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# THE INFLUENCE OF SOIL AMENDMENTS (COAL FLY ASH, AND STABILIZED BIOSOLIDS) ON THE PLANT PARASITIC NEMATODE Meloidogyne hap\a\ INCLUDING FREE LIVING NEMATODES, MICROBES AND SOIL CHEMICAL PROPERTIES

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# THE INFLUENCE OF SOIL AMENDMENTS (COAL FLY ASH, AND STABILIZED BIOSOLIDS) ON THE PLANT PARASITIC NEMATODE *Meloidogyne hapla*; INCLUDING FREE LIVING NEMATODES, MICROBES AND SOIL CHEMICAL PROPERTIES

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## ABSTRACT

Field and greenhouse soil studies were designed to test the effect of stabilized biosolids and coal fly ash as management on the plant-parasitic nematode *Meloidogyne hapla*. The trials were conducted in a Bryanston silt loam in Southwestern Ontario formed on a calcareous substrate. Fly ash was obtained from the Lambton Generating station in Sarnia, Ontario and stabilized biosolids from lagoons at Glencoe, Ontario. Field and greenhouse crops included carrot (*Daucus carota* L.) and tomato (*Lycopersicon esculentum* Mill), each grown from seeds.

Amendment ratios of biosolids and fly ash and application rates were established based on international guidelines and previous studies for the use of biosolids and ash in soils. Field trials included amendments of 1.5%, 3%, 4.5%, 6%, and 7.5% (w/w) of each raw material. Greenhouse trials included additions of 2.5%, 5%, 7.5%, 10%, and 12.5% (w/w) of each raw material. Changes in parameters that might affect nematode viability were monitored including electrical conductivity, pH, biocontrol agents (bacterial and fungal colony forming units), selected heavy metals (As, Cd, Cr, Cu, Ni, Pb, Zn), and plant yield.

Carrot yield was improved with increased amendments while infested carrots did not show visible nematode damage. Tomatoes grown with amendments showed improved yields relative to controls in the presence of nematode infection. None of the amendment application rates altered EC, pH, CFU, or metals enough to impact nematode populations. It can be concluded that the amendment application rates tested in this study for the raw materials used were below threshold levels that impact nematode populations. Future work should take into consideration the impact of the raw materials on parameters that control nematodes so that application rates include levels (upper and lower) that affect nematode variability.

Keywords: Fly ash, biosolids, soil amendments, *Meloidogyne hapla*, crop yield

En mi parcela sembré unas coles unas cebollas y un perejil,
si en enero quedan plantadas cosecharemos en el abril,
un cierto día, muy de mañana a mis plantitas a verlas fui,
y me encontré hermosas perlas que relucían cual un rubí
traté cogerlas, se disolvieron, y mi vestido quedó mojado
eran las lágimas de mis plantitas que por mi ausencia habían llorado

Anonymous
Oral Tradition - Ecuador

## DEDICATION

**To the people from Glencoe, Ontario who indirectly provided the materials necessary to conduct this study.**

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| | |
|---|---|
| AH | Fly ash |
| A50 | Mixture of 50% biosolids and 50% fly ash |
| A75 | Mixture of 75% biosolids and 25% fly ash |
| BFN | Bacterial feeding nematodes |
| BS | Biosolids |
| cm | centimeter |
| °C | Degrees Celsius |
| CCB | Coal combustion by-products |
| CT | Control treatment (Soil only) |
| CT-N | Control treatment without nematode inoculum |
| CT+N | Control treatment with nematode inoculum |
| CTF-N | Control treatment with fertilizer without nematode inoculum |
| CTF+N | Control treatment with fertilizer with nematode inoculum |
| cv. | Cultivar |
| EC | Electrical conductivity |
| ESW | Environmental Sciences Western Field Station |
| FFN | Fungal feeding nematodes |
| g | gram |
| ha | hectare |
| km | Kilometer |
| l | Liter |
| $m^2$ | Square meter |
| m.a.s.l | Meters above sea level |
| mg | milligram |
| ml | milliliter |
| $mm^2$ | Square millimeter |
| mm | millimeter |
| PFN | Plant feeding nematodes |
| µm | micrometer |
| µS | microSiemen |
| µl | microliter |
| pH | Acidity |

# 1 INTRODUCTION

This project was inspired by observations of a field experiment in Ecuador where an amendment made from a mixture of biosolids and ash was applied to a ginger crop. Preliminary results from that experiment showed that ginger plants grown in plots that were planted with the same crop the previous year and had received the amendments, had no apparent nematode infestation. As stated by Mai (1985) crops that are extremely susceptible to nematodes cannot be planted in the same field after the crop has been harvested. This becomes especially important in tropical regions where environmental conditions are ideal for nematode propagation. The constant search for "virgin" plots is not economically feasible, especially in areas where agricultural land is becoming more and more scarce. Applying mixtures of biosolids and fly ash became an important alternative to nematode management as well as an alternative for their disposal.

In this study, carrot and tomato plants were grown under field and greenhouse conditions using mixtures of stabilized biosolids and coal combustion fly ash. Both plant species are affected by plant-parasitic nematodes and therefore were selected as hosts. The application rates selected for this project were designed to improve crop yield and to alter the populations of the plant-parasitic nematode *Meloidogyne hapla*. Amendment application rates were designed not to breach any application guideline regarding heavy metals in soils.

The plant-parasitic northern root-knot nematode (*Meloidogyne hapla*) is a serious threat to several crops in temperate regions, causing important yield losses related to root malformation and galling, which inhibit nutrient absorption by the plant (Bélair and Fournier, 1996). This parasite causes severe damage to carrots including reduced weight and length of the primary taproot. Extreme infestation can cause forking of the carrots, making them unmarketable (Bélair, 1992; Kimpinski and Sanderson, 2003). Additionally, it is well known that this nematode species also affects tomato plants by infesting its roots and causing it to absorb

nutrients poorly, which ultimately results in reduced yields. The degree of damage that *M. hapla* causes carrots and tomatoes will depend on how resistant or susceptible the cultivars are. Carrot appears to be more susceptible (Wang and Goldman, 1996) than tomato where a few resistant cultivars have been determined (Oka et al., 1999; Roberts, 1992). Carrot and tomato cultivars selected for this study were susceptible to *M. hapla* infestation because the same cultivars were used to propagate the inoculum used for all experiments. Degree of infestation was determined by a quantitative method of egg extraction and not by a qualitative method such as gall rating. Eggs extracted from infested roots in the treatments result from egg-laying females that are living inside the host plant, reflecting positive infection and quantitatively accounting for differences among treatments.

Soil nematode populations are affected by direct (e.g., metal concentrations or changes in pH) and indirect (e.g., availability of food and predation) parameters in soils in which they live. Determining direct or indirect effects is complicated. For instance, changes in pH can have direct effects on nematode populations by influencing metal availability. Indirect effects can be related to shifts in nematode species dominance, which in turn control nematode abundance. Soil metal concentrations can have both direct and indirect effects on nematode populations. There is significant variability as to how direct and indirect effects control nematode populations due to changes in soil types, ranges of pH, EC, and other soil parameters.

### 1.1 Agriculture, Chemical Inputs, and Pest Control Management

Humans have domesticated plants and used them for their benefit for millennia but this interaction has changed in many ways, especially during the last 50 years. The phenomenon know as the "Green Revolution", which is heavily dependent on chemical compounds, has improved agricultural processes relying on the use of external inputs to increase yields, reduce labor, and reduce disease

incidence (Evenson and Gollin, 2003). The use of chemical compounds has been preferred over naturally occurring compounds because of their reliability and efficiency, but the consequences of their use have affected the environment and human health (Edwards, 1993).

Most modern agricultural practices, which employ chemical fertilizers, pesticides and herbicides, have affected the soil's biota by reducing its biodiversity. Biodiversity promotes homeostasis between the biological and non-biological components of soils, promoting essential processes, and hence supplying adequate amounts of nutrients required for sustainable plant growth. A healthy soil has the ability to control populations of pathogens, which can be reduced when the soil is sterilized by means of a physical or chemical treatment (Westphal, 2005).

Several chemical compounds that affect the environment directly or indirectly have been banned from agricultural use after the 1997 Montreal Protocol (UNEP, 2000). One such compound is Methyl Bromide, one of the most important compounds for controlling nematode infestation (Duncan, 1991). This compound was widely used to control soilborne pathogens, but it also caused the depletion of the ozone layer, contributing to global climate change (Noling and Becker, 1994).

Stabilized biosolids and coal combustion fly ash have been applied to soils, improving crop yield as well as soil health (Parkpian et al., 2002; Punshon et al., 2002). However, these compounds should be analyzed prior to their application to soils because they may contain concentrations of heavy metals that might enter the food chain altering the balance in the soil ecosystem. After the concentration of these elements is determined, an appropriate amount of these by-products can be applied to soils, enhancing the beneficial effects mentioned above. Soil amendments made from biosolids and fly ash can be seen not only as beneficial to the crop, but also as a way to use them rather than land filling.

## 1.2 Nematodes

### 1.2.1 Kinds, Distribution, and Physical Attributes

An increase in the populations of free-living species of nematodes can be an indicator of soil health, reflecting the availability of nutrients, water-holding capacity, soil structure, pH, and buffering capacity (Widmer et al., 2002; Magdoff, 2001). Fluctuations in nematode communities can be due to addition of fertilizers, tillage practices, plant species and their physiological state, heavy metals, and pesticides (Neher, 2001).

Only a few nematode taxa show parasitic behavior, but they can cause severe damage to their hosts, including death. In crop plants, parasitic nematode damage can be economically quantified and is estimated at approximately 100 billion dollars per year worldwide (Zasada et al., 2008).

