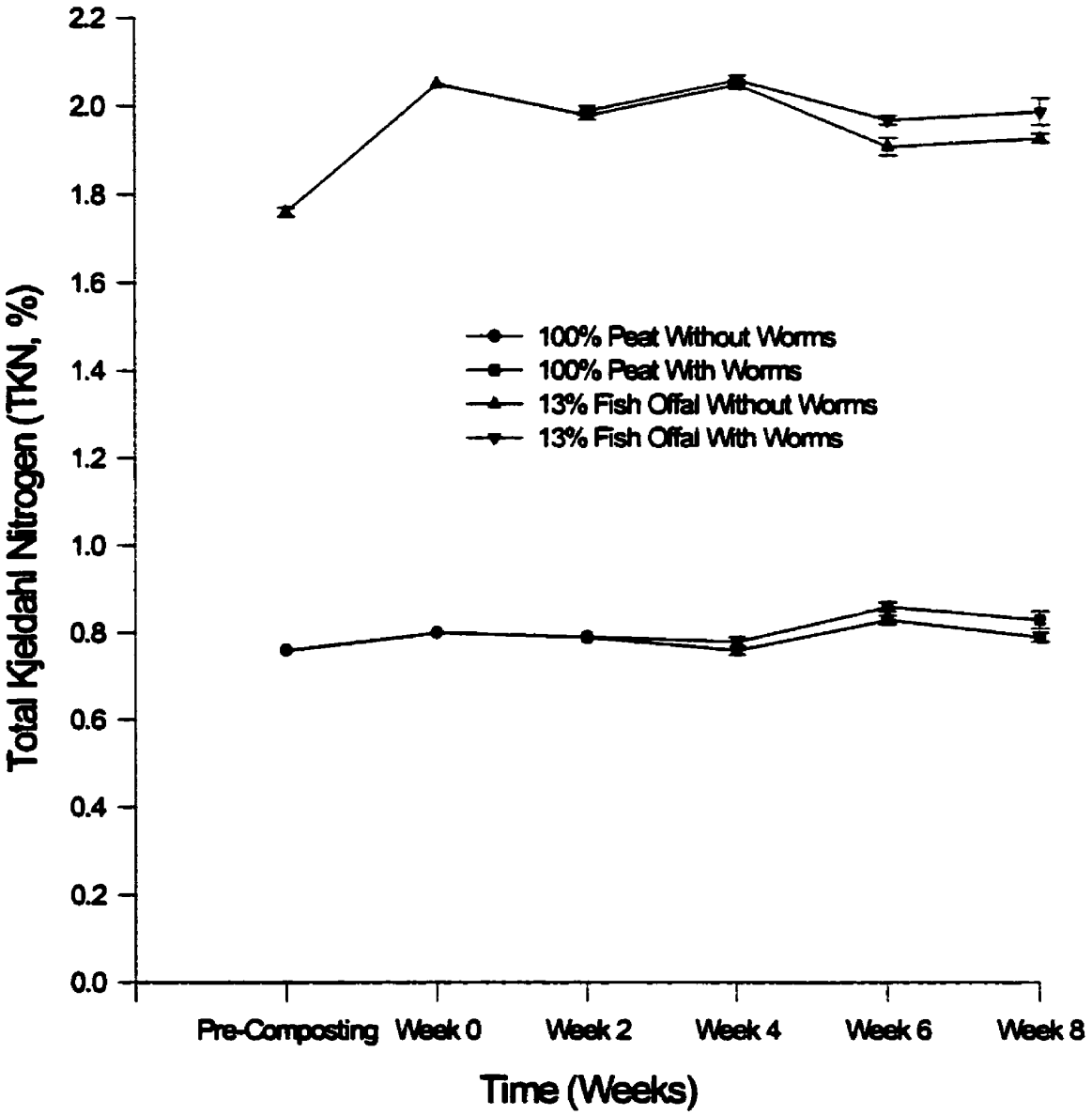

Figure 10. Changes in Total Kjeldahl Nitrogen (TKN, %) during composting in the presence and absence of *Eisenia fetida* for samples with 100% *Sphagnum* peat and for samples with 13% cod (*Gadus morhua*) offal and 87% *Sphagnum* peat over a 10 week period (including 2 weeks of pre-composting prior to adding the earthworms) (dry wt.).

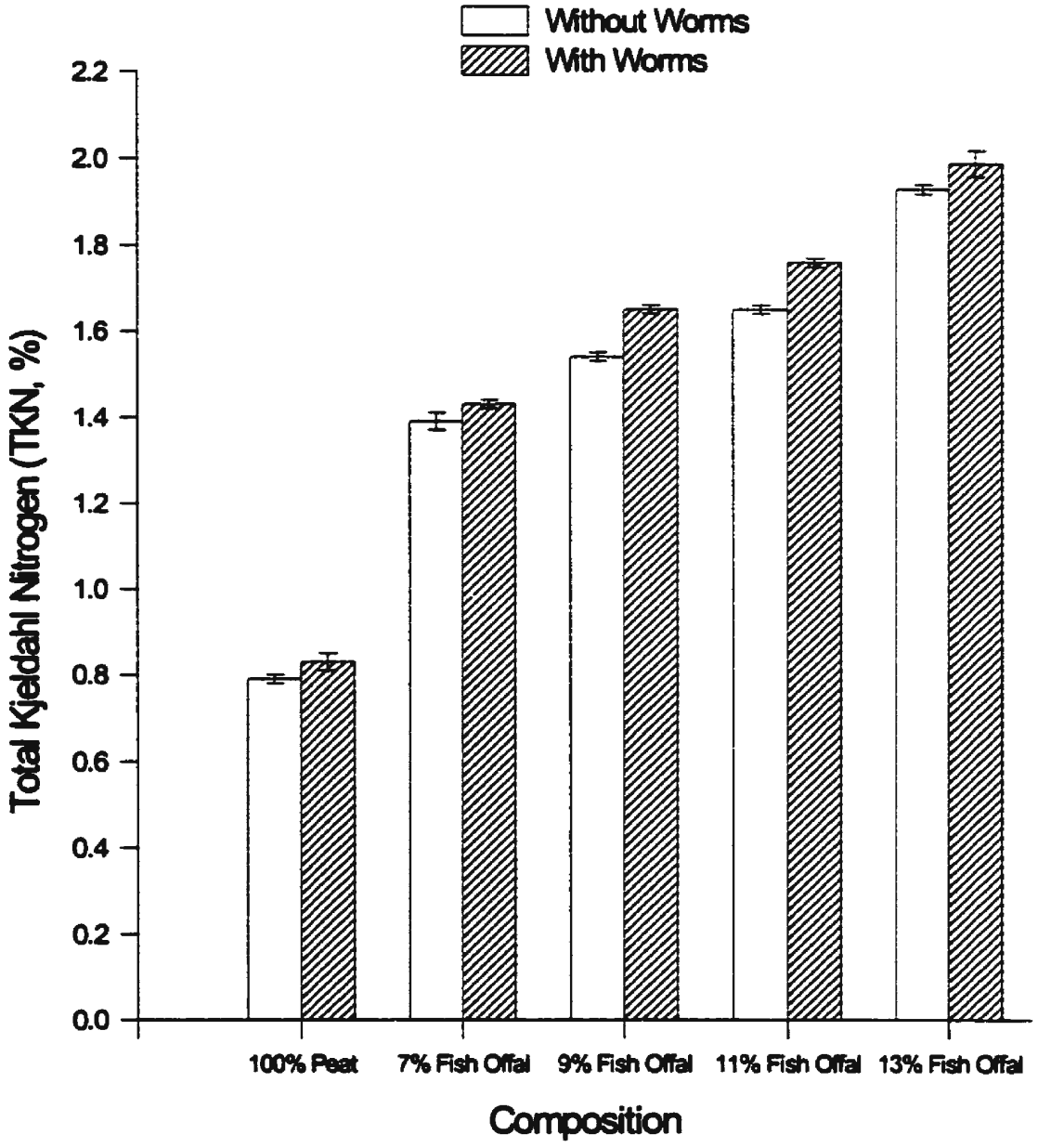

Figure 11. Changes in Total Kjeldahl Nitrogen (TKN, %) during composting in the presence and absence of *Eisenia fetida* for samples with 0%, 7%, 9%, 11%, and 13% cod (*Gadus morhua*) offal mixed with *Sphagnum* peat at the 8th week of vermicomposting (there was 2 weeks of pre-composting prior to adding the earthworms) (dry wt.).

4.5 C:N Ratio

The results of this study show that vermicomposting results in a lower C:N ratio than samples without earthworms (Figures 12 and 13). However, there is only a significant difference between samples during week 8 of the experiment (Table 8). Lowering of C:N ratio is achieved mainly by bio-oxidation of carbon during respiration. The results for C:N ratio are consistent with previous results that there was a significant decrease in percentage TOC in samples with earthworms. The lower C:N ratio from vermicomposting also indicates that there is a high rate of humification in the castings. This is in agreement with findings by Riffaldi and Levi-Minzi (1983). They showed that earthworms increase the transformation of organic substances to stable humic compounds.

Table 8. Changes in C:N Ratio during composting of cod (*Gadus morhua*) offal mixed with *Sphagnum* peat in the presence and absence of *Eisenia fetida* over a 10 week period (including 2 weeks of pre-composting).*

Time (Weeks)	Pre-Composting	0**	2		4		6		8	
	No Worms	No Worms	No Worms	With Worms	No Worms	With Worms	No Worms	With Worms	No Worms	With Worms
100% Peat	72.99 ± 2.59	68.86 ± 1.87	69.31 ± 2.37[a]	68.18 ± 3.41[a]	72.09 ± 3.38[a]	66.15 ± 1.26[a]	69.92 ± 2.74[a]	65.53 ± 0.76[a]	70.37 ± 1.41[a]	64.12 ± 1.50[b]
7% Cod Offal, 93% Peat	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	39.79 ± 1.32[a]	37.02 ± 0.33[b]
9% Cod Offal, 91% Peat	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	35.09 ± 1.89[a]	31.81 ± 1.29[a]
11% Cod Offal, 89% Peat	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	33.57 ± 0.38[a]	28.97 ± 2.04[b]
13% Cod Offal, 87% Peat	30.63 ± 3.49	27.10 ± 1.09	27.58 ± 0.53[a]	26.19 ± 0.66[a]	27.19 ± 2.10[a]	24.97 ± 1.12[a]	27.83 ± 1.92[a]	24.70 ± 1.45[a]	28.33 ± 2.07[a]	24.14 ± 0.44[b]

* All results are expressed in dry weight. Mean values of three determinations ± standard deviations.

** Week 0 occurs after two weeks of pre-composting and is also time period at which the earthworms were added to the containers for vermicomposting.

[a,a] Values in the same row for the same week with the same superscript are not statistically different ($P > 0.05$).