Nematodes are unsegmented roundworms found in almost every environment on Earth. Their ubiquitous distribution is due to the fact that they have very diverse and specialized feeding habits contributing to soil processes related to nutrient cycling. There is an estimated half a million species of nematodes that feed on a wide range of organisms, including bacteria, fungi, and other nematodes (Strange, 2003). Due to the interactions among nematodes and so many different organisms, they have become key players in various food webs at several trophic levels (Ingham et al., 1985).

The "mouth" parts, or stoma, of nematodes are specialized according to the prey or food source. Bacterial feeding nematodes have an "open-mouth" that, in some species, is equipped with external appendages that help them graze on bacteria. Fungal feeding nematodes have a hollow needle-like structure that helps them puncture the hyphae of the fungi that they feed on. Predatory nematodes have tooth-like structures known as denticles in their stoma that allow them to cut the cuticles of other nematodes as well as other soil microorganisms (Yeates et al.,

1993). Taken together, these free-living nematodes form part of the soil food web and feed on and are food for other soil microorganisms. As a result of these interactions, many elements are released in the soil matrix, serving as nutrients for plants that grow in those environments (Neher, 2001). For example, an estimated 8 to 19% of the nitrogen mineralization in soils is attributed to the activity of predatory and bacterial feeding nematodes (Beare, 1997).

Plant-parasitic nematodes have a hollow "needle-like" structure called a stylet that is used to puncture the cell wall of plant tissue and feed on the cell contents. Stylets are classified as stomatostylets or odontostylets depending on their structure. Stylet kind, size, and shape are commonly used to classify plant-parasitic nematodes (Eisenback, 1985a).

The outermost layer of the nematode is called the cuticle, which protects the organism from many physical, chemical, and biological forces, and selectively regulates the flow of fluids through the body wall (Eisenback, 1985c).

Depending on their life cycle, plant-parasitic nematodes are classified as either ecto or endo parasites (sedentary/migratory). Each classification is related to where the nematode will complete its life cycle. Once they infect their host, they complete their reproductive cycle in the host (sedentary) or they move from one root system to the next (migratory). Endoparasitic nematodes that exhibit sedentary behavior have an infective (mobile) stage and then become immobile after establishing a parasitic relationship with its host and start feeding. Plants can be subjected to both types of nematodes and their negative effects can be additive (Eisenback, 1985b). Sedentary endoparasites (such as *Meloidogyne hapla*) usually enter the root tip and establish a feeding site by creating an enlarged multinucleated (giant) cell. At this stage, infective juveniles become sedentary females and begin laying eggs (Hussey and Grundler, 1998). Formation of numerous giant cells results in root galls, which reduce nutrient absorption by the plant. At this stage of infection, the plant becomes stunted or

chlorotic (Ellis et al., 2008). Often, this is misdiagnosed as nutrient deficiency by the farmer rather than nematode infection.

Ectoparasites cause direct and indirect damage. Direct damage may be wounds in the root, resulting in galleries or tunnels, where the nematodes have fed. The root system is weakened and the plant has to expend more energy producing new root shoots (Endo, 1975). Indirect damage is caused when nematodes transmit viruses from one plant to another. Additionally, the open wounds serve as an entry point for other soilborne pathogens such as bacteria and fungi (Mai, 1985). It has been shown that endoparasites are much more harmful to plants than ectoparasites (Trudgill, 1991).

### 1.2.2 Parameters That Control Nematodes

#### 1.2.2.1 Soil Texture

Distribution and abundance of nematodes in soils varies according to biotic and abiotic factors. Temperature and moisture act as extrinsic abiotic factors, while soil texture determines nematode distribution intrinsically. Van Gundy (1985) determined that the root-knot nematode *Meloidogyne* favors specific environmental conditions, which are complicated by interactions between soil moisture and temperature at different parts of their life cycle. For example, the optimal temperature range for adults of *M. javanica* is higher than the optimal temperature for their eggs and juveniles. This means that eggs and juveniles can resist lower temperatures and still be active to infest the plant. Such is the case of *M. hapla*, which can survive soil temperatures as low as 0 °C, remaining dormant during the winter season and becoming active soon after the crop is reestablished the following spring (Van Gundy, 1985).

Nematode distribution in soils is partly controlled by porosity and soil water/air oxygen content. Attempts have been made to relate soil textural data with nematode distribution, resulting in general considerations to determine nematode

prone areas. O'Bannon and Reynolds (1961) conducted an experiment under greenhouse and field conditions and found that cotton plants cultivated in sandier soils were more affected by *M. incognita* than those grown in soils with a higher clay content. Additionally, the authors suggested that *M. incognita* reproduced more rapidly in sandier soils. Monfort et al. (2007) carried out a 3 year experiment which included the use of GPS generated data and soil texture maps for a cotton field infested with *M. incognita*. Their results suggest that *M. incognita* had more detrimental effects in cotton yield in plots that had higher sand content.

#### 1.2.2.2 Soil Electrical Conductivity

Electrical conductivity can have both direct and indirect effects on nematode populations. Direct effects are related to absorption of elements into the nematode's body in soils with high osmotic potential. In a review, Norton (1979) reported evidence on how different species of nematodes can adapt to soil environments with different osmotic pressures partly due to increasing egg hatching in some species. Indirect effects are mainly related to how EC controls plant growth, increasing or decreasing host accessibility. Edongali et al. (1982) suggested that higher salinity levels in the soil might cause the plant to reduce calcium translocation inhibiting the development of meristematic tissue, hence reducing potential infection sites for nematodes. Direct and indirect effects have to be considered as a whole because ideal soil electrical conductivity can improve plant growth, but might also favor nematode activity.

#### 1.2.2.3 Soil pH

Soil pH can be changed by addition of chemical fertilizers, pesticides, herbicides or almost any agricultural amendment. The most important effect of pH change might be the impact on the host more than the nematodes (Norton, 1979). Brzeski (1969) found that *Tylenchorhynchus dubius* and *Pratylenchus crenatus*

were more numerous in acid than neutral or alkaline soils and that *Heterodera schachtii* was found in both neutral and acidic soils. Burns (1970) found that the greatest number of *Pratylenchus alleni* in soybean roots was found at pH 6.0. Morgan and MacLean (1968, cited by Norton 1979) reported that *Pratylenchus penetrans* was more abundant in vetch roots at pH 5.5-5.8 and declined when the pH was higher than 6.6. Siddiqui (2005) showed that nematode populations increased at pH values from 5.10-6.20 and decreased at pH from 7.80-8.40.

In forage yield experiments in alfalfa, the interaction of nematode infection and soil pH had a negative effect on the yield of alfalfa at soil pH 5.2 and 6.4 but no significant effect at pH 4.4 and 7.3 (Willis, 1972). Zasada and Tenuta (2004) demonstrated that after applying N-Viro soil (alkaline-stabilized municipal biosolid) high pH levels were a major factor in nematode mortality.

Korthals et al. (1996a) demonstrated that nematode communities were affected in an agroecosystem by the application of copper and change in pH. With lower pH levels, the total biomass of bacteria was reduced, which resulted in a lower population of bacterial-feeding nematodes. Conversely, fungal-feeding nematodes then exhibited an increase in their population, probably due to an increment in the fungal biomass. Plant feeding nematode populations were related to root biomass, which at lower pH levels was reduced. Nematodes use diffusion to breathe and for circulation of substances around their bodies. At lower pH enhanced metal bioavailability and increased ion exchange through the nematode cuticle can cause an accumulation of copper to toxic concentrations. Soil pH might have indirect effects on nematode communities by reducing food availability, competition among nematode species, and by affecting the abiotic environment (Korthals et al., 1996a). Most plant-parasitic nematodes favor a pH level of 5.10 – 6.40, which is also the level where most plants have the highest nutrient uptake, hence producing more root biomass. Conversely, pH levels that can cause high nematode mortality are also levels in which some elements might cause toxicity to the plant.

### 1.2.2.4 Microbial Control of Nematodes

The application of organic amendments has been shown to increase soil biodiversity, enhancing the development of potential plant-parasitic nematode biocontrol agents. Thorne (1961) noted increases in predatory nematodes, sporozoan parasites, nematophagous fungi, and other natural enemies of phytoparasitic nematodes with increasing applications of organic matter (OM). The application of OM to agricultural soils may also have an effect on various components of the food web, increasing the propagule density of nematophagous fungi (Venette et al., 1997) and the adhesive network of traps and knobs that allow nematophagous fungi to reduce plant-parasitic nematode populations (Jaffee, 2004). Akhtar and Alam (1993) reviewed the direct or indirect stimulation of biocontrol agents that reduce phytoparasitic nematodes.

Furthermore, the application of organic amendments that are high in N may have a negative effect on plant-parasitic nematodes because they produce compounds such as ammonia, and nitrous acid that have nematicidal properties. These properties are enhanced by certain physical and chemical changes such as soil buffering capacity, pH, and organic matter content resulting from the application of organic amendments (Lazarovits, 2001). Amendments such as meat and bone meal have shown promising results in reducing some soilborne diseases, but their efficacy depends on the characteristics of the soil to which the amendments are applied (Tenuta and Lazarovits, 2004). Soils with low organic carbon and to some extent the amount of sand present were very important in ammonia accumulation which in turn resulted in effective control for some of these soilborne diseases (Tenuta and Lazarovits, 2002).

Several studies have shown the potential of parasitic and non-parasitic bacteria to control plant-parasitic nematodes (Trudgill et al., 2000; Meyer et al., 2000). Chen et al. (1994) documented that *Pasteuria penetrans* can act as a biocontrol agent for the suppression of plant-parasitic nematodes. Non-parasitic bacteria

such as Rhizobacteria have the potential of reducing plant-parasitic nematode populations by means of colonizing the roots of the host plant and producing certain compounds that have nematicidal properties (Siddiqui and Mahmood, 1999).

## 1.3 Amendments

### 1.3.1 Biosolids

Stabilized biosolids can be a good source of organic matter and nutrients when applied to soils. The nutrient concentration of biosolids is typically equivalent to animal manures, but may be high in heavy metals, which may be harmful to a soil environment (OMAFRA, 1996).

The main benefits of the addition of biosolids to soils are their ability to increase bacterial/fungal populations, which may help control plant-parasitic nematodes.

### 1.3.2 Fly Ash

Coal combustion by-products ("ash" - material remaining after burning coal for energy (AH)) have been shown to improve the physical and chemical characteristics of soils in many parts of the World (Bilski et al., 1995). Coal combustion byproducts are typically divided into "fly ash" (material which leaves the boiler after combustion), and "bottom ash" (material which remains in the boiler). These two CCB are very different in size and elemental composition. They are mixtures of inorganic constituents found in the coal (mineral matter) and material that is not completely combusted (Bilski et al., 1995). Like biosolids, CCB may contain high concentrations of heavy metals. The application of CCB to a soil environment may increase the availability of carbonates, bicarbonates, sulphates, chlorides, B, P, K, Ca, Mg, Mn, Cu, Zn, and many essential trace elements as well as improve structure, water–holding capacity, pH, conductivity, and cation exchange capacity. When coal combustion byproducts are used as a soil amendment, they have enhanced plant growth, chlorophyll content, fruit

production, and fruit weight (Khan et al., 1997). Additionally, CCB have a negative effect on root invasion by plant-parasitic nematodes, severity of root-knot disease, and the numbers and reproductive cycles of phytoparasitic nematodes (Khan et al., 1997). In addition, numerous studies have shown that ash or ash and biosolids mixtures can be important controls for various microbes, which might act as biocontrol agents on nematodes (Wong and Wong, 1986; Pichtel, 1990; Wong et al., 1995).

### 1.4 Hypothesis and Objectives

This study will determine if addition of soil amendments composed of stabilized biosolids mixed with coal fly ash can reduce the population of plant-parasitic nematodes. The null hypothesis is that amended soils and non-amended soils will have the same population levels of plant-parasitic nematodes.

#### 1.4.1 Objectives:

- Determine if the physical and chemical changes in the amended soils produce nematicidal effects.
- Determine the effects of the amendments on fungal and bacterial activity.
- Determine if the amendments have a positive effect on plant growth.

#### 1.4.2 Experiments:

- Field experiment to test the effects of the amendments on naturally occurring nematode populations and carrot yield.
- Field experiment with naturally occurring nematodes, carrots, and *M. hapla* inoculum.
- Field experiment with tomatoes and *M. hapla* inoculum
- Greenhouse experiments with and without vegetation and *M. hapla* inoculum.

# 2 Materials and Methods

## 2.1 Field Area

All field experiments were conducted at the Environmental Sciences Western (ESW) Field Station located on Wonderland Rd. North and Ten Mile Road, Ilderton, Ontario, Canada (43°4'47"N, 81°20'24"W, and 292 m.a.s.l.). The plot chosen for experiments is close to an artificial pond from which water was used when needed. The pH and EC of the water in the pond, when sampled, were 8.4 and 235 µS/cm respectively. Precipitation for the summer months is shown in Appendix 1.

## 2.2 Materials Used for Treatments

### 2.2.1 Soil

The soil in the plot is a Bryanston silt loam with 11% sand, 76% silt, and 4% clay, 3% organic matter (Diner, 2004). Texture was determined by the hydrometer method. In order to promote nematode activity (Monfort et al., 2007) soil texture was modified to a sandy loam by adding 600 g of brick sand (AAROC Aggregates, 1460 Fanshawe Park. Rd., London, Ontario, Canada) for every 1000 g of ESW soil. The resulting soil mixture was a sandy loam with 46% sand, 46% silt, and 8% clay. Predetermination of nematode species present in the soil showed absence of the plant-parasitic nematode *Meloidogyne hapla*.

### 2.2.2 Biosolids

The biosolids used were obtained from the municipal sewage settling ponds from the town of Glencoe, Ontario, Canada. The biosolids were dewatered, transported to ESW, placed on a concrete pad for drying and subsequently crushed with a roller attached to a tractor. After crushing, large clumps (mostly clay fragments from the lining of the pond) were discarded and then the biosolids were air dried. The material was screened using a 0.635 cm screen and the

<0.635 cm fraction was used to prepare the treatments (chemical analyses shown in Appendix 13).

### 2.2.3 Fly Ash

The fly ash used came from the Lambton coal-fuelled generating station (St. Clair River, St. Clair Township, 26 km. South of Sarnia, Ontario, Canada). The ash was stockpiled at the ESW and used as received (chemical analyses shown in Appendix 13).

### 2.2.4 Preparation of Treatments

Amendment ratios and application rates were determined based on previous studies and the effects of these amendments on crop yield (Christie et al., 2001; Canadian International Development Agency, 2002).

All treatments (2006, 2007, and 2008) were prepared from soil+ash+biosolids and mixed using an electric cement mixer according to the ratios in Table 2.1. The ratios shown were added to the hopper of a rotating cement mixer in the order: soil-biosolids-ash in order to prevent clumping. The treatments were mixed until homogeneous, approximately 5 minutes.

**Table 2.1 Treatment ratios used to produce growth media for all field experiments. (Summer 2006, 2007, and 2008).**

| Treatment | Description |
|---|---|
| CT | Control (soil only)[1] |
| CTF | Control (soil only) with chemical fertilizer[1] |
| CT-N | Control (soil only) without inoculum[2] |
| CT+N | Control (soil only) with inoculum[2] |
| CTF-N | Control with chemical fertilizer without inoculum[2] |
| CTF+N | Control with chemical fertilizer with inoculum[2] |
| AH-3 | 3 % fly ash with 97 % soil[3] |
| AH-6 | 6 % fly ash with 94 % soil[3] |
| AH-9 | 9 % fly ash with 91 % soil[3] |
| AH-12 | 12 % fly ash with 88 % soil[3] |
| AH-15 | 15 % fly ash with 85 % soil[3] |
| BS-3 | 3 % biosolids with 97 % soil[4] |
| BS-6 | 6 % biosolids with 94 % soil[4] |

Table 2.1 (cont.)

| | |
|---|---|
| BS-9 | 9 % biosolids with 91 % soil[4] |
| BS-12 | 12 % biosolids with 88 % soil[4] |
| BS-15 | 15 % biosolids with 85 % soil[4] |
| A50-3 | 3 % of a 50% biosolids: 50% fly ash mixture and 97 % soil[4] |
| A50-6 | 6 % of a 50% biosolids: 50% fly ash mixture and 94 % soil[4] |
| A50-9 | 9 % of a 50% biosolids: 50% fly ash mixture and 91 % soil[4] |
| A50-12 | 12 % of a 50% biosolids: 50% fly ash mixture and 88 % soil[4] |
| A50-15 | 15 % of a 50% biosolids: 50% fly ash mixture and 85 % soil[4] |
| A75-3 | 3 % of a 75% biosolids: 25% fly ash mixture and 97 % soil[4] |
| A75-6 | 6 % of a 75% biosolids: 25% fly ash mixture and 94 % soil[4] |
| A75-9 | 9 % of a 75% biosolids: 25% fly ash mixture and 91 % soil[4] |
| A75-12 | 12 % of a 75% biosolids: 25% fly ash mixture and 88 % soil[4] |
| A75-15 | 15 % of a 75% biosolids: 25% fly ash mixture and 85 % soil[4] |

1 - Treatments used in the 2006 field season only
2 - Treatments used in the 2007 and 2008 field seasons only
3 - Treatments used in the 2006 and 2007 field seasons only
4 - Treatments used in the 2006, 2007, and 2008 field seasons

### 2.2.5 Experimental Plot Design, Carrot Seeding, and Tomato Planting for 2006, 2007, and 2008

Treatments for 2006 and 2007 are given in Table 2.1. A composite sample was taken from triplicate microplots (12 l pots filled with 14 kg of treatment each) for baseline analyses. Each composite consisted of 150 g of sample from each of the three replicates. The term baseline is used for samples taken after amendments were mixed with soils. An area of approximately 120 $m^2$ was prepared by removing vegetation and leveling. Pits for placement of the microplots were dug using a tractor auger and arranged in random order (Appendix 2) with a spacing of 0.75 m between pots and 1.5 m between rows. Fertilized carrot controls (CTF) received a mixture of 0.7 g of ammonium nitrate, 1.4 g of triple superphosphate, and 1.4 g of muriate of potash per pot (personal communication, Keith McKell, Soil Smith Ltd.). Carrot (*Daucus carota* L.) seeds (Carrot Baby Finger, OSC seeds, Waterloo, Ontario, N2J 3Z6) were sown in 6 equidistant holes in each pot. Each of the 6 holes received 2 to 3 seeds and sprouts were thinned 2 weeks later, leaving 3 plants per pot. Pots were watered and kept inside a shed for 8 days until being transferred to the field pits.

Treatments for 2008 are given in Table 2.1. Each pot was sampled at the beginning of the experiment for baseline analyses. Tomato (*Lycopersicon*

*esculentum* P. Mill.) seeds (cv. Basket vee, Stokes seeds, Buffalo, New York) were sown in Promix© and kept in the greenhouse for three weeks prior to transplanting into 12 l pots (microplots) filled with treatments. One tomato seedling was transplanted to each pot and the pots buried in pits dug in the same way as previous years. Pots were arranged in random order (Appendix 3).

Tomato controls (CTF) were fertilized with a mixture of 0.77 g of urea, 1.75 g of triple superphosphate, and 1.61 g of muriate of potash per pot. The pots were fertilized with the same dose two more times; one at the middle of the growing season and one after the plants had fruited. (Personal communication, Keith McKell, Soil Smith, Ltd.).