[a,b] Values in the same row for the same week with a different superscript are statistically different ($P > 0.05$).

n.d. Not Determined

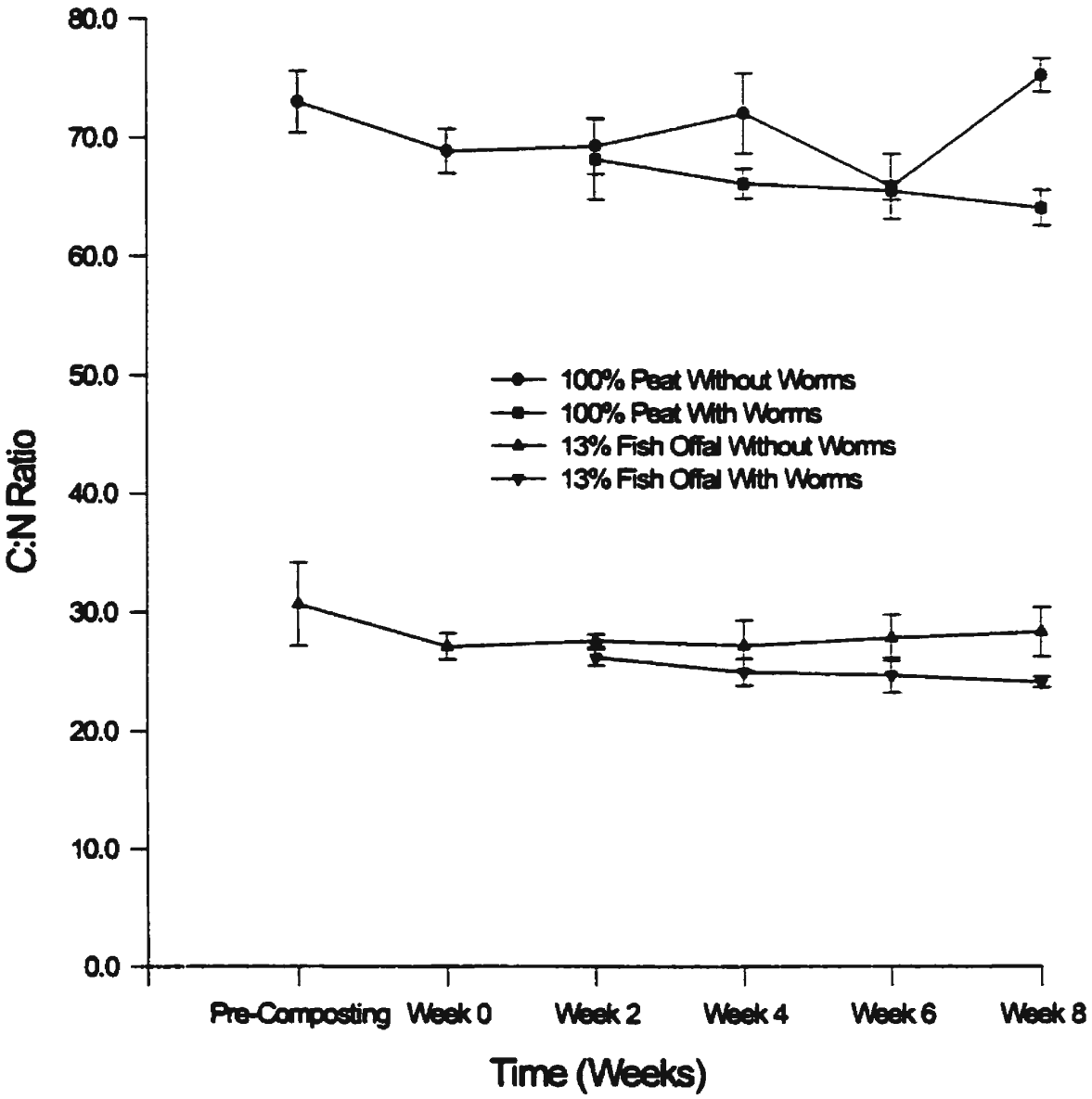

Figure 12. Changes in C:N Ratio during composting in the presence and absence of *Eisenia fetida* for samples with 100% *Sphagnum* peat and for samples with 13% cod (*Gadus morhua*) offal and 87% *Sphagnum* peat over a 10 week period (including 2 weeks of pre-composting prior to adding the earthworms) (dry wt.).

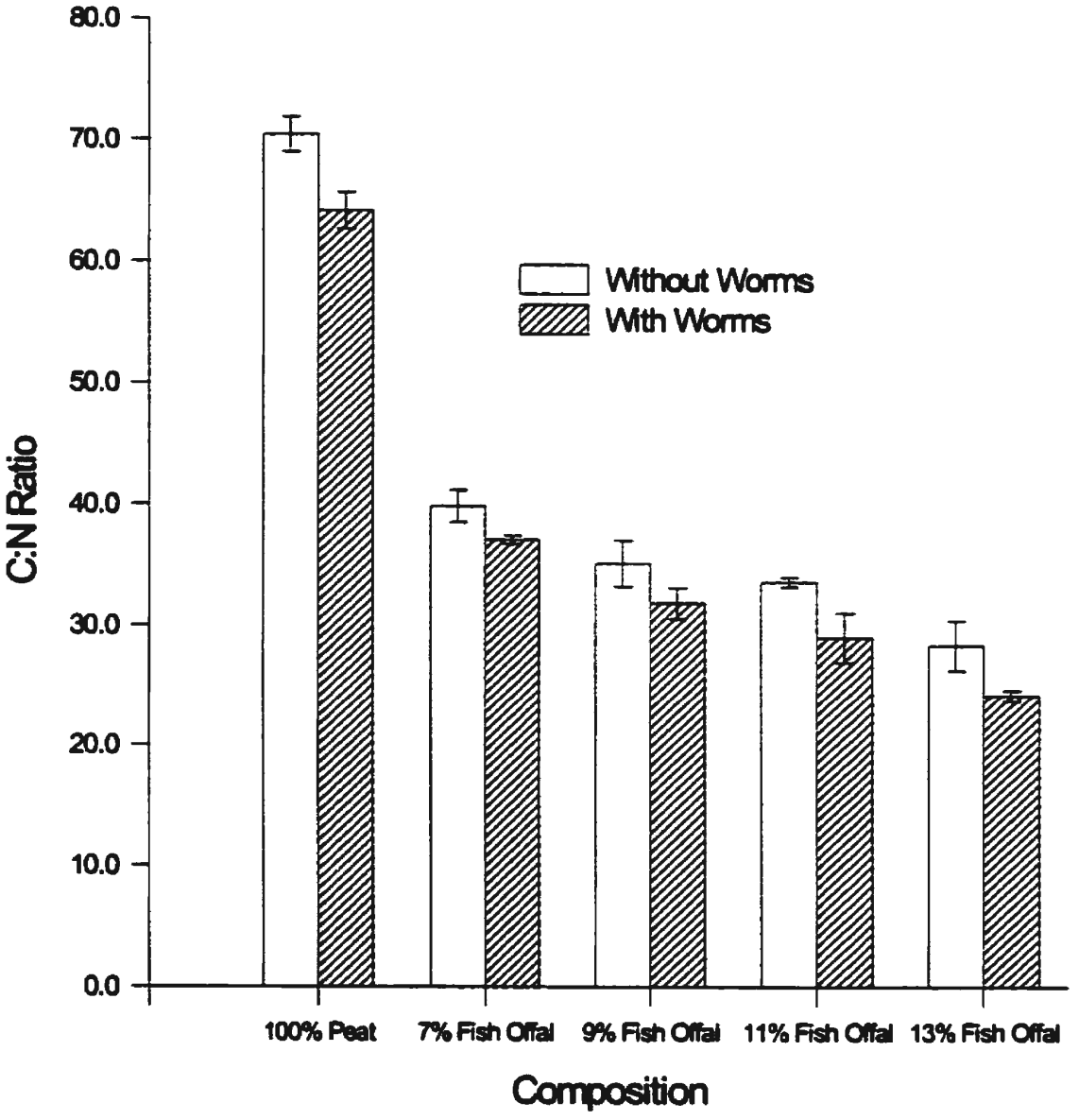

Figure 13. Changes in C:N Ratio during composting in the presence and absence of *Eisenia fetida* for samples with 0%, 7%, 9%, 11%, and 13% cod (*Gadus morhua*) offal mixed with *Sphagnum* peat at the 8th week of vermicomposting (there was an additional 2 weeks of pre-composting prior to adding the earthworms) (dry wt.).

4.6 Exchangeable Ammonium

This study attempted to find out the maximum proportion of ammonium that the earthworms can tolerate to achieve a 100% survival rate. It was concluded that when adding earthworms to compost the initial amount of ammonium should be no more than approximately 1.0 mg/kg (Figure 14). There appears to be a very sharp cutoff point at which the worms will survive 100% of the time. There are containers in which the worms survived higher levels than 1.0 mg/kg of ammonium, yet these levels of ammonium occurred at later stages of the composting process, at weeks 2, 4, 6, and 8.

Results also showed that there was a significant difference in the levels of exchangeable ammonium (NH_4^+) between samples in the presence and the absence of *E. fetida* (Table 9). Figures 15 - 19 show the ammonium levels in the presence and the absence of earthworms at 0%, 7%, 9%, 11%, and 13% cod offal during the 8 weeks of vermicomposting, as well as the pre-composting period. During the pre-composting phase, the amount of ammonium increased dramatically in the samples which contained cod offal, confirming the fact that when peat is used as a bulking agent nearly all of the NH_3 produced during the composting of cod offal is adsorbed as NH_4^+ by the moist, acidic peat. It has been reported that the high exchangeable acidity of *sphagnum*-dominated peats enables them to adsorb NH_3 and convert it to NH_4^+, equivalent to as much as 3% of their dry weights (Mathur *et al.*, 1986). However, after the initial increase in NH_4^+, the samples without worms remained relatively constant over the rest of the composting period. Meanwhile, the amount

of ammonium continued to increase in the vermicomposting samples. This indicated that the earthworms can increase ammonium levels in compost as they further break down the material in the containers. This is because earthworms assimilate organic nitrogen and excrete approximately equal amounts of nitrogen as ammonium and muco-proteins (Needham, 1957). At approximately week 6 the rate of ammonium increase peaked, which may be a result of the earthworms having digested all of the material. Thus, the amount of ammonium began to decrease. This may be due to the conversion of excreted ammonium-N to nitrate, as has been noted in previous studies (Parle, 1963; Syers *et al.*, 1979).

These results are in agreement with Parle (1963) who observed that freshly-deposited casts were high in NH_4^+, but with time, NH_4^+ decreased with a concomitant increase in NO_3^-, indicating high nitrification. Similar findings were reported by Syers *et al.* (1979), who investigated the mineral-N dynamics of earthworm casts and found that 87% of the NH_4^+ initially present in casts was lost, but that increases in the NO_3^- pool did not match losses in NH_4^+. It was suggested that the resulting N deficit was due to a combination of immobilization and denitrification (Parkin and Berry, 1994). According to Barley and Jennings (1959) there was an increase in exchangeable nitrate and ammonium ions in the soil with or without earthworms, but the increase was more than 20% greater in cultures with earthworms than in cultures without earthworms (Lee, 1985). In summary, the concentrations of inorganic nitrogen in fresh earthworm casts is usually greater than in bulk soil, with ammonium usually being the dominant form of inorganic nitrogen in casts (Scheu, 1987; Lavelle *et al.*, 1992).

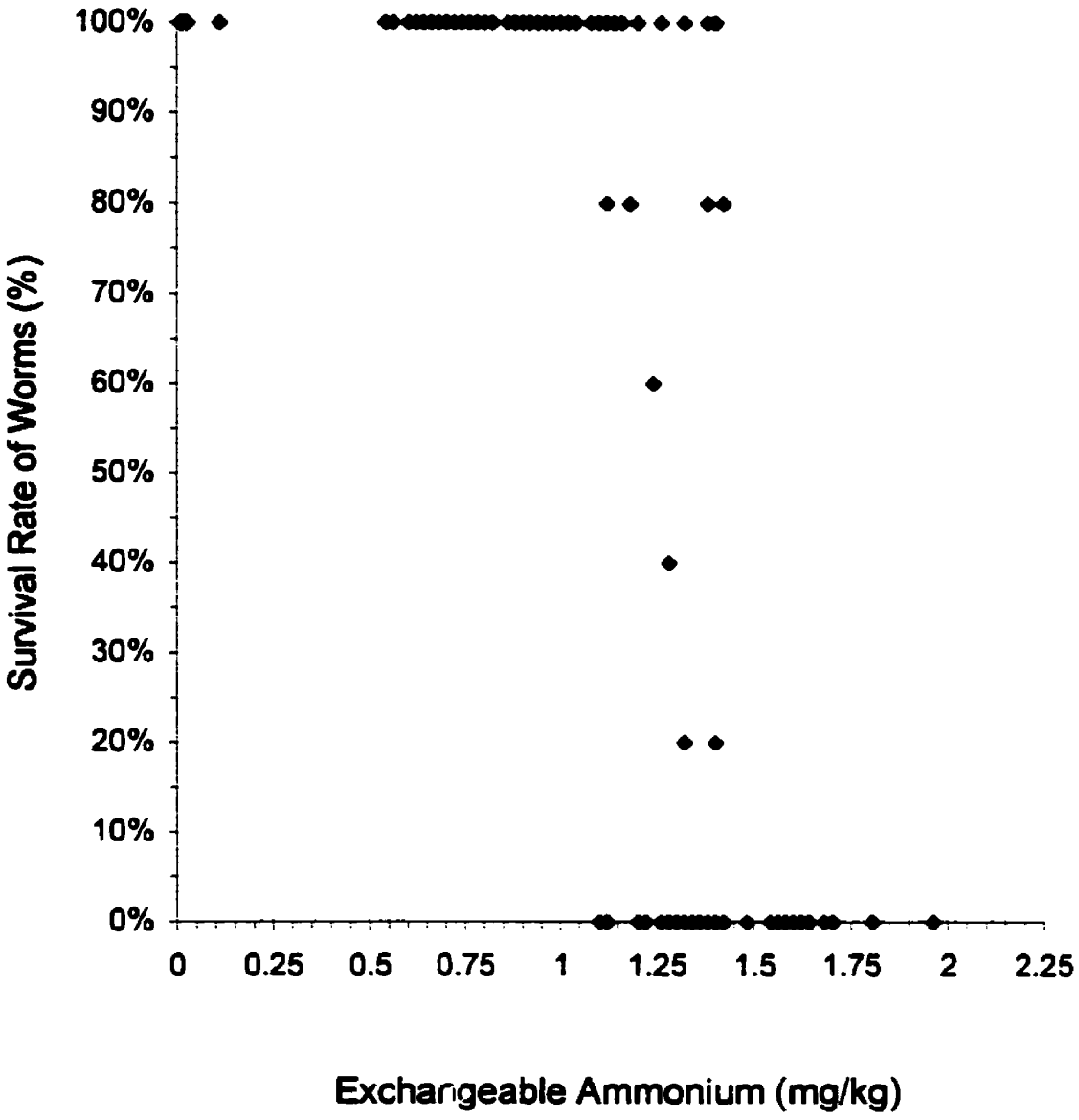

Figure 14. Comparison of the amount of exchangeable ammonium (mg/kg) in each container with survival rate of earthworms.