## 2.3 Laboratory Protocols

For 2006, soil samples were taken at 3 sampling times designated as: baseline, middle (34 days after sowing) and harvest (68 days after sowing). For 2007, soil samples were taken at 4 sampling times designated as: baseline, middle (34 days after sowing), first harvest (73 days after first sowing), and second harvest (83 days after second sowing).
For 2008, soil samples were taken at the beginning and at the end of the experiment. All soil samples were processed and analyzed for each variable as described below.

### 2.3.1 Electrical Conductivity (EC) and pH

A 20 g aliquot of the composite sample for each treatment was transferred to a 100 ml beaker and 40 ml of deionized water were added. The beakers were shaken in a rotary shaker for 30 minutes and allowed to settle for 30 minutes. The pH was measured using an Accumet model 10 pH meter (Fisher Scientific) with an ORION 9172 BN probe (Thermo Electron Corporation, Sure flow combination pH) calibrated at room temperature with standard buffers of pH 4, 7,

and 10. Subsequently, EC was determined on a 15 ml aliquot of the suspension using a conductivity meter probe for the range 0-1999 µS (HI 8033 Handheld EC/TDS Meter, HANNA Instruments, India, Pvt. Ltd). EC and pH protocols were the same for all experiments.

### 2.3.2 Nematode Extraction and Counting, 2006 and 2007 (Baermann Tray Technique)

A 100 ml aliquot of the composite sample for each treatment was placed in 2 l of water and stirred using a metal spatula for 2 minutes to disaggregate the sample. The suspension was allowed to settle for 30 seconds and then passed through a series of 60 and 325 mesh sieves (openings of 250 and 45 µm, respectively). The 60 mesh fraction was discarded. The 325 mesh fraction was transferred onto a Baermann tray apparatus consisting of a 15 cm plastic ring covered with a plastic mesh (0.1 mm) and then a coffee filter was carefully spread onto the hollow side of the plastic ring (Fig. 2.1). The ring was put on an aluminum pie plate and enough water was added under the plastic ring so that the water touched the coffee filter. The Baermann trays were incubated at room temperature for 24 hours and then the suspension under the plastic ring was transferred into a 100 ml beaker and the volume adjusted to 100 ml. Two 5 ml aliquots were counted in a counting chamber. Nematodes were counted using a dissecting microscope and categorized into 4 feeding groups (bacterial feeders, fungal feeders, plant feeders, and predators (Yeates et al., 1993)). Counts were standardized for 100 ml of suspension by multiplying the counts made in 5 ml by a factor of 20. Nematode populations will be given per 100 ml of soil.

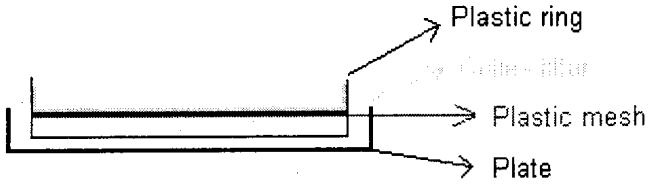


Fig. 2.1. Baermann tray

### 2.3.3 Carrot and Tomato Harvesting (2006, 2007, and 2008)

In 2006, carrots were harvested 68 days after sowing. Carrots from all three replicates for each treatment were combined and weighed in order to determine both above and below ground (root) biomass.

In 2007, carrots were harvested 73 days after sowing. All carrots from each treatment were combined and fresh weights were measured; above ground biomass was not weighed because of grazing by a flock of Canada Geese on July 20$^{th}$, 2007. Soil from each pot was screened using a 0.635 cm screen in order to collect root fragments and determine the number of nematode eggs per gram of fresh feeder root. After screening, the soil was placed back into the pot and new carrot seeds were sown to produce a second harvest. Pots that served as controls with chemical fertilizer were fertilized again with the same amount used at the beginning of the experiment (see Section 2.2.5). Carrots for the second harvest were collected 83 days after sowing. All carrots from each treatment were combined and the fresh weights of above and below ground biomass were measured. After the second harvest, the soil was screened and roots collected in the same way as the first harvest.

In 2008, tomatoes were harvested weekly as they ripened. A total of 5 harvests were collected and total fresh weight per plant was determined. After all tomatoes were harvested, microplots were pulled out of the ground and placed in a shed. Above ground biomass was weighed and pots were taken to the laboratory for processing and extraction of nematode eggs.

### 2.3.4 Bacterial and Fungal Colony Forming Units

Bacterial and fungal colony forming units were determined for 2007 and 2008 as follows.

An LB broth with Nystatin (0.5 g/liter) was used to culture bacterial CFU and a PDA agar with Streptomycin (0.1 g/liter) and Tetracycline (0.01 g/liter) was used to measure fungal CFU (Riegel et al., 1996). A sample of each treatment (0.5 g) was mixed with 4.5 ml of autoclaved, deionized water and vortexed for 2 minutes. One hundred µl of the suspension were transferred to a test tube containing 9.9 ml of a peptone blank and vortexed for 30 seconds. One hundred µl of final suspension were plated onto each medium. Triplicates of each media were plated for either bacteria or fungi. Peptone blanks were prepared by dissolving 10 g of peptone, 5 g of NaCl, and 80 µl of 4M NaOH in one liter of water and pH was adjusted to 7-7.5 using NaOH when necessary. Subsequently, 9.9 ml of solution were transferred to test tubes, autoclaved for 15 min at 121 °C and allowed to cool. All plating was done in a laminar flow hood. Plates were incubated at 25 °C in the dark. Bacterial CFU were counted 3 days after plating and fungal CFU were counted 5 days after plating.

### 2.4 *Meloidogyne hapla* Inoculum

Roots and soils infested with *M. hapla* obtained from Guy Belair (Agriculture Canada CRDH, 430 Gouin Blvd Saint-Jean-sur-Richelieu, Quebec, Canada J3B 3E6) were used to start nematode cultures in the greenhouses, Dept. of Biology, University of Western Ontario, London, Ontario, Canada.

A mixture of soil (sandy loam used for all experiments) and Promix© (Premier Tech Ltd.) was prepared at a 2:1 ratio and 700 g of the resulting mixture were added to 15 cm diameter pots in which the bottom holes were covered with cotton batting. Subsequently, 200 g of infested roots and soil were added to the top of the pots. One three-week old tomato (*Lycopersicon esculentum* Mill.) seedling (cv. Basket vee, Stokes seeds, Buffalo, New York) was transplanted into the layer of infested soil, and then a layer of sandy soil-Promix© was added to cover the seedling and infested soil. Plants were allowed to grow in the greenhouse for 3 months before using them as a source for inoculum.

Soil from the root ball of the tomatoes was used to extract nematode eggs. Excess soil was carefully shaken from roots, trying not to break the root ball. Subsequently, roots and remaining soil were placed on a 2mm sieve and carefully washed with a mild stream of cold tap water until most of the roots were free of soil. The cleaned root ball was then patted dry using paper towels and cut into 1-2 cm pieces. Approximately 300 ml of root pieces were put into a 600 ml beaker with 250 ml of a 1% NaOCl solution (bleach) (Hussey and Barker, 1973). The beaker was covered with Parafilm® and shaken by hand for 4 minutes. The resulting slurry was poured over nested sieves (140 mesh over 500 mesh [openings of 105 and 25 µm, respectively]) and rinsed with water. After all root particles were thoroughly rinsed and the fraction collected in the 140 mesh sieve was discarded, the fraction collected in the 500 mesh sieve was further rinsed with water for an extra 3 minutes to remove traces of bleach and transferred to a 100 ml beaker.

Aliquots of the egg suspension were transferred to conical polypropylene centrifuge tubes (50 ml) and water was added to balance the tubes prior to centrifugation. Tubes were centrifuged using a Beckman GS-6R tabletop centrifuge at 600 g for 10 minutes and then allowed to stop without using the brake. After centrifugation, the supernatant from each tube was removed by pipette, leaving approximately 10 ml of supernatant on top of the pellet.

Subsequently, 30% sugar solution was added to each tube and the pellet was resuspended by stirring, centrifuged at 600 g for 10 minutes, and the rotor allowed to stop without using the brake. The supernatant was poured into a 500 mesh sieve taking care not to include pellet material. Sieve contents were rinsed for 3 minutes to wash the sugar from the eggs. The egg suspension was then transferred to a beaker for further counting and use. Egg counts were estimated using a counting chamber and a compound microscope at 40 X.

### 2.4.1 Inoculation of Carrot and Tomato Plants with *M. hapla* Inoculum

For 2007, *M. hapla* eggs were extracted following the procedure described in section 2.4 and aliquots were placed on Baermann funnels for hatching. Hatched juveniles were collected daily from funnels and transferred to a 500 ml beaker, agitated for aeration, and kept in a refrigerator (4 °C). After 6 days of collecting the juveniles they were added to a suspension of eggs that had been kept in the refrigerator. The population from the whole suspension of eggs and juveniles was estimated by counting ten 10 µl aliquots. Carrot plants were allowed to grow 29 days prior to inoculation. All treatments were inoculated with the exception of the control (soil only) without inoculum and control with fertilizer without inoculum. Each pot received a 6 ml aliquot of nematode (eggs and juveniles) suspension delivered with a 1 ml pipette in 6 equidistant holes (7 cm deep). Three of the holes were made beside the carrot plants and 3 holes between plants. Each pot received approximately 12,600 eggs and 2,400 juveniles, which translates to 90 eggs and 17 juveniles per 100 $cm^3$ of soil.