Table 9. Changes in Exchangeable Ammonium (NH_4^+, mg/kg) during composting of cod (*Gadus morhua*) offal mixed with *Sphagnum* peat in the presence and absence of *Eisenia fetida* over a 10 week period (including 2 weeks of pre-composting).*

Time (Weeks)	Pre-Composting	0**	2		4		6		8	
	No Worms	No Worms	No Worms	With Worms	No Worms	With Worms	No Worms	With Worms	No Worms	With Worms
100% Peat	0.0028 mg/kg ± 0.0003	0.0028 mg/kg ± 0.0002	0.0029 mg/kg ± 0.0003^a	0.0263 mg/kg ± 0.0032^b	0.0098 mg/kg ± 0.0007^a	0.0467 mg/kg ± 0.0042^b	0.0171 mg/kg ± 0.0018^a	0.1146 mg/kg ± 0.0049^b	0.0161 mg/kg ± 0.0021^a	0.1257 mg/kg ± 0.0100^b
7% Cod Offal, 93% Peat	0.0078 mg/kg ± 0.0011	0.5667 mg/kg ± 0.0305	0.6467 mg/kg ± 0.0757^a	0.6000 mg/kg ± 0.0400^a	0.6333 mg/kg ± 0.0577^a	0.7466 mg/kg ± 0.0577^a	0.6200 mg/kg ± 0.0200^a	0.8667 mg/kg ± 0.0945^b	0.6667 mg/kg ± 0.0115^a	0.7933 mg/kg ± 0.0310^b
9% Cod Offal, 91% Peat	0.0300 mg/kg ± 0.0288	0.6333 mg/kg ± 0.0305	0.7133 mg/kg ± 0.0416^a	0.6733 mg/kg ± 0.0460^a	0.7333 mg/kg ± 0.0503^a	0.7866 mg/kg ± 0.0501^a	0.7067 mg/kg ± 0.0416^a	0.9267 mg/kg ± 0.0306^b	0.6667 mg/kg ± 0.0305^a	0.8800 mg/kg ± 0.0600^b
11% Cod Offal, 89% Peat	0.0163 mg/kg ± 0.0076	0.6733 mg/kg ± 0.0116	0.8467 mg/kg ± 0.0462^a	0.9200 mg/kg ± 0.0200^a	0.9000 mg/kg ± 0.0200^a	0.9667 mg/kg ± 0.0231^b	0.9233 mg/kg ± 0.0115^a	1.1533 mg/kg ± 0.0416^b	0.9333 mg/kg ± 0.0416^a	0.7267 mg/kg ± 0.0305^b
13% Cod Offal, 87% Peat	0.0111 mg/kg ± 0.0017	0.8667 mg/kg ± 0.1172	0.9933 mg/kg ± 0.0757^a	1.0867 mg/kg ± 0.0500^a	0.9733 mg/kg ± 0.0231^a	1.1533 mg/kg ± 0.0500^b	0.9333 mg/kg ± 0.0231^a	1.2000 mg/kg ± 0.0529^b	1.0333 mg/kg ± 0.0305^a	0.9400 mg/kg ± 0.0200^b

* All results are expressed in dry weight. Mean values of three determinations ± standard deviations.

** Week 0 occurs after two weeks of pre-composting and is also time period at which the earthworms were added to the containers for vermicomposting.

^{a,a} Values in the same row for the same week with the same superscript are not statistically different ($P > 0.05$).

^{a,b} Values in the same row for the same week with a different superscript are statistically different ($P > 0.05$).

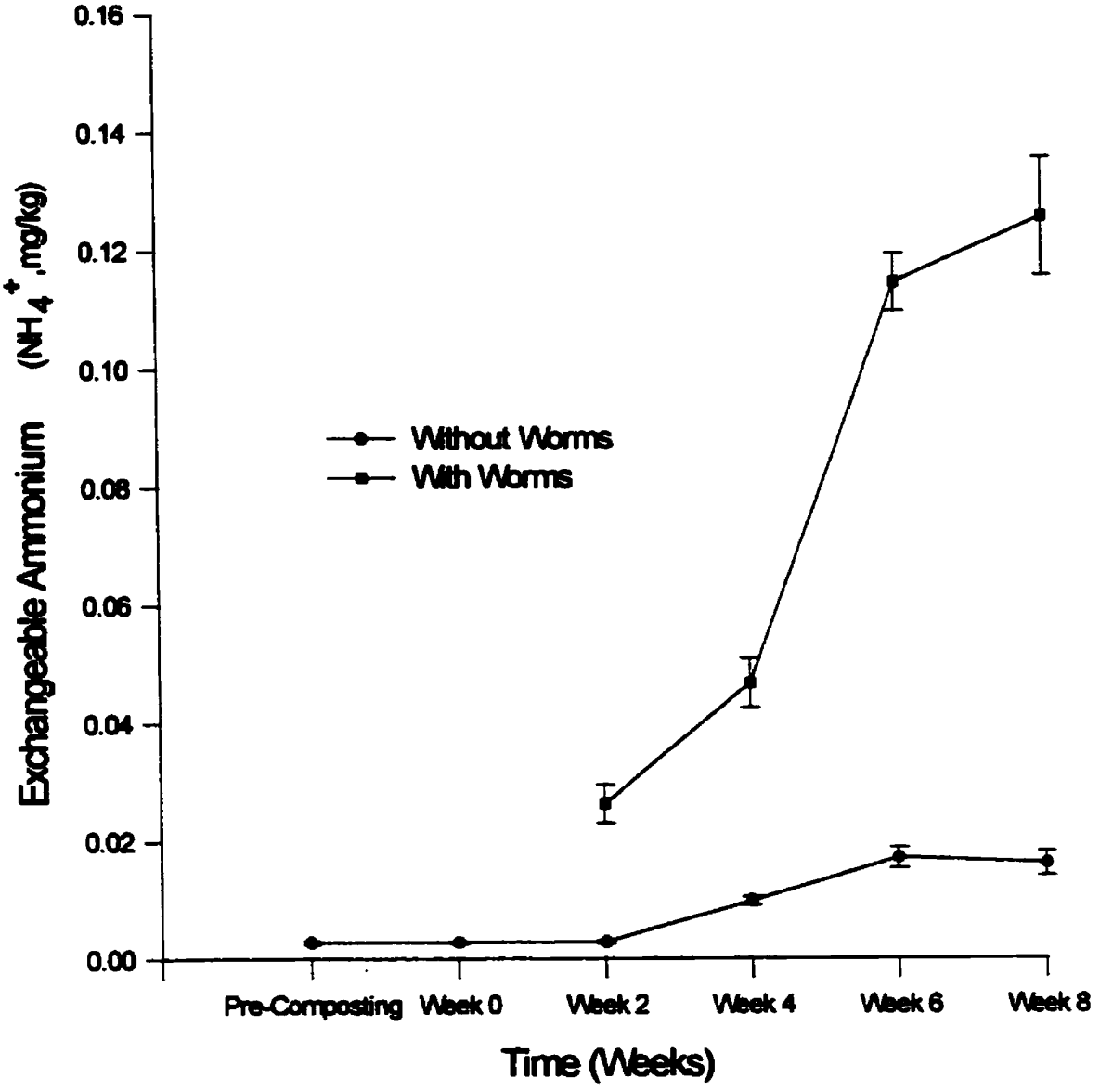

Figure 15. Changes in Exchangeable Ammonium (NH_4^+, mg/kg) during composting of samples with 100% *Sphagnum* peat in the presence and absence of *Eisenia fetida* over a 10 week period (including 2 weeks of pre-composting prior to adding the earthworms) (dry wt.).

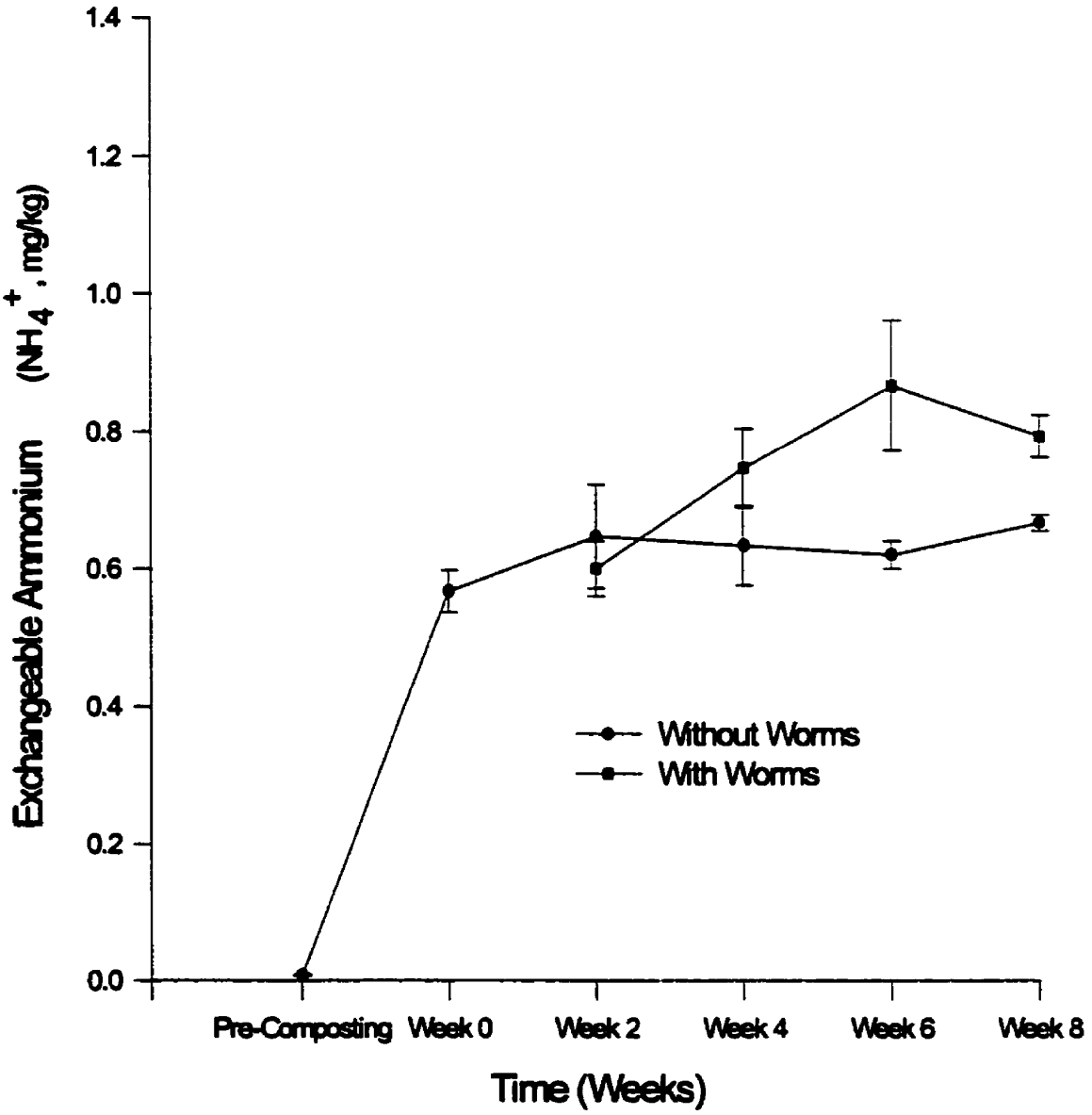

Figure 16. Changes in Exchangeable Ammonium (NH_4^+, mg/kg) during composting of samples with 7% cod (*Gadus morhua*) offal and 93% *Sphagnum* peat in the presence and absence of *Eisenia fetida* over a 10 week period (including 2 weeks of pre-composting prior to adding the earthworms) (dry wt.).

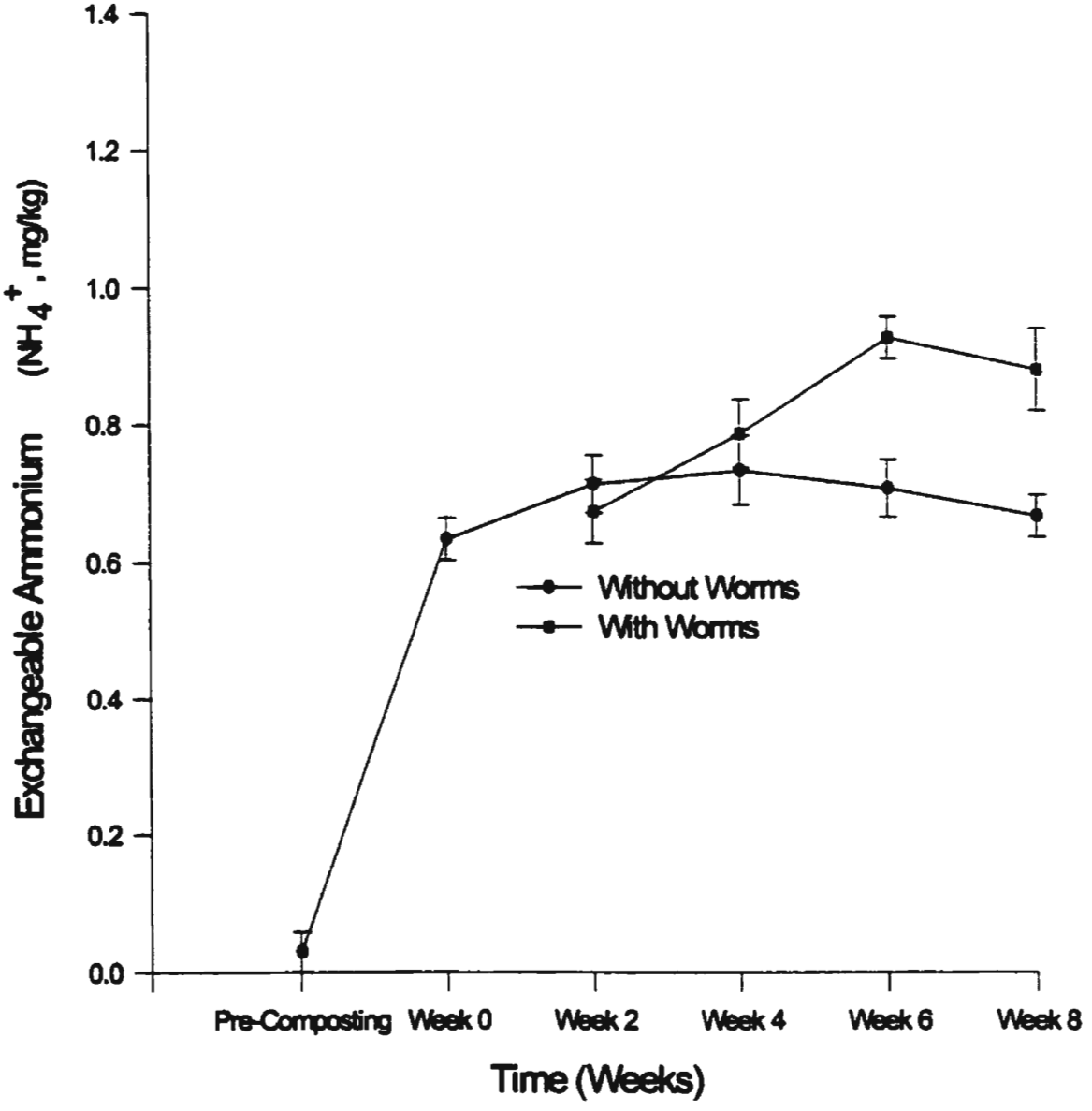

Figure 17. Changes in Exchangeable Ammonium (NH_4^+, mg/kg) during composting of samples with 9% cod (*Gadus morhua*) offal and 91% *Sphagnum* peat in the presence and absence of *Eisenia fetida* over a 10 week period (including 2 weeks of pre-composting prior to adding the earthworms) (dry wt.).

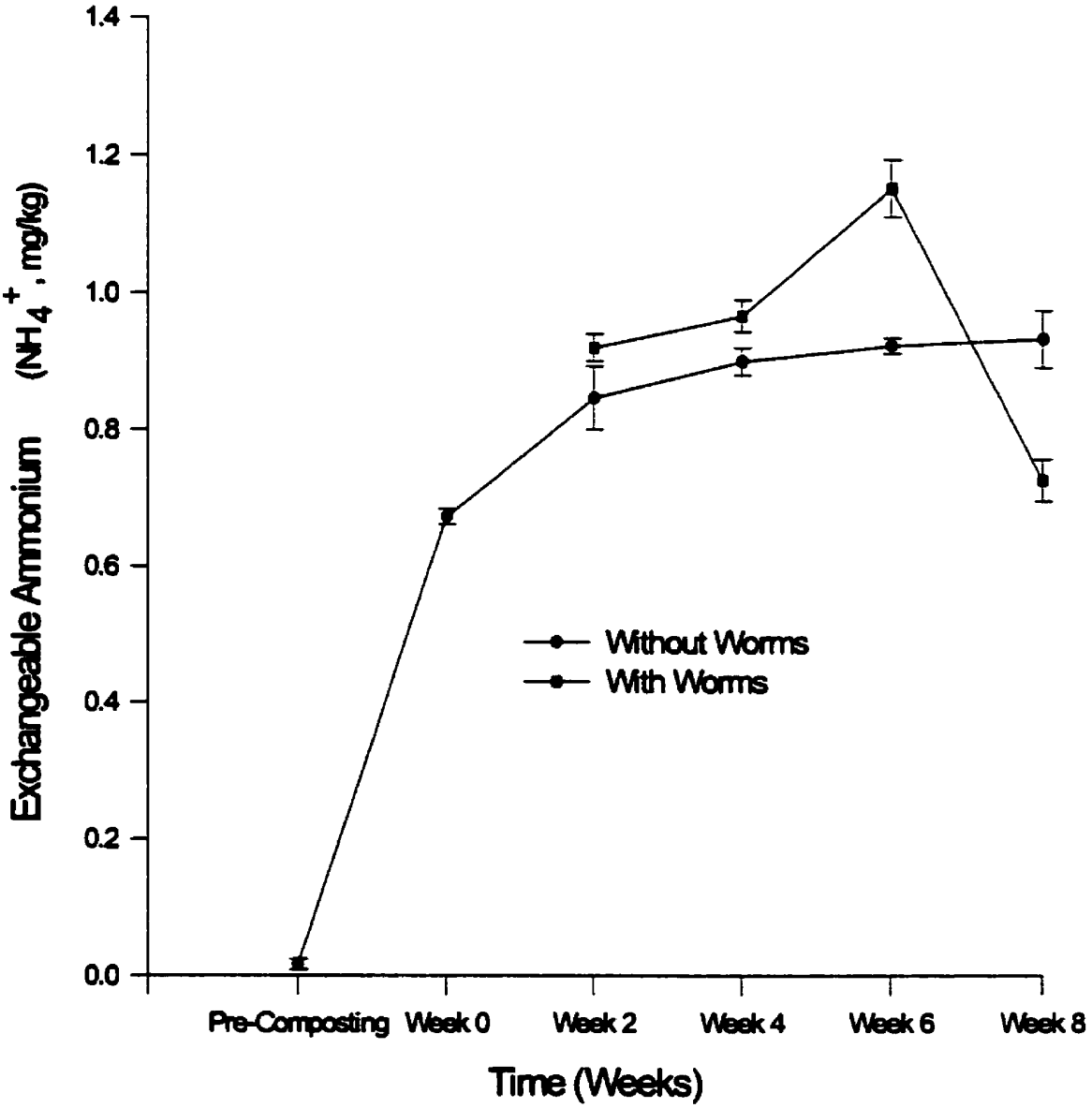

Figure 18. Changes in Exchangeable Ammonium (NH_4^+, mg/kg) during composting of samples with 11% cod (*Gadus morhua*) offal and 89% *Sphagnum* peat in the presence and absence of *Eisenia fetida* over a 10 week period (including 2 weeks of pre-composting prior to adding the earthworms) (dry wt.).

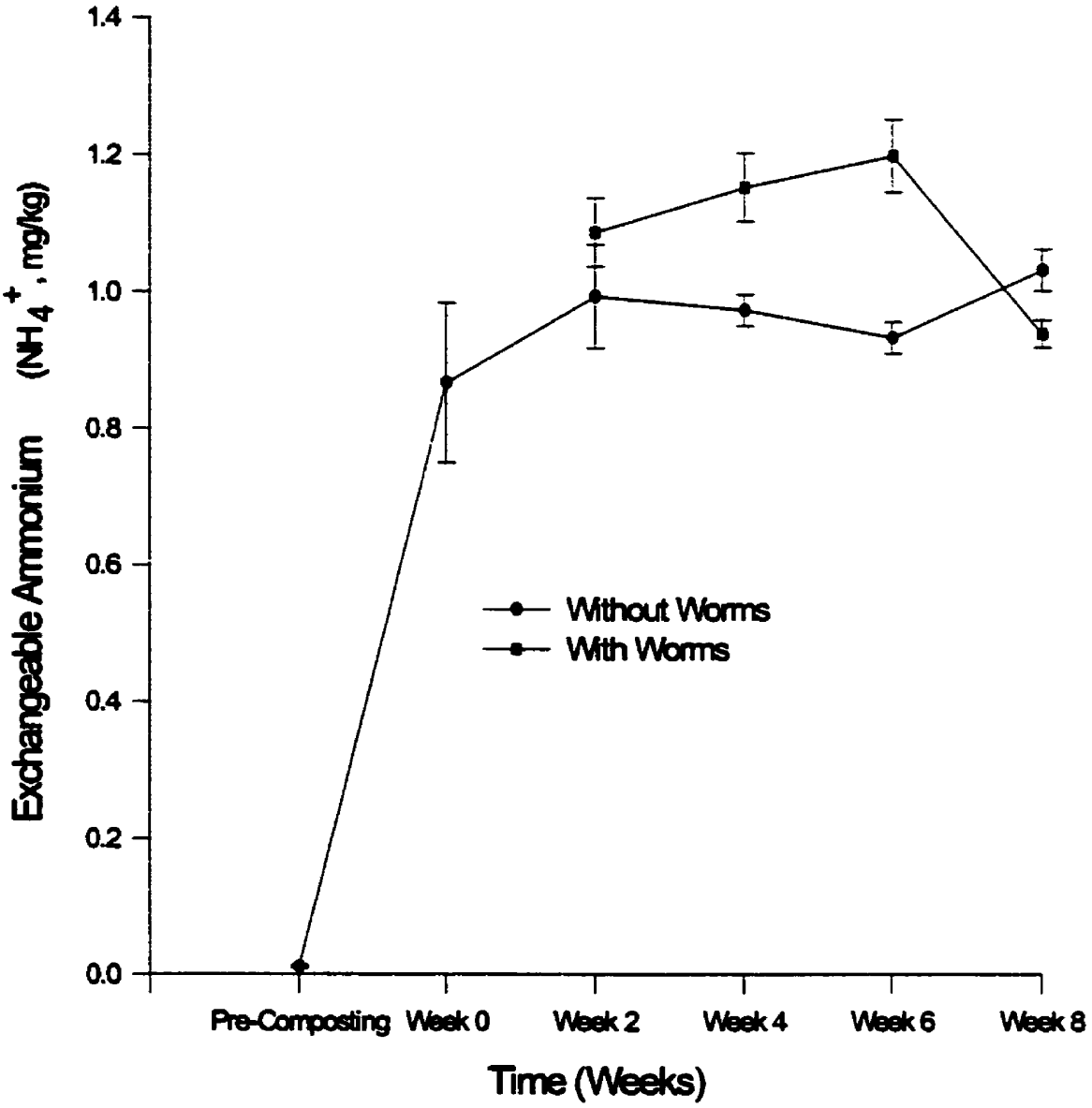

Figure 19. Changes in Exchangeable Ammonium (NH_4^+, mg/kg) during composting of samples with 13% cod (*Gadus morhua*) offal and 87% *Sphagnum* peat in the presence and absence of *Eisenia fetida* over a 10 week period (including 2 weeks of pre-composting prior to adding the earthworms) (dry wt.).