For 2008, *M. hapla* inoculum was extracted following the procedure described in section 4.4. Transplanted tomato plants were allowed to grow for 20 days before initial inoculation. All treatments were inoculated with the exception of the control (soil only) without inoculum and control with fertilizer without inoculum. Each pot received two 5 ml aliquots of nematode (eggs and juveniles) suspension delivered with a 5 ml pipette in 2 holes (7 cm deep). The holes were made 5 cm away from the main stem of the plant. Each pot received approximately 11,320 eggs and 270 juveniles. Pots (14000 $cm^3$) were reinfested 71 days after the tomato plants were transplanted. Infected roots from an *M. hapla* culture were homogenized prior to inoculation. Infected roots were used directly as inoculum without processing them using NaOCl solution. A 15 cm deep hole was made 5 cm away from the main tomato stem. One hundred ml of infested roots were put into each hole. Soil taken from the hole was used to cover the inoculated site. To determine the number of eggs and juveniles that were placed in each pot, 100 ml

of homogenized infected roots processed using a 1% NaOCl solution. The procedure for extracting eggs was the same as described in Section 2.4 but the concentration of sucrose was changed from 30% to 45% (McClure et al., 1973) and tubes were centrifuged for 5 minutes instead of 10. Each 100 ml infected roots contained approximately 2.9 million *M. hapla* eggs.

### 2.4.2 Extraction of Eggs from Carrot and Tomato Roots

For 2007, carrot feeder roots recovered after screening the soil were rinsed with tap water in order to remove soil particles. Special care was taken not to wash the roots too aggressively to prevent loss of nematode egg masses. The combined sample was weighed and then cut into 1- 2 cm pieces and placed into a 200 ml blender cup. Two hundred ml of a 1% bleach solution were added to the blender cup and then blended for 1 minute. The resulting slurry was passed through stacked sieves (120 mesh over a 500 mesh [openings of 125 and 25 µm, respectively]) and rinsed using tap water. The fraction collected in the 120 mesh sieve was discarded after washing. The fraction collected in the 500 mesh sieve was further rinsed for 3 minutes with tap water in order to completely remove all traces of bleach. The resulting egg suspension was transferred to a 100 ml beaker covered with Parafilm® and stored in the refrigerator (4 °C) until counted. *Meloidogyne hapla* eggs were counted from 2 aliquots of 5 ml for each treatment. The aliquots were transferred to counting chambers and counted using a compound microscope at 40 X. The average egg count was then multiplied by the dilution factor for each suspension and standardized to express eggs per gram of fresh root. The extraction and counting procedure was performed for the first and second harvests. Number of eggs will be expressed as eggs per gram of fresh root.

For 2008, after weighing and measuring above ground biomass for tomato plants, each pot was emptied into a plastic tub and the root ball was carefully separated from the soil so that root length and weight could be determined.

Subsequently, all feeder roots were cut from the main roots and weighed. One gram of feeder roots was stained for 15 minutes using a 20% red food dye solution (Thies et al., 2002). After 15 minutes, the roots were placed in a kitchen strainer and rinsed with tap water. Roots were patted dry using paper towels and stained egg masses were counted using a dissecting microscope at 40 X magnification. Remaining feeder roots were cut into 1-2 cm pieces and put in a blender cup. Two hundred ml of a 0.525% bleach solution were added to the blender cup and manually shaken for 3.5 minutes.

The remaining steps were carried out as previously described for carrot feeder roots. Volume of extracted egg suspension was standardized to 100 ml and a 15 ml aliquot was transferred onto an 85 mm gridded Petri plate. The pipette tip was rinsed from the inside and the outside using a wash bottle to make sure no eggs were retained in the tip. The Petri plate was placed under a compound microscope at 40 X magnification and 72 (9 $mm^2$ each) squares from the grid were counted. The average number of eggs from the 72 counts was extrapolated to the total area of the counting plate. That value represents the number of eggs in 15 ml of egg suspension. The number of eggs/15 ml of suspension was then extrapolated to the total volume of suspension, resulting in number of eggs/100 ml. The final value represents the total number of eggs extracted from the feeder roots of each plant. Number of eggs will be expressed as eggs per gram of fresh root.

### 2.5 Preparation of Treatments for Greenhouse Experiment Conducted without Plant Material (2007)

Treatments were prepared using the same materials as for previous experiments. Materials were mixed by hand until homogeneous according to the ratios in Table 2.2. Each treatment was transferred into Dixie® cups (approximately 150 ml) to be inoculated with *M. hapla* eggs.

**Table 2.2 Treatment ratios used to produce growth media for greenhouse experiment. Winter 2007.**

| Treatment | Description |
|---|---|
| Control | Control (soil only) |
| AH-2.5 | 2.5 % fly ash with 97.5 % soil |
| AH-5.0 | 5 % fly ash with 95 % soil |
| AH-7.5 | 7.5 % fly ash with 92.5 % soil |
| AH-10.0 | 10 % fly ash with 90 % soil |
| AH-20.0 | 20 % fly ash with 80 % soil |
| BS-2.5 | 2.5 % biosolids with 97.5 % soil |
| BS-5.0 | 5 % biosolids with 95 % soil |
| BS-7.5 | 7.5 % biosolids with 92.5 % soil |
| BS-10.0 | 10 % biosolids with 90 % soil |
| BS-20.0 | 20 % biosolids with 80 % soil |
| A50-5.0 | 5 % of a 50% biosolids: 50% fly ash mixture and 95 % soil |
| A50-10.0 | 10 % of a 50% biosolids: 50% fly ash mixture and 90 % soil |
| A50-15.0 | 15 % of a 50% biosolids: 50% fly ash mixture and 85 % soil |
| A50-20.0 | 20 % of a 50% biosolids: 50% fly ash mixture and 80 % soil |
| A50-40.0 | 40 % of a 50% biosolids: 50% fly ash mixture and 60 % soil |
| X50-50.0 | 50 % biosolids and 50 % fly ash mixture |

Nine replicates of each treatment were prepared so that they could be analyzed in sets of 3 at the beginning, middle, and end of the experiment. All cups were randomly arranged on a bench top in the greenhouse until analyzed. Cups were kept moist throughout the experiment.

### 2.5.1. Infestation of Dixie® Cups with *M. hapla* Inoculum

*Meloidogyne hapla* inoculum was extracted following the procedure described in section 4.4. Each Dixie® cup received 150 µl of *M. hapla* egg suspension containing approximately 1,750 eggs pipetted into a hole (3 cm deep) in the center of the cup.

### 2.5.2. Nematode Extraction and Counts

Nematodes from the first 2 sets of samples were extracted as described in Section 2.3.2. Nematodes from the final set of samples were extracted in the same way as the first 2 sets then further subjected to sugar centrifugation. The fraction collected on the 325 mesh was transferred to conical polypropylene centrifuge tubes (50 ml), spun at 600 g for 2 minutes, and the centrifuge was

stopped using the brake. The supernatant was discarded and a 45% sugar solution was added to the remaining pellet which was resuspended by stirring and spun at 600 g for an additional 2 minutes before stopping using the break.

## 2.6 Preparation of Treatments for Greenhouse Experiment with Tomato Plants (Spring 2008)

Treatments were prepared using the same materials as for previous experiments. Materials were mixed by hand until homogeneous according to the ratios in Table 2.3. Each treatment was transferred into 15 cm diameter pots in which the bottom holes were covered with cotton batting. A three-week old tomato seedling (cv. Basket vee) was transplanted into each pot and allowed to grow for 7 days before inoculation. Treatments were divided into 4 sets and each set included a control (soil only). Each group of pots from each set was put on a bench in the greenhouse and arranged in a complete randomized design. Each set was handled separately for all laboratory analyses.

### 2.6.1 Inoculation of Tomato Plants with *M. hapla* Inoculum

*Meloidogyne hapla* inoculum was extracted following the procedure described in Section 2.4. Transplanted tomato plants were inoculated with 20 ml of nematode egg suspension delivered in 4 holes (7 cm deep) made 5 cm away from the main stem of the plant. Each hole received 5 ml of inoculum containing approximately 2,500 *M. hapla* eggs, resulting in 10,000 eggs/pot.

**Table 2.3 Treatment ratios used to produce growth media for greenhouse experiment. Spring 2008.**

| Set # | Treatment | Description |
|---|---|---|
| 1 | CTAH | Control for AH treatments: soil only |
| 1 | AH-2.5 | 2.5 % fly ash and 97.5 % soil |
| 1 | AH-5.0 | 5 % fly ash and 95 % soil |
| 1 | AH-7.5 | 7.5 % fly ash and 92.5 % soil |
| 1 | AH-10.0 | 10 % fly ash and 90 % soil |
| 1 | AH-12.5 | 12.5 % fly ash and 87.5 % soil |
| 2 | CTBS | Control for BS treatments: soil only |
| 2 | BS-2.5 | 2.5 % biosolids and 97.5 % soil |
| 2 | BS-5.0 | 5 % biosolids and 95 % soil |

Table 2.3 (cont.)

| | | |
|---|---|---|
| 2 | BS-7.5 | 7.5 % biosolids and 92.5 % soil |
| 2 | BS-10.0 | 10 % biosolids and 90 % soil |
| 2 | BS-12.5 | 12.5 % biosolids and 87.5 % soil |
| 3 | CTA50 | Control for A50 treatments: soil only |
| 3 | A50-2.5 | 2.5 % of a 50% biosolids: 50% fly ash mixture and 97.5 % soil |
| 3 | A50-5.0 | 5 % of a 50% biosolids: 50% fly ash mixture and 95 % soil |
| 3 | A50-7.5 | 7.5 % of a 50% biosolids: 50% fly ash mixture and 92.5 % soil |
| 3 | A50-10.0 | 10 % of a 50% biosolids: 50% fly ash mixture and 90 % soil |
| 3 | A50-12.5 | 12.5 % of a 50% biosolids: 50% fly ash mixture and 87.5 % soil |
| 4 | CTA75 | Control for A75 treatments: soil only |
| 4 | A75-2.5 | 2.5 % of a 75% biosolids: 25% fly ash mixture and 97.5 % soil |
| 4 | A75-5.0 | 5 % of a 75% biosolids: 25% fly ash mixture and 97.5 % soil |
| 4 | A75-7.5 | 7.5 % of a 75% biosolids: 25% fly ash mixture and 92.5 % soil |
| 4 | A75-10.0 | 10 % of a 75% biosolids: 25% fly ash mixture and 90 % soil |
| 4 | A75-12.5 | 12.5 % of a 75% biosolids: 25% fly ash mixture and 87.5 % soil |

### 2.6.2 Processing Tomato Plants after Growing Season

Tomato plants were allowed to grow until they clearly showed signs of nematode infestation, including stunted growth and chlorotic leaves. Each set was processed individually and measurements for weight and length of above ground biomass were determined The roots were further processed to determine their weight, length, eggs/gram of fresh root, and egg masses/gram of fresh root as described in Section 2.4.2.