4.7 Available Phosphorus, Potassium, Calcium, and Magnesium

This study showed that the concentrations of available phosphorus, potassium, calcium, and magnesium are significantly greater in earthworm casts than in uningested soil. These results are consistent with many other studies which demonstrate that earthworms increase the level of mineral nutrients available for plant growth, and therefore are important in improving soil fertility. As well, earthworms may increase the quantity of nutrients available by increasing the rate of nutrient cycling (Sharpley and Syers, 1977).

4.7.1 Phosphorus

Results showed that there was a significant difference in the levels of available phosphorus between samples in the presence and absence of *E. fetida* (Table 10). There was an initial increase in the amount of available phosphorus in samples with cod offal during pre-composting. Then, the amount of phosphorus remained constant in the samples without worms. However, there was a higher level of available phosphorus in the samples with worms. The earthworms increased the available phosphorus mainly between week 2 and week 4 (Figure 20). Earthworms significantly increased the amount of available phosphorus for all samples in which the earthworms survived (Figure 21).

Similar results were also found in other studies showing that vermicomposting may prove to be an efficient bio-technological tool for providing better phosphorus nutrition from different organic wastes (Ghosh *et al.*, 1999). It is hypothesized that casts usually have more

available phosphorus than soils without worms, due to enhanced phosphatase activity in the casts, although it is not known whether the increase in activity is due to earthworm-derived enzymes or to increased microbial activity (Syers and Springett, 1984).

4.7.2 Potassium

This study also showed that *E. fetida* can significantly increase the levels of available potassium in comparison to samples without worms (Table 11). In the control with 100% peat the level of potassium remained constantly low throughout the study period. Due to a very low initial amount of potassium in peat, the earthworms are only able to increase the amount of available potassium slightly. However, in the samples with 13% cod offal the amount of potassium is much higher. The amount of available potassium increased during the pre-composting period as the cod offal broke down. Then, the amount of potassium remained constant in the samples without worms but continued to increase in vermicomposted samples (Figure 22).

Control and vermicomposted samples of 7%, 9%, 11%, and 13% cod offal at week 8 were also compared and showed that earthworms can increase the amount of available potassium by as much as 40% (Figure 23). These results are in agreement with a study by Basker *et al.* (1992) which indicated that the exchangeable potassium content increased significantly due to earthworm activity. It is inferred that earthworms increase the availability of potassium by shifting the equilibrium among the forms of potassium from relatively unavailable forms to more available forms in the soil (Basker *et al.*, 1992).

Table 10. Changes in Available Phosphorus (P, mg/kg) during composting of cod (*Gadus morhua*) offal mixed with *Sphagnum* peat in the presence and absence of *Eisenia fetida* over a 10 week period (including 2 weeks of pre-composting).*

Time (Weeks)	Pre-Composting	0**	2		4		6		8	
	No Worms	No Worms	No Worms	With Worms	No Worms	With Worms	No Worms	With Worms	No Worms	With Worms
100% Peat	14.00 mg/kg ± 3.00	15.50 mg/kg ± 2.12	17.67 mg/kg ± 0.58[a]	20.67 mg/kg ± 4.16[a]	19.00 mg/kg ± 4.20[a]	27.00 mg/kg ± 4.29[a]	20.33 mg/kg ± 3.04[a]	28.67 mg/kg ± 3.06[b]	19.00 mg/kg ± 3.29[a]	37.00 mg/kg ± 3.36[b]
7% Cod Offal, 93% Peat	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	265.33 mg/kg ± 28.38[a]	400.00 mg/kg ± 45.58[b]
9% Cod Offal, 91% Peat	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	340.00 mg/kg ± 20.00[a]	586.67 mg/kg ± 60.42[b]
11% Cod Offal, 89% Peat	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	400.00 mg/kg ± 20.00[a]	736.67 mg/kg ± 74.23[b]
13% Cod Offal, 87% Peat	200.00 mg/kg ± 40.00	506.67 mg/kg ± 45.06	486.67 mg/kg ± 62.40[a]	630.00 mg/kg ± 26.46[b]	500.00 mg/kg ± 65.83[a]	793.33 mg/kg ± 35.12[b]	516.67 mg/kg ± 45.08[a]	750.00 mg/kg ± 65.20[b]	543.33 mg/kg ± 47.67[a]	770.00 mg/kg ± 45.19[b]

* All results are expressed in dry weight. Mean values of three determinations ± standard deviations.

** Week 0 occurs after two weeks of pre-composting and is also time period at which the earthworms were added to the containers for vermicomposting.

[a,a] Values in the same row for the same week with the same superscript are not statistically different (P > 0.05).

[a,b] Values in the same row for the same week with a different superscript are statistically different (P > 0.05).

n.d. Not Determined

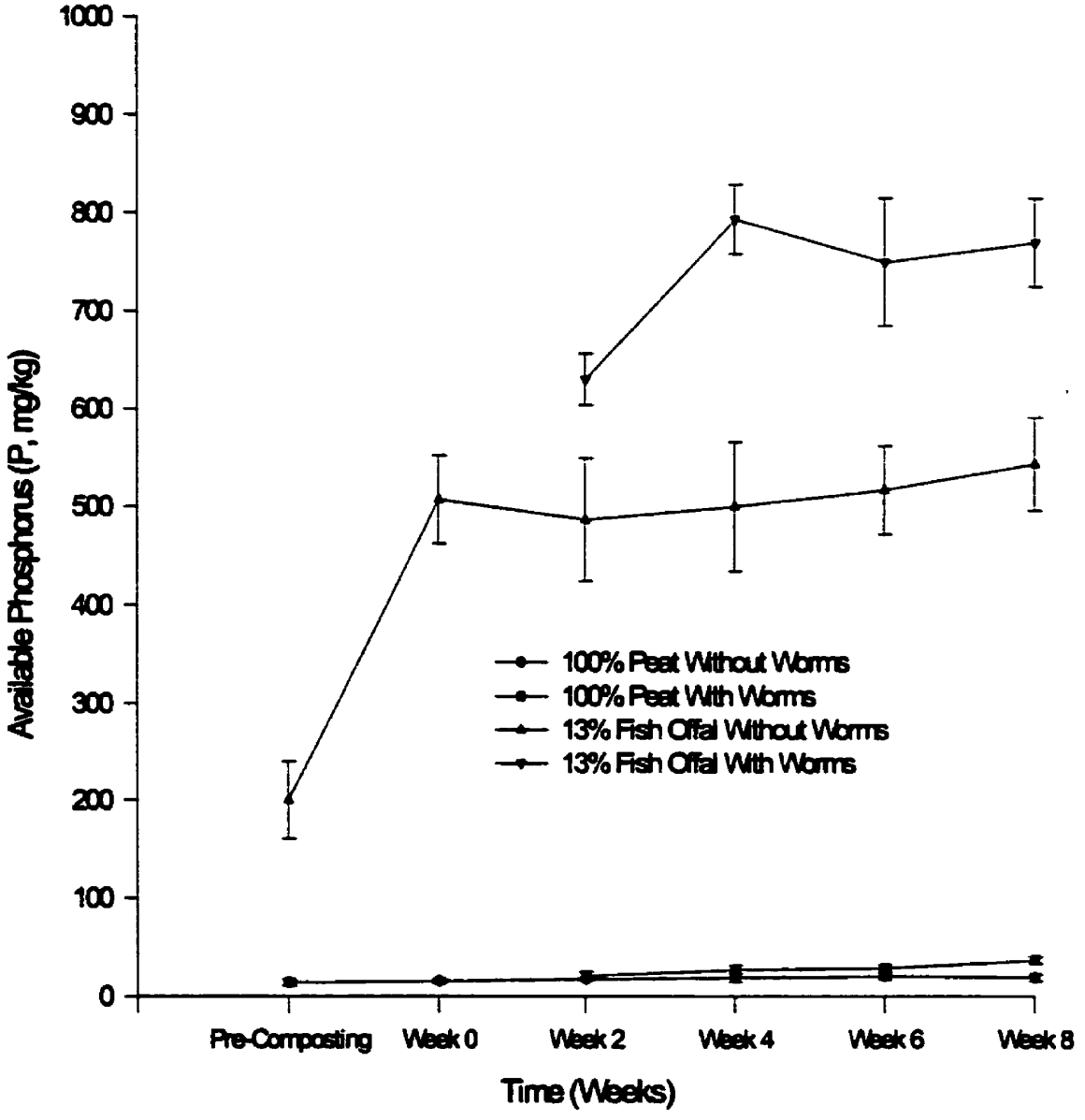

Figure 20. Changes in Available Phosphorus (P, mg/kg) during composting in the presence and absence of *Eisenia fetida* for samples with 100% *Sphagnum* peat and for samples with 13% cod (*Gadus morhua*) offal and 87% *Sphagnum* peat over a 10 week period (including 2 weeks of pre-composting prior to adding the earthworms) (dry wt.).

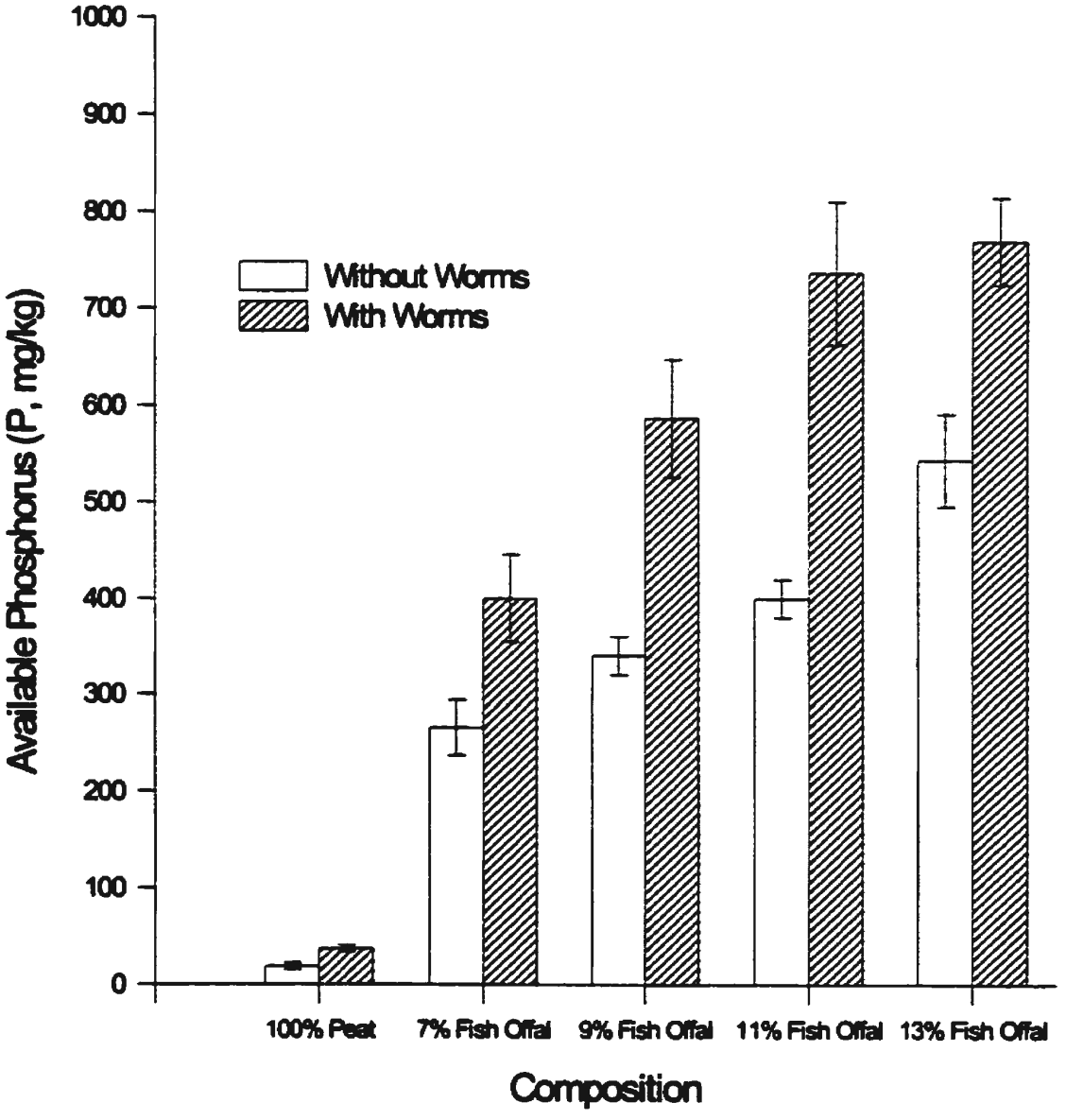

Figure 21. Changes in Available Phosphorus (P, mg/kg) during composting in the presence and absence of *Eisenia fetida* for samples with 0%, 7%, 9%, 11%, and 13% cod (*Gadus morhua*) offal mixed with *Sphagnum* peat at the 8th week of vermicomposting (there was 2 weeks of pre-composting prior to adding the earthworms) (dry wt.).

Table 11. Changes in Available Potassium (K, mg/kg) during composting of cod (*Gadus morhua*) offal mixed with *Sphagnum* peat in the presence and absence of *Eisenia fetida* over a 10 week period (including 2 weeks of pre-composting).*

Time (Weeks)	Pre-Composting	0**	2		4		6		8	
	No Worms	No Worms	No Worms	With Worms	No Worms	With Worms	No Worms	With Worms	No Worms	With Worms
100% Peat	31.21 mg/kg ± 5.07	35.75 mg/kg ± 2.47	35.21 mg/kg ± 4.86^{a}	41.80 mg/kg ± 9.43^{a}	33.29 mg/kg ± 6.70^{a}	42.63 mg/kg ± 0.76^{a}	34.17 mg/kg ± 4.98^{a}	48.08 mg/kg ± 2.50^{b}	32.17 mg/kg ± 1.13^{a}	55.13 mg/kg ± 5.84^{b}
7% Cod Offal, 93% Peat	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	181.67 mg/kg ± 10.02^{a}	247.08 mg/kg ± 25.04^{b}
9% Cod Offal, 91% Peat	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	207.08 mg/kg ± 28.07^{a}	313.83 mg/kg ± 37.61^{b}
11% Cod Offal, 89% Peat	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	319.58 mg/kg ± 6.41^{a}	389.17 mg/kg ± 46.35^{a}
13% Cod Offal, 87% Peat	223.33 mg/kg ± 10.41	340.00 mg/kg ± 56.97	334.58 mg/kg ± 16.27^{a}	420.42 mg/kg ± 32.80^{b}	323.33 mg/kg ± 26.73^{a}	482.50 mg/kg ± 15.61^{b}	335.42 mg/kg ± 14.81^{a}	511.25 mg/kg ± 56.44^{b}	351.25 mg/kg ± 44.60^{a}	494.58 mg/kg ± 19.38^{b}

* All results are expressed in dry weight. Mean values of three determinations ± standard deviations.

** Week 0 occurs after two weeks of pre-composting and is also time period at which the earthworms were added to the containers for vermicomposting.

^{a,a} Values in the same row for the same week with the same superscript are not statistically different ($P > 0.05$).

^{a,b} Values in the same row for the same week with a different superscript are statistically different ($P > 0.05$).

n.d. Not Determined

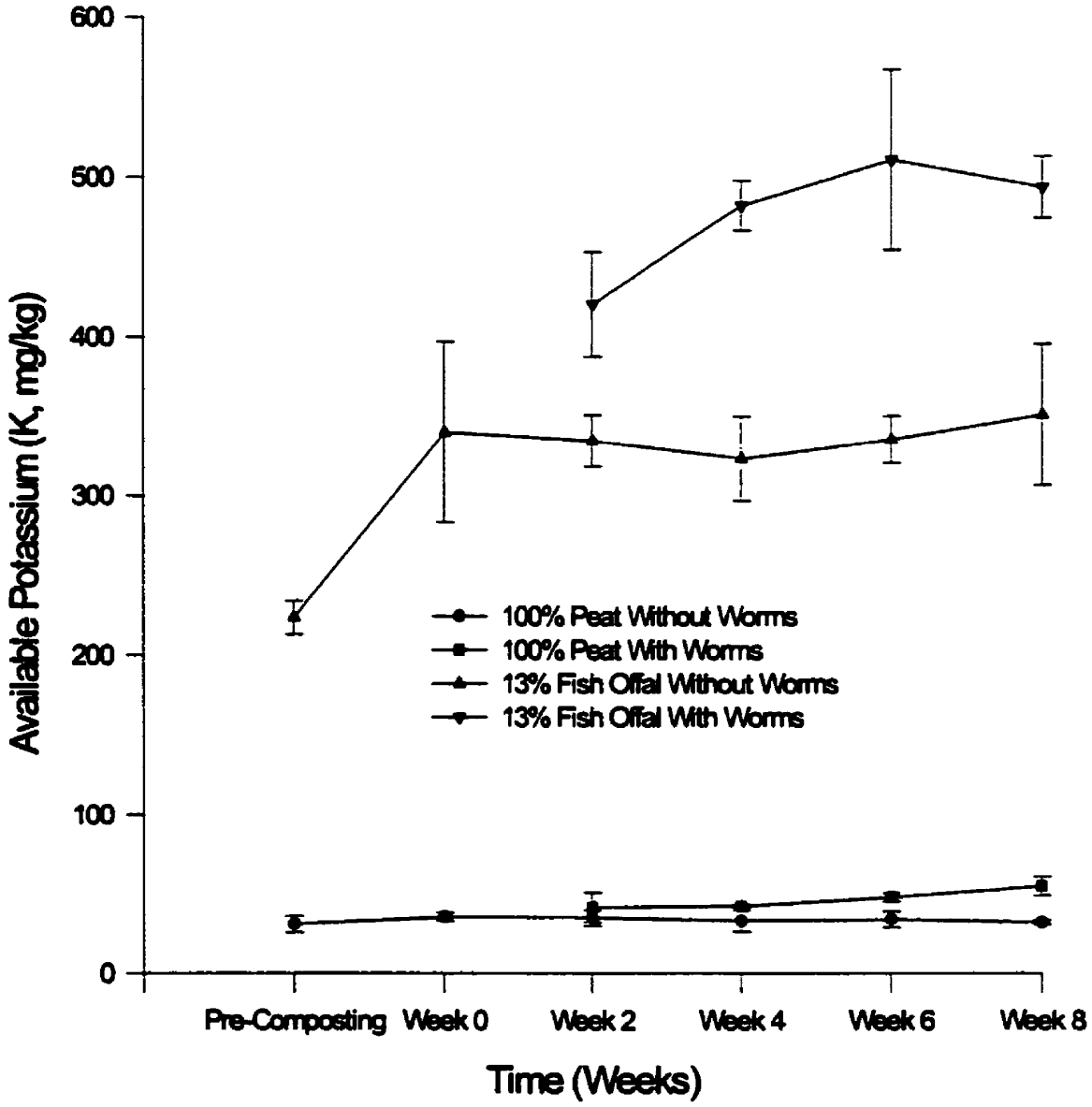

Figure 22. Changes in Available Potassium (K, mg/kg) during composting in the presence and absence of *Eisenia fetida* for samples with 100% *Sphagnum* peat and for samples with 13% cod (*Gadus morhua*) offal and 87% *Sphagnum* peat over a 10 week period (including 2 weeks of pre-composting prior to adding the earthworms) (dry wt.).

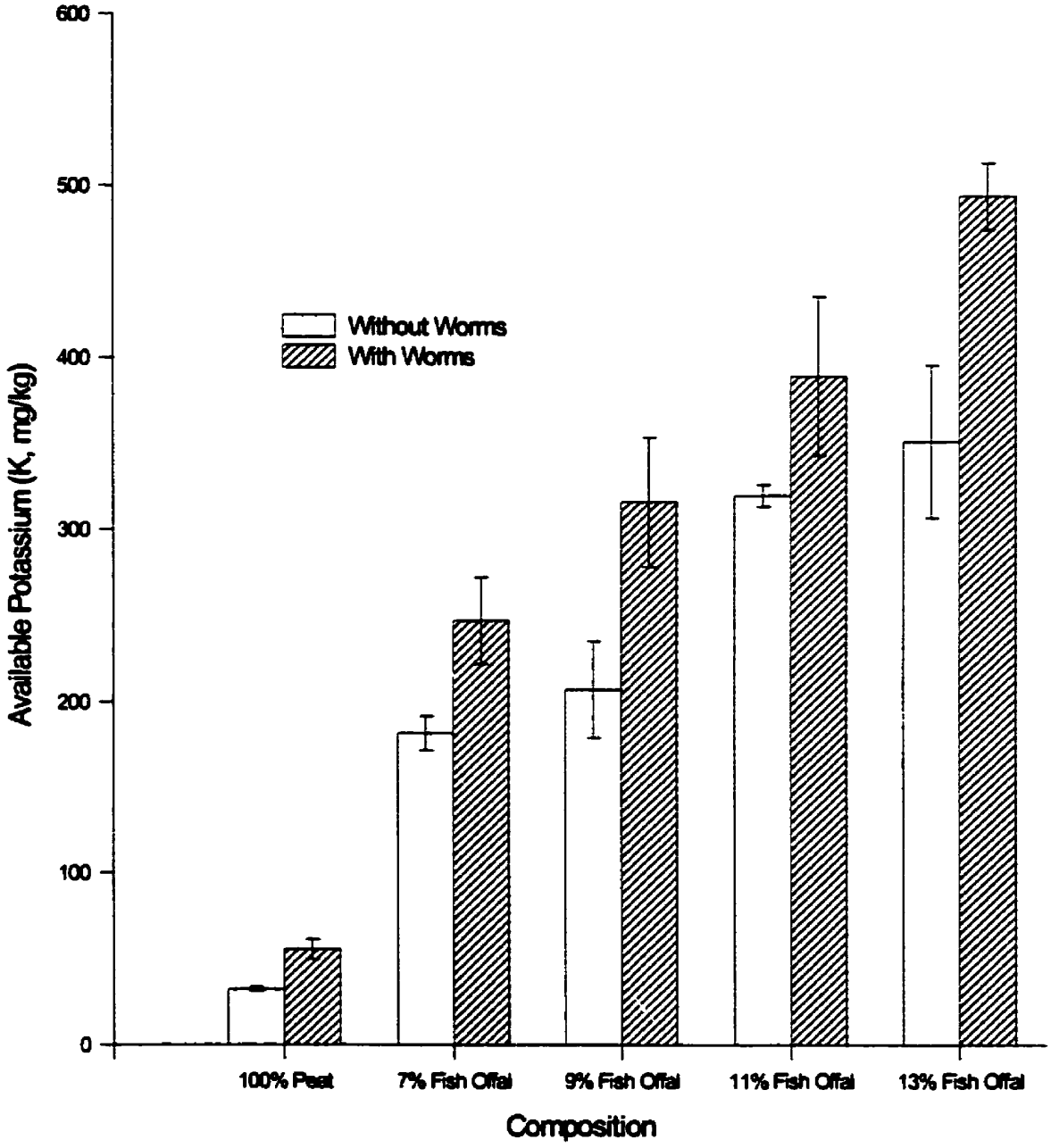

Figure 23. Changes in Available Potassium (K, mg/kg) during composting in the presence and absence of *Eisenia fetida* for samples with 0%, 7%, 9%, 11%, and 13% cod (*Gadus morhua*) offal mixed with *Sphagnum* peat at the 8th week of vermicomposting (there was 2 weeks of pre-composting prior to adding the earthworms) (dry wt.).

4.7.3 Calcium

Results showed that there was a significantly greater amount of available calcium in earthworm casts in comparison to controls without worms (Table 12). The amount of available calcium in the samples without worms remained constant throughout the study period. However, the amount of available calcium continued to increase as the earthworms continued to digest the material in the containers (Figure 24). In all samples in which earthworms had a survival rate of 100% there was a significant increase in the amount of available calcium (Figure 25).