## 2.7 Satistical Analyses

Amendment ratio data for 2006 and 2007 were combined per set of treatments and subjected to (one-way) ANOVA and mean differences for each set were compared by Tukey's range test. Data for individual ratios for the 2006 and 2007 seasons were combined due to a lack of replication and length of sample processing. One sample per treatment ratio for 5 ratios of each amendment were used as replicates to show the overall effect of each amendment on the parameters measured, therefore statistical analyses were performed on a pooled sample (n=5). It should be noted that the pooled data includes the effects of 60 – 600 tons/ha amendment ratios and while it is not appropriate to compare this vast range together this eventuality was imperative because of the lack of

replicates. Data for 2008 were subjected to (one-way) ANOVA followed by Tukey's range test. Correlation analyses were performed to compare variables (SPSS Statistics 17.0, Chicago, Illinois).

## 2.8 Concentration of Plant Available Elements

Concentration of plant available elements was determined by Mehlich III extraction (Mehlich, 1984) followed by ICP-AES analysis. Mehlich III procedure is detailed in Appendix 11. All ICP-Analysis were done at the Biotron experimental climate change research facility at the University of Western Ontario.

# 3 RESULTS

## 3.1 Impact of Biosolids and Fly Ash on Natural Nematode Populations and Carrot Yield (2006)

Average data for each treatment were used to determine the impact of the amendment on soils and nematodes. The composition of each amendment is given in Table 3.1. Average data collected for the 2006 field season, and from which figures in this section are made, are shown in Table 3.2 (raw data are shown in Appendix 6).

**Table 3.1 Composition of amendments used in the 2006 and 2007 field seasons.**

| Treatment | Composition |
|---|---|
| CT | Soil only |
| CTF | Soil + fertilizer |
| AH | Average of fly ash treatments for 3, 6, 9, 12, and 15% (w/w) fly ash added to soil |
| BS | Average of biosolids treatments for 3, 6, 9, 12, and 15% (w/w) biosolids added to soil |
| A50 | Average of 3, 6, 9, 12, and 15% (w/w) of an amendment made of 50% biosolids and 50% fly ash |
| A75 | Average of 3, 6, 9, 12, and 15% (w/w) of an amendment made of 75% biosolids and 25% fly ash |

**Table 3.2 Average data collected for various treatments on nematode populations (nematodes/100 ml of soil; BF=bacterial feeding nematodes; FF= fungal feeding nematodes; PF= plant feeding nematodes); pH; EC= electrical conductivity (µS/cm); Top=weight of above ground biomass (g); Root=weight of roots (g) (n=2 for CT and CTF; n=5 for AH, BS, A50. and A75). Baseline (July 10/06), Middle (Aug. 16/06), and Harvest (Sept. 15/06), (P values for differences among treatment groups).**

| | Baseline | | | | | Middle | | | | | Harvest | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Treat.* | BF | FF | PF | pH | EC | BF | FF | PF | pH | EC | BF | FF | PF | pH | EC |
| CT | 30 | 15 | 120 | 7.73 | 250 | 35 | 25 | 120 | 7.91 | 180 | 0 | 70 | 95 | 8.06 | 165 |
| CTF | 0 | 30 | 60 | 7.77 | 265 | 90 | 10 | 60 | 7.96 | 210 | 35 | 70 | 95 | 8.08 | 170 |
| AH | 0 | 6 | 72 | 7.82 | 648 | 76 | 26 | 62 | 8.14 | 204 | 8 | 30 | 88 | 8.09 | 286 |
| BS | 30 | 24 | 132 | 7.70 | 620 | 54 | 20 | 88 | 7.90 | 202 | 34 | 12 | 76 | 7.99 | 206 |
| A50 | 42 | 0 | 96 | 7.78 | 624 | 116 | 58 | 84 | 7.92 | 186 | 44 | 48 | 76 | 8.03 | 228 |
| A75 | 36 | 30 | 102 | 7.76 | 656 | 132 | 58 | 112 | 7.95 | 182 | 40 | 42 | 52 | 7.98 | 222 |
| P value | >.05 | >.05 | >.05 | >.05 | >.05 | >.05 | >.05 | .004 | <.001 | >.05 | >.05 | .03 | >.05 | >.05 | >.05 |

* Description of each treatment is given in Table 3.1

**Table 3.2 (cont.)**

| | Carrot Yield | |
|---|---|---|
| Treat * | Top | Root |
| CT | 53 | 137 |
| CTF | 56 | 189 |
| AH | 42 | 83 |
| BS | 103 | 415 |
| A50 | 82 | 285 |
| A75 | 93 | 378 |
| P value | <.001 | <.001 |

* Description of each treatment is given in Table 3.1

### 3.1.1 Bacterial Feeding Nematodes

At baseline, no bacterial feeding nematodes (BFN) were found in the CTF and AH treatments. The other treatments had mean BFN populations ranging from 30-42 BFN/100 ml of soil, with the highest found in the A50 treatments (Fig. 3.1, "Baseline"). Midway through the growing season (Fig. 3.1, "Middle") mean BFN populations ranged from 35-132 BFN/100 ml of soil, with the highest found in the A75 treatments. At harvest (Fig. 3.1, "Harvest") no BFN were found in the CT treatments. The other treatments had mean BFN populations ranging from 8-44 BFN/100 ml of soil, with the highest found in the A50 treatments.

No significant differences were found among groups at any of the sampling times. Lack of BFN in the CTF and AH treatments at baseline might be attributed to inconsistencies in nematode extraction procedure.

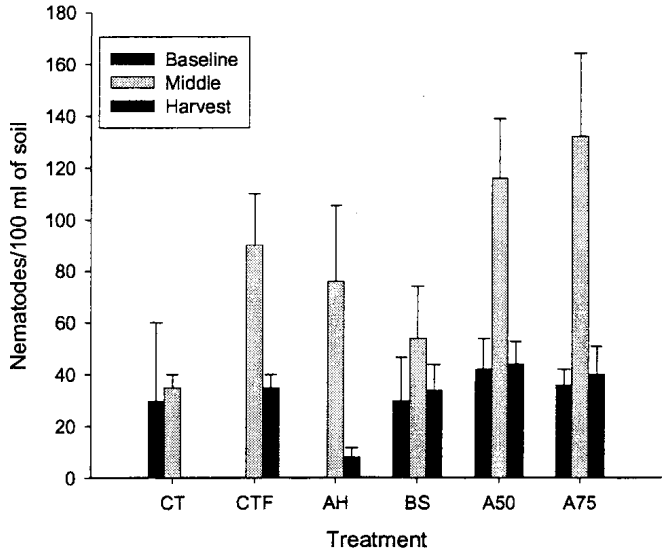


**Fig. 3.1 Bacterial feeding nematode populations in treatments (CT= soil; CTF= soil+fertilizer; AH= fly ash; BS= biosolids; A50= 50:50 mixture of biosolids and fly ash; A75= 75:25 mixture of biosolids and fly ash); (n=5, values +/- SE) at 3 sampling times (Baseline, Middle, and Harvest). Summer 2006.**

### 3.1.2 Fungal Feeding Nematodes

At baseline, no fungal feeding nematodes (FFN) were found in the A50 treatments. The other treatments had mean FFN populations ranging from 6-30 FFN/100 ml of soil, with the highest found in the CTF and A75 treatments (Fig. 3.2, "Baseline"). Midway through the growing season (Fig. 3.2, "Middle") mean FFN populations ranged from 10-58 FFN/100 ml of soil, with the highest found in the A50 and A75 treatments. At harvest (Fig. 3.2, "Harvest") mean FFN populations ranged from 12-70 FFN/100 ml of soil, with the highest found in the CT and CTF treatments. Significant differences among groups were found only at harvest ($F=3.1$, $df=5,18$, $P=0.034$). Lack of FFN in the A50 treatments at baseline might be attributed to inconsistencies in nematode extraction procedure.

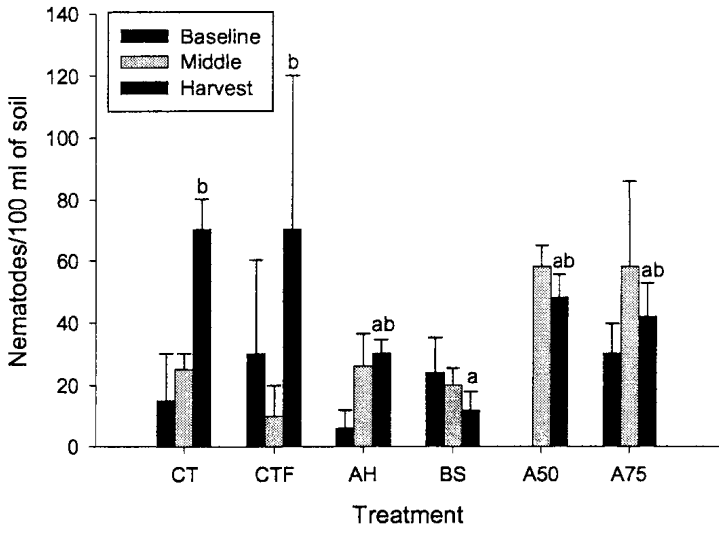


**Fig. 3.2 Fungal feeding nematode populations in treatments (CT= soil; CTF= soil+fertilizer; AH= fly ash; BS= biosolids; A50= 50:50 mixture of biosolids and fly ash; A75= 75:25 mixture of biosolids and fly ash); (n=5, values +/- SE) at 3 sampling times (Baseline, Middle, and Harvest). Bars with the same letter are not significantly different (Tukey's range test, P<0.05). Summer 2006.**

### 3.1.3 Plant Feeding Nematodes

At baseline (Fig. 3.3, "Baseline") mean plant feeding nematode (PFN) populations ranged from 60-132 PFN/100 ml of soil, with the highest found in the BS treatments. Midway through the growing season (Fig. 3.3, "Middle") mean PFN populations ranged from 60-120 PFN/100 ml of soil, with the highest found in the CT treatments. At harvest (Fig. 3.3, "Harvest") mean PFN populations ranged from 52-95 PFN/100 ml of soil, with the highest found in the CT and CTF treatments. Significant differences among groups were found only midway through the growing season ($F=5.3$, $df=5,18$, $P=0.004$).

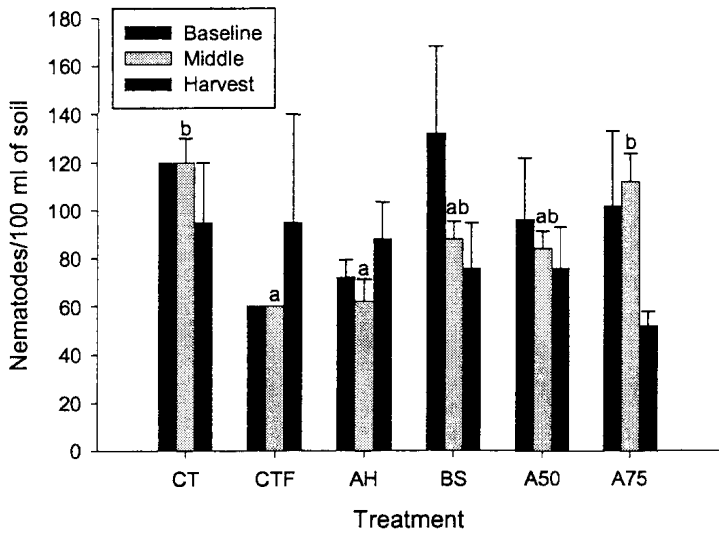


**Fig. 3.3 Plant feeding nematode populations in treatments (CT= soil; CTF= soil+fertilizer; AH= fly ash; BS= biosolids; A50= 50:50 mixture of biosolids and fly ash; A75= 75:25 mixture of biosolids and fly ash); (n=5, values +/- SE) at 3 sampling times (Baseline, Middle, and Harvest). Bars with the same letter are not significantly different (Tukey's range test, P<0.05). Summer 2006.**

### 3.1.4 Electrical Conductivity

Even though no statistically significant differences in EC were found for the summer 2006 field season, at baseline, EC for the amended treatments was much higher than the controls (Fig. 3.4, "Baseline"). Mean electrical conductivity ranged from 250-656 µS/cm, with the highest found in the A75 treatments. Midway through the growing season (Fig. 3.4, "Middle") mean EC ranged from 180-210 µS/cm, with the highest found in the CTF treatments. At harvest (Fig. 3.4, "Harvest") mean EC ranged from 165-286 µS/cm, with the highest found in the AH treatments. No significant differences were found among groups at any of the sampling times.

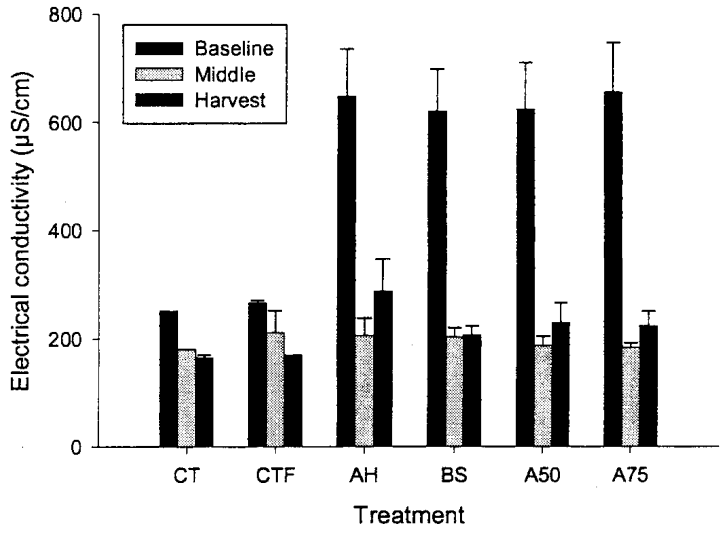


**Fig. 3.4 Electrical conductivity of treatments (CT= soil; CTF= soil+fertilizer; AH= fly ash; BS= biosolids; A50= 50:50 mixture of biosolids and fly ash; A75= 75:25 mixture of biosolids and fly ash); (n=5, values +/- SE) at 3 sampling times (Baseline, Middle, and Harvest). Summer 2006.**

### 3.1.5 pH

At baseline (Fig. 3.5, "Baseline") mean pH ranged from 7.70-7.82. Midway through the growing season (Fig. 3.5, "Middle") mean pH ranged from 7.90-8.14. At harvest (Fig. 3.5, "Harvest") mean pH ranged from 7.98-8.09. At all 3 sampling times, the treatments with the highest pH values were the AH. A significant difference among groups was found only midway through the growing season ($F=9.2$, $df=5,18$, $P<0.001$).

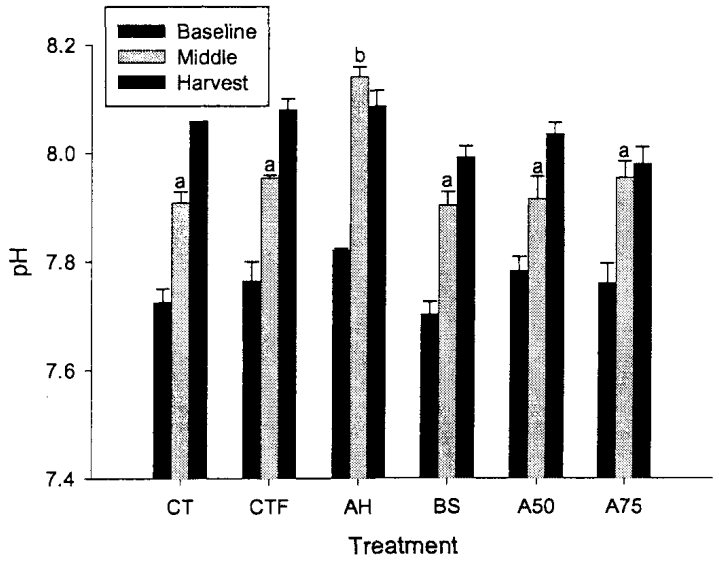


**Fig. 3.5 pH of treatments (CT= soil; CTF= soil+fertilizer; AH= fly ash; BS= biosolids; A50= 50:50 mixture of biosolids and fly ash; A75= 75:25 mixture of biosolids and fly ash); (n=5, values +/- SE) at 3 sampling times (Baseline, Middle, and Harvest). Bars with the same letter are not significantly different (Tukey's range test, P<0.05). Summer 2006.**

### 3.1.6 Carrot Yield

There was a significant difference among treatment groups for carrot root yield ($F=29.3$, df = 5,18, $P<0.001$). Mean yield ranged from 83-415 g/treatment with the highest obtained from the BS treatments (Fig. 3.6).

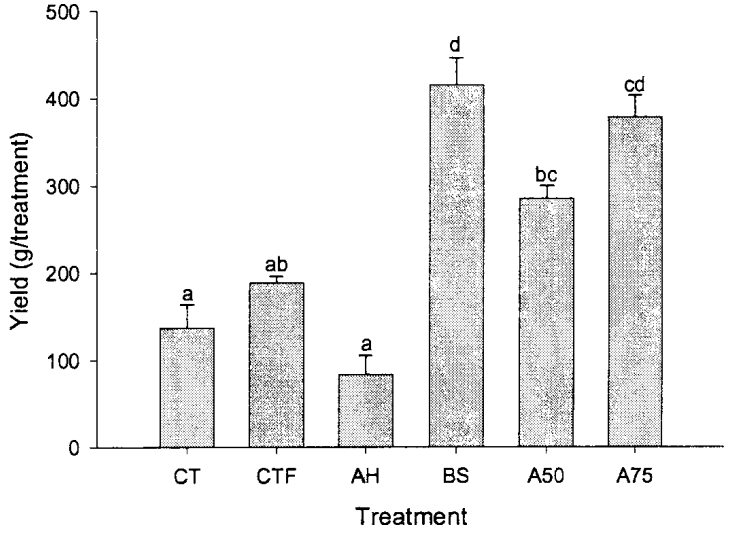


**Fig. 3.6 Carrot (primary tap root) yield of treatments (CT= soil; CTF= soil+fertilizer; AH= fly ash; BS= biosolids; A50= 50:50 mixture of biosolids and fly ash; A75= 75:25 mixture of biosolids and fly ash); (n=5, values +/- SE). Bars with the same letter are not significantly different (Tukey's range test, P<0.05). Summer 2006.**

There was a significant difference among treatment groups in carrot top weights (F=15.1, df = 5,18, *P*<0.001). Mean carrot top weights ranged from 42-103 g/treatment with the highest obtained from the BS treatments (Fig. 3.7).