4.7.4 Magnesium

Results showed that earthworms can increase the levels of available magnesium in comparison to controls without earthworms (Table 13). In all the samples the amount of available magnesium increased during the pre-composting period and the first 2 weeks of composting as the cod offal was still breaking down. After all the cod offal was digested the available magnesium levels remained constant in the samples without worms. However, for the vermicomposting samples the available magnesium levels continued to increase as the earthworms were still digesting the raw materials (Figure 26). As well, in a comparison of containers with different percentages of cod offal during the final week of the study it was shown that there is a difference in the amount of available magnesium (Figure 27).

Table 12. Changes in Available Calcium (Ca, mg/kg) during composting of cod (*Gadus morhua*) offal mixed with *Sphagnum* peat in the presence and absence of *Eisenia fetida* over a 10 week period (including 2 weeks of pre-composting).*

Time (Weeks)	Pre-Composting	0**	2		4		6		8	
	No Worms	No Worms	No Worms	With Worms	No Worms	With Worms	No Worms	With Worms	No Worms	With Worms
100% Peat	232.92 mg/kg ± 40.65	320.63 mg/kg ± 6.19	410.00 mg/kg ± 18.92^{a}	894.17 mg/kg ± 81.01^{b}	427.50 mg/kg ± 19.41^{a}	883.75 mg/kg ± 62.16^{b}	542.08 mg/kg ± 64.20^{a}	1094.92 mg/kg ± 4.03^{b}	440.00 mg/kg ± 63.87^{a}	1101.12 mg/kg ± 54.98^{b}
7% Cod Offal, 93% Peat	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	610.42 mg/kg ± 120.29^{a}	1257.50 mg/kg ± 104.65^{b}
9% Cod Offal, 91% Peat	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	653.75 mg/kg ± 153.46^{a}	1587.50 mg/kg ± 250.00^{b}
11% Cod Offal, 89% Peat	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	810.83 mg/kg ± 53.99^{a}	1720.83 mg/kg ± 222.32^{b}
13% Cod Offal, 87% Peat	432.50 mg/kg ± 23.35	697.50 mg/kg ± 33.75	804.17 mg/kg ± 77.17^{a}	1266.25 mg/kg ± 94.38^{b}	688.33 mg/kg ± 41.63^{a}	1591.67 mg/kg ± 91.41^{b}	760.83 mg/kg ± 106.73^{a}	1684.58 mg/kg ± 163.13^{b}	816.25 mg/kg ± 105.02^{a}	1812.50 mg/kg ± 219.02^{b}

* All results are expressed in dry weight. Mean values of three determinations ± standard deviations.

** Week 0 occurs after two weeks of pre-composting and is also time period at which the earthworms were added to the containers for vermicomposting.

^{a,a} Values in the same row for the same week with the same superscript are not statistically different ($P > 0.05$).

^{a,b} Values in the same row for the same week with a different superscript are statistically different ($P > 0.05$).

n.d. Not Determined

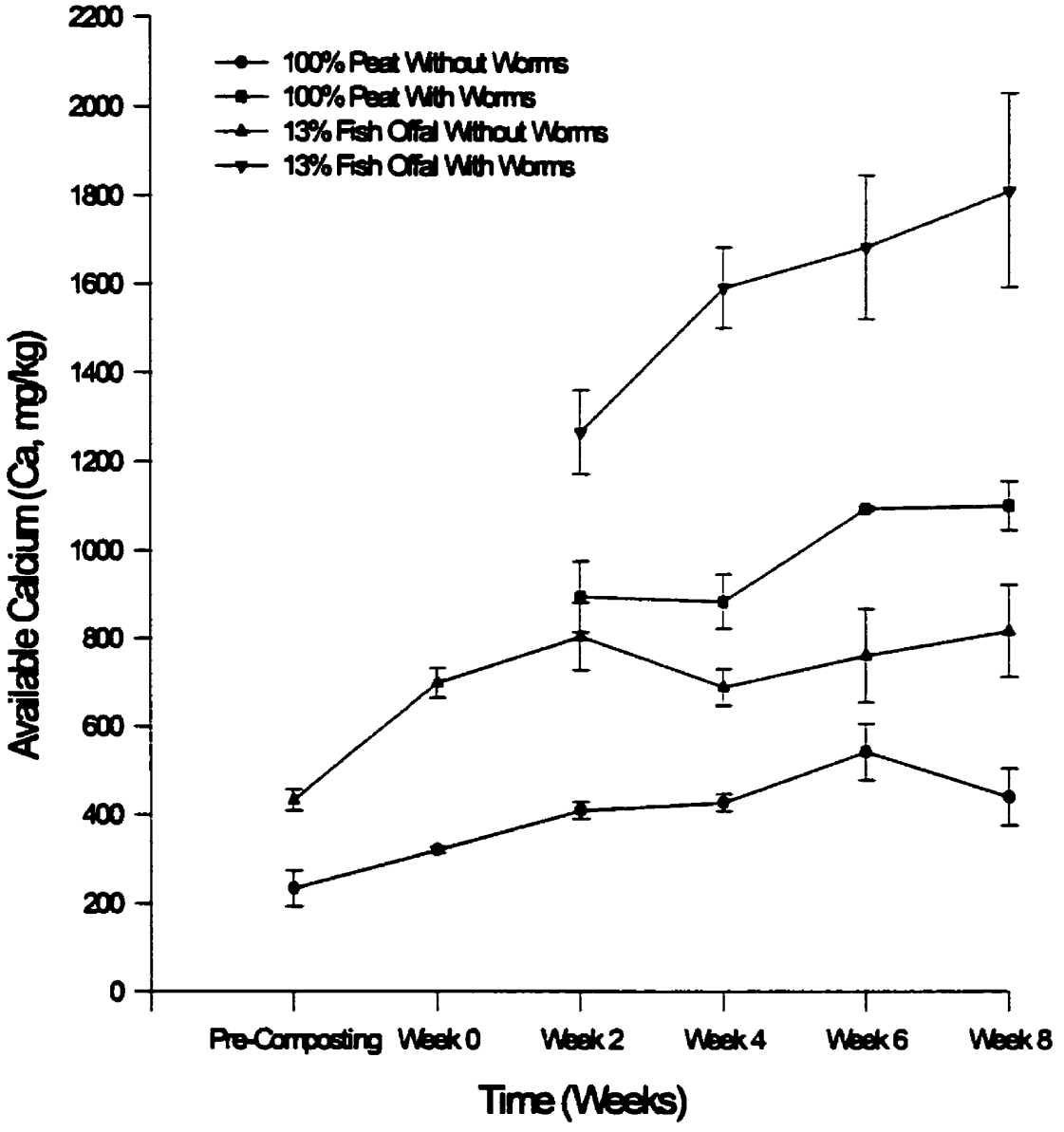

Figure 24. Changes in Available Calcium (Ca, mg/kg) during composting in the presence and absence of *Eisenia fetida* for samples with 100% *Sphagnum* peat and for samples with 13% cod (*Gadus morhua*) offal and 87% *Sphagnum* peat over a 10 week period (including 2 weeks of pre-composting prior to adding the earthworms) (dry wt.).

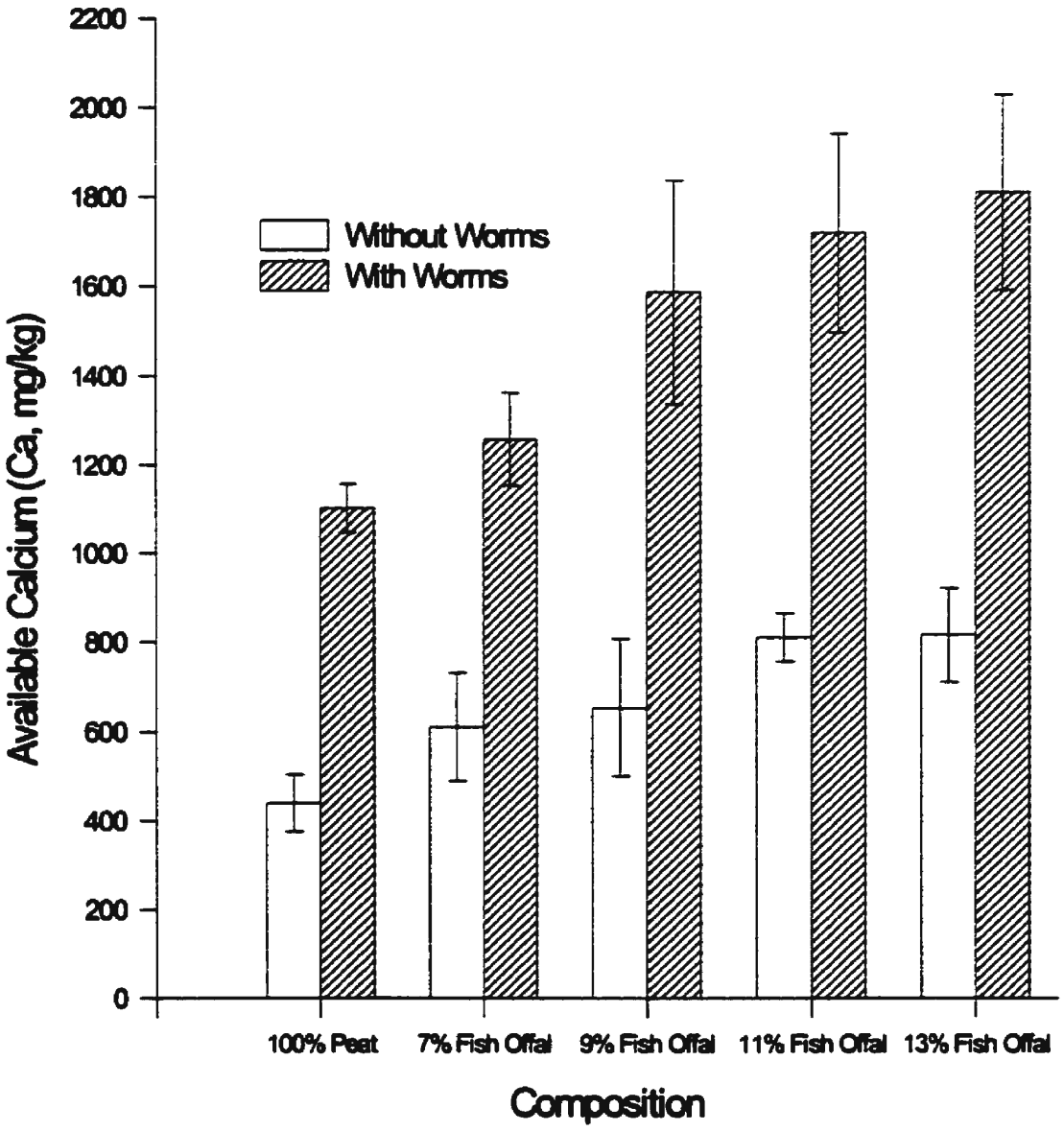

Figure 25. Changes in Available Calcium (Ca, mg/kg) during composting in the presence and absence of *Eisenia fetida* for samples with 0%, 7%, 9%, 11%, and 13% cod (*Gadus morhua*) offal mixed with *Sphagnum* peat at the 8th week of vermicomposting (there was 2 weeks of pre-composting prior to adding the earthworms) (dry wt.).

Table 13. Changes in Available Magnesium (Mg, mg/kg) during composting of cod (*Gadus morhua*) offal mixed with *Sphagnum* peat in the presence and absence of *Eisenia fetida* over a 10 week period (including 2 weeks of pre-composting).*

Time (Weeks)	Pre-Composting	0**	2		4		6		8	
	No Worms	No Worms	No Worms	With Worms	No Worms	With Worms	No Worms	With Worms	No Worms	With Worms
100% Peat	169.17 mg/kg ± 20.97	236.88 mg/kg ± 18.56	293.33 mg/kg ± 14.05^{a}	299.17 mg/kg ± 26.28^{a}	289.17 mg/kg ± 16.31^{a}	295.42 mg/kg ± 27.52^{a}	292.92 mg/kg ± 13.22^{a}	345.00 mg/kg ± 9.92^{b}	285.42 mg/kg ± 27.51^{a}	363.33 mg/kg ± 24.30^{b}
7% Cod Offal, 93% Peat	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	277.50 mg/kg ± 12.37^{a}	377.50 mg/kg ± 21.21^{b}
9% Cod Offal, 91% Peat	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	289.17 mg/kg ± 39.92^{a}	412.08 mg/kg ± 30.66^{b}
11% Cod Offal, 89% Peat	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	311.25 mg/kg ± 9.92^{a}	425.00 mg/kg ± 28.75^{b}
13% Cod Offal, 87% Peat	167.50 mg/kg ± 16.54	275.00 mg/kg ± 40.64	290.00 mg/kg ± 13.05^{a}	353.75 mg/kg ± 27.07^{b}	292.92 mg/kg ± 34.13^{a}	434.58 mg/kg ± 19.38^{b}	294.58 mg/kg ± 11.88^{a}	442.08 mg/kg ± 56.45^{b}	300.83 mg/kg ± 21.30^{a}	463.75 mg/kg ± 22.98^{b}

* All results are expressed in dry weight. Mean values of three determinations ± standard deviations.

** Week 0 occurs after two weeks of pre-composting and is also time period at which the earthworms were added to the containers for vermicomposting.

^{a,a} Values in the same row for the same week with the same superscript are not statistically different ($P > 0.05$).

^{a,b} Values in the same row for the same week with a different superscript are statistically different ($P > 0.05$).

n.d. Not Determined

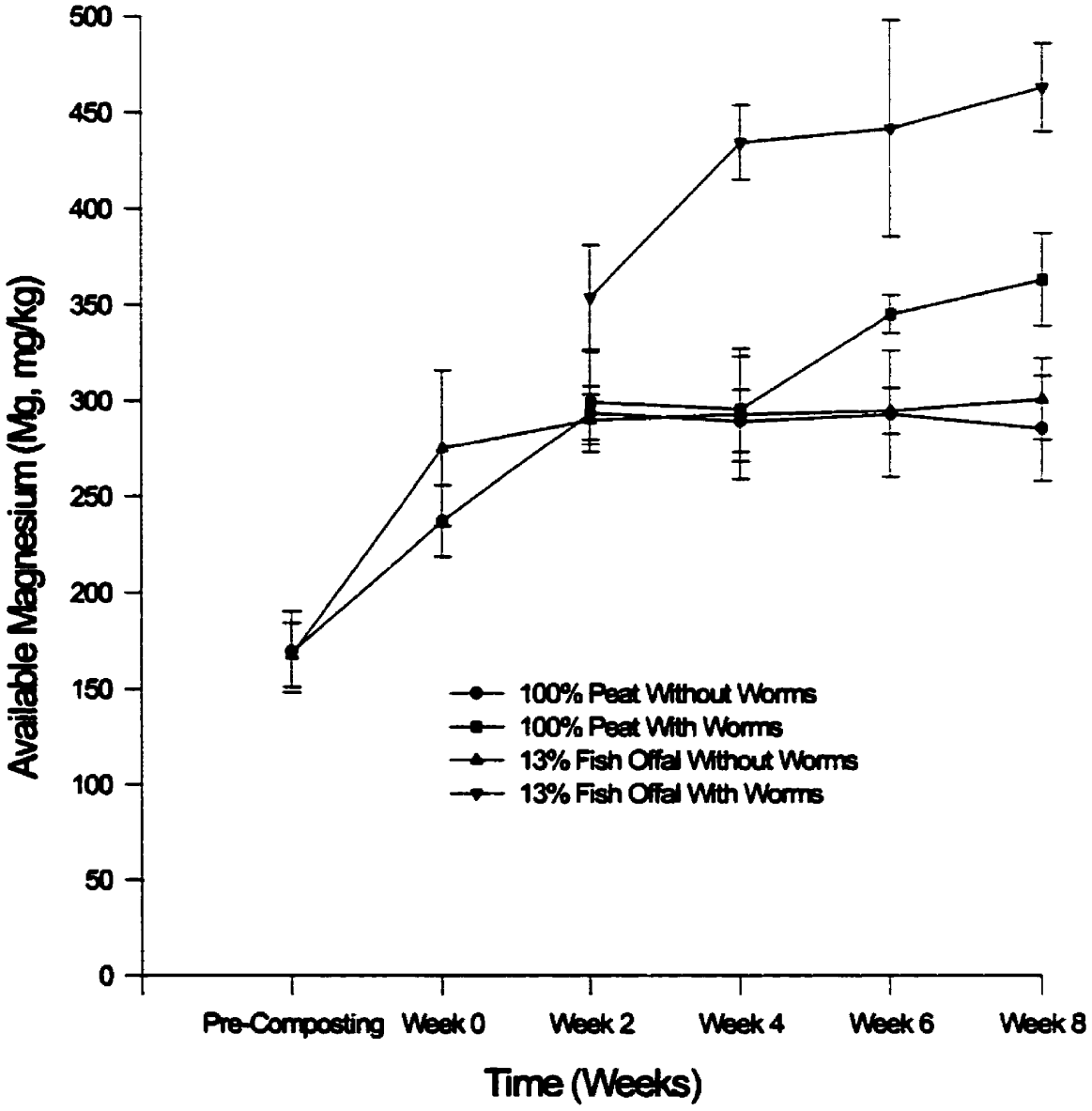

Figure 26. Changes in Available Magnesium (Mg, mg/kg) during composting in the presence and absence of *Eisenia fetida* for samples with 100% *Sphagnum* peat and for samples with 13% cod (*Gadus morhua*) offal and 87% *Sphagnum* peat over a 10 week period (including 2 weeks of pre-composting prior to adding the earthworms) (dry wt.).

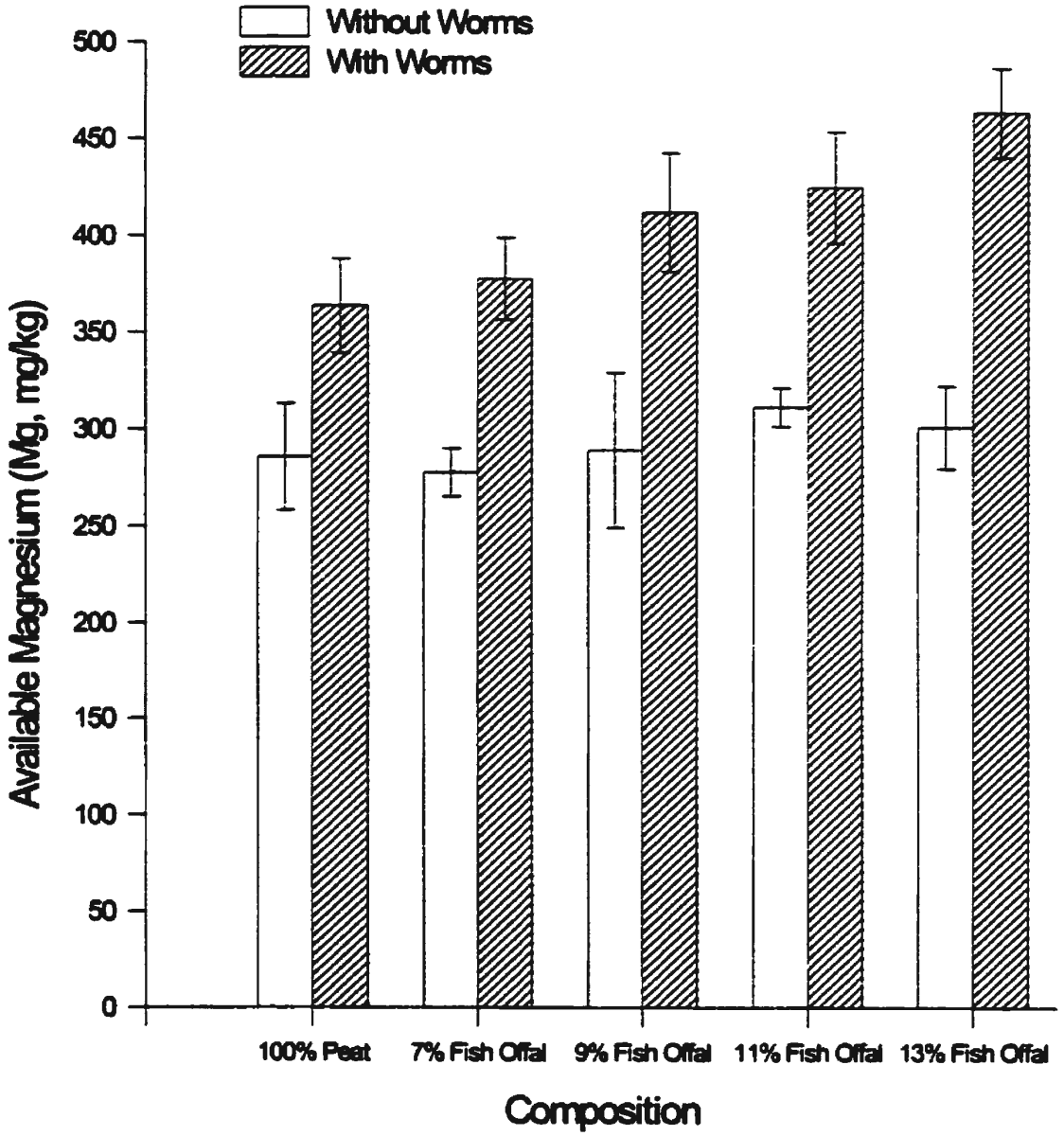

Figure 27. Changes in Available Magnesium (Mg, mg/kg) during composting in the presence and absence of *Eisenia fetida* for samples with 0%, 7%, 9%, 11%, and 13% cod (*Gadus morhua*) offal mixed with *Sphagnum* peat at the 8th week of vermicomposting (there was 2 weeks of pre-composting prior to adding the earthworms) (dry wt.).

4.8 Seed Germination

If there are no plant inhibitors, small seeds placed on a filter paper and soaking up an aqueous extrantant of the compost in a Petri dish should germinate to the same percentage level as those placed on the paper soaked in distilled water (Mathur, 1991; Mathur, 1998). Results showed that the percentage of seed germination using distilled water was 92%. For all of the samples both in the presence and the absence of earthworms the seed germination ranged between 90% - 98% germination (Table 14). This indicates that all of the samples are free of plant inhibitors such as water-extractable aliphatic acids.

Table 14. Percent germination (± standard error of the mean) of radish seeds in the composts and vermicomposts with cod (*Gadus morhua*) offal during the final week of the study (week 8).

	Week 8	
	No Worms	With Worms
Only Distilled Water	92.00% ± 0.06%	n.d.
100% Peat Extract	90.00% ± 0.11%[a]	93.00% ± 0.08%[b]
7% Cod Offal and 93% Peat Extract	95.00% ± 0.00%[a]	92.00% ± 0.06%[b]
9% Cod Offal and 91% Peat Extract	95.00% ± 0.05%[a]	93.00% ± 0.03%[b]
11% Cod Offal and 89% Peat Extract	93.00% ± 0.03%[a]	95.00% ± 0.05%[b]
13% Cod Offal and 87% Peat Extract	95.00% ± 0.05%[a]	98.00% ± 0.03%[b]

[a,a] Values in the same row for the same week with the same superscript are not statistically different (P > 0.05).
[a,b] Values in the same row for the same week with a different superscript are statistically different (P > 0.05).
n.d. Not Determined

5.0 CONCLUSIONS

Vermicomposting has been shown to be an effective method for stabilizing cod offal. These are the conclusions of this study:

1) To vermicompost with peat and cod offal the maximum proportion of cod offal that can be used to ensure 100% survival of *Eisenia fetida* is 13% cod offal (dry wt.).

2) Vermicomposting can increase the stabilization of organic matter in comparison to controls without earthworms.

3) Ammonium (NH_4^+) levels should not exceed 1.0 mg/kg in a mixture when earthworms are being added for vermicomposting.

4) Results also indicated that composting with worms increased the amount of available nutrients (phosphorus, potassium, calcium, magnesium) for plants which makes vermicomposting a beneficial option for future composting operations.

Other than vermicomposting with just cod offal, it is also suggested to include seaweed in the vermicompost mixture. Fish offal is a good source of nitrogen and phosphorus. However, seaweeds, such as the kelps, are rich in potassium and are also good sources of such elements as iodine, boron, copper, magnesium, calcium, and phosphorus. Thus, a compost containing both fish offal and seaweed would have a good well-balanced N-P-K level.

In conclusion, this experiment was conducted in small containers under controlled laboratory conditions and the results may not be exactly the same as those that would occur

under field conditions. However, the experiment did evaluate fundamental factors affecting the activity of earthworms and the results should be indicative of the impact of field conditions on the vermistabilization process. It would be difficult to reproduce this experiment at a large scale due to the fact that only 13% cod offal can be used to ensure survival of the earthworms. As a result, too much peat would be required and the project would not be economically feasible on a large scale.

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