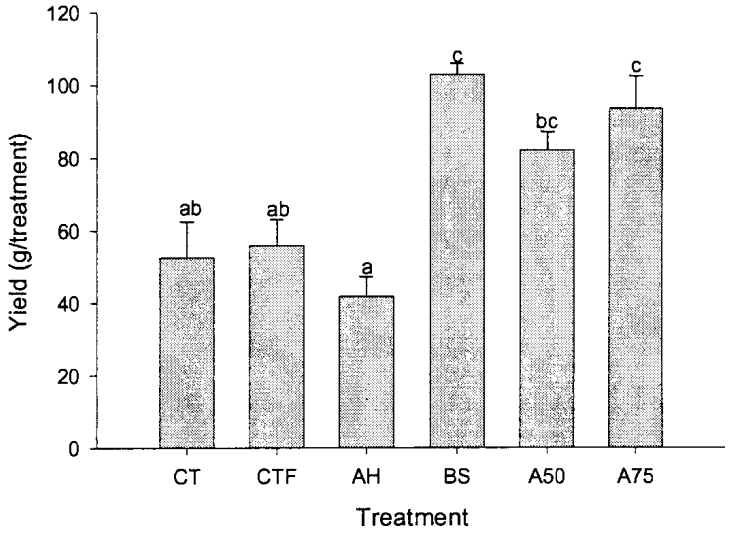


**Fig. 3.7 Carrot (above ground biomass) yield of treatments (CT= soil; CTF= soil+fertilizer; AH= fly ash; BS= biosolids; A50= 50:50 mixture of biosolids and fly ash; A75= 75:25 mixture of biosolids and fly ash); (n=5, values +/- SE). Bars with the same letter are not significantly different (Tukey's range test, P<0.05). Summer 2006.**

### 3.1.7 Concentration of Elements in Soils and Amendments

The concentration of 14 elements detected in each replicate of soil and treatments for the 2006, 2007, and 2008 seasons is shown in Appendix 12. Data for fly ash and biosolids alone are given in Appendix 13. Table 3.3 includes average elemental data for each set of treatments.

**Table 3.3 Concentration of elements in various treatments used in the 2006, 2007, and 2008 field seasons. Concentration in mg $kg^{-1}$.**

| | Element concentration (mg $kg^{-1}$) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Treatment* | As | B | Ca | Cd | Cr | Cu | Fe | K | Mg | Mn | Ni | P | Pb | Zn |
| CT | 0.1 | 1.3 | 3759 | 0.1 | 0.1 | 1.3 | 72 | 40 | 119 | 72 | 0.5 | 11.4 | 1.8 | 1.3 |
| CTF | 0.1 | 1.2 | 3380 | 0.1 | 0.2 | 1.2 | 72 | 37 | 110 | 68 | 0.4 | 13.0 | 1.7 | 1.2 |
| AH | 0.9 | 8.1 | 3856 | 0.1 | 0.4 | 1.4 | 100 | 45 | 127 | 68 | 0.5 | 25.0 | 1.9 | 1.5 |
| BS | 0.3 | 1.3 | 4273 | 0.1 | 0.2 | 2.4 | 85 | 53 | 168 | 69 | 0.3 | 53.9 | 2.2 | 3.3 |
| A50 | 0.6 | 4.3 | 3864 | 0.1 | 0.3 | 1.8 | 92 | 48 | 146 | 60 | 0.4 | 44.1 | 1.9 | 2.7 |
| A75 | 0.5 | 3.1 | 4239 | 0.1 | 0.2 | 2.3 | 92 | 53 | 165 | 62 | 0.4 | 56.4 | 2.2 | 3.2 |

*Description of each treatment is given in Table 3.1

The Ontario government (OMAFRA, 1996) gives limits for metal addition to soil (mg $kg^{-1}$) as As (14), Cd (1.6), Cr (120), Co (20), Cu (100), Pb (60), Ni (32), and Zn (220). The guidelines indicate that none of the metals in the amended soils in the present study breach ministry regulations regarding the use of waste materials in soils. The impact of these metals on nematode populations is covered in the discussion section of this thesis.

## 3.2 Impact of Biosolids and Fly Ash on Natural Nematode Populations, *Meloidogyne hapla*, and Carrot Yield (2007)

Average data for each treatment were used to determine the impact of the amendment on soils and nematodes. The composition of each amendment is given in Table 3.1. Average data collected for the 2007 field season, and from which figures in this section are made, are shown in Tables 3.4 and 3.5. Raw data are shown in Appendix 7.

**Table 3.4 Average data collected for various treatments on nematode populations (nematodes/100 ml of soil; BF=bacterial feeding nematodes; FF= fungal feeding nematodes; PF= plant feeding nematodes); pH; EC= electrical conductivity (µS/cm) at Baseline (June 11/07), Middle (July 9/07), 1st. Harvest (July 23/07) and 2nd. Harvest (Oct. 8/07). Carrot yield for 1st. and 2nd. Harvests (g). 1st. Harvest (July 23/07), and 2nd. Harvest (Oct. 8/07), (P values for differences among treatment groups).**

| | Baseline | | | | | Middle | | | | | 1st. Harvest | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Treatment* | BF | FF | PF | pH | EC | BF | FF | PF | pH | EC | BF | FF | PF | pH | EC |
| CT | 29 | 11 | 203 | 7.91 | 200 | 28 | 11 | 245 | 8.09 | 115 | 63 | 21 | 156 | 8.23 | 120 |
| CTF | 43 | 12 | 266 | 7.77 | 1050 | 24 | 32 | 227 | 8.15 | 122 | 50 | 16 | 125 | 8.13 | 152 |
| AH | 44 | 24 | 166 | 7.93 | 548 | 40 | 32 | 186 | 8.13 | 350 | 49 | 24 | 106 | 8.19 | 471 |
| BS | 65 | 14 | 142 | 7.77 | 688 | 86 | 33 | 190 | 8.03 | 271 | 87 | 26 | 95 | 7.96 | 234 |
| A50 | 49 | 19 | 225 | 7.81 | 691 | 85 | 27 | 245 | 8.05 | 317 | 51 | 33 | 104 | 8.12 | 270 |
| A75 | 72 | 23 | 187 | 7.7 | 773 | 157 | 19 | 210 | 8.05 | 260 | 48 | 22 | 140 | 8.07 | 281 |
| P value | >.05 | >.05 | >.05 | .008 | .021 | <.001 | .023 | >.05 | >.05 | >.05 | >.05 | >.05 | >.05 | .009 | >.05 |

* Description of each treatment is given in Table 3.1

**Table 3.4(cont.)**

| | 2nd. Harvest | | | | | Carrot Yield | |
|---|---|---|---|---|---|---|---|
| Treatment* | BF | FF | PF | pH | EC | 1st. Harvest | 2nd. Harvest |
| CT | 25 | 18 | 126 | 8.28 | 115 | 64 | 30 |
| CTF | 31 | 27 | 159 | 8.42 | 106 | 111 | 115 |
| AH | 12 | 14 | 89 | 8.41 | 206 | 63 | 51 |
| BS | 51 | 26 | 203 | 8.17 | 186 | 169 | 280 |
| A50 | 23 | 13 | 150 | 8.28 | 218 | 182 | 198 |
| A75 | 24 | 19 | 171 | 8.21 | 207 | 204 | 218 |
| P value | <.001 | >.05 | >.05 | >.05 | >.05 | <.001 | .004 |

* Description of each treatment is given in Table 3.1

**Table 3.5 Average data collected for various treatments on bacterial and fungal colony forming units (CFU) per gram of soil at Baseline (June 11/07), Middle (July 9/07), 1st. Harvest (July 23/07), and 2nd. Harvest (Oct. 8/07), (P values for differences among treatment groups).**

| | Baseline | | Middle | | 1st. Harvest | | 2nd. Harvest | |
|---|---|---|---|---|---|---|---|---|
| | Bacterial | Fungal | Bacterial | Fungal | Bacterial | Fungal | Bacterial | Fungal |
| Treatment* | CFU*$10^6$ | CFU*$10^4$ | CFU*$10^6$ | CFU*$10^4$ | CFU*$10^6$ | CFU*$10^4$ | CFU*$10^6$ | CFU*$10^4$ |
| CT | 1.2 | 4.2 | 1.3 | 4.2 | 0.9 | 2.7 | 1.6 | 3.8 |
| CTF | 1.2 | 3.7 | 1.3 | 3.3 | 1.2 | 3.2 | 1.6 | 4.5 |
| AH | 1.1 | 4.5 | 0.8 | 2.8 | 1.1 | 2.9 | 1.3 | 3.3 |
| BS | 1.8 | 7.9 | 1.7 | 8.8 | 1.3 | 6.3 | 2.0 | 8.4 |
| A50 | 1.6 | 5.9 | 2.5 | 5.2 | 1.8 | 3.7 | 1.7 | 5.3 |
| A75 | 2.2 | 6.5 | 1.8 | 5.9 | 1.8 | 5.1 | 4.8 | 5.9 |
| P value | .009 | .005 | .015 | <.001 | .004 | >.05 | >.05 | .002 |

* Description of each treatment is given in Table 3.1

### 3.2.1 Bacterial Feeding Nematodes

At baseline, mean bacterial feeding nematode (BFN) populations ranged from 29-72 BFN/100 ml of soil, with the highest found in the A75 treatments (Fig. 3.8, "Baseline"). Midway through the growing season (Fig. 3.8, "Middle") mean BFN populations ranged from 24-157 BFN/100 ml of soil, with the highest found in the A75 treatments. At first harvest (Fig. 3.8, "1st. Harvest") mean BFN populations ranged from 49-87 BFN/100 ml of soil, with the highest found in the BS treatments. At second harvest (Fig. 3.8, "2nd. Harvest") mean BFN populations ranged from 12-51 BFN/100 ml of soil, with the highest found in the BS treatments. Significant differences among groups were found midway through the growing season ($F=8.1$, $df=5,18$, $P<0.001$) and at second harvest ($F=7.4$, $df=5,18$, $P=0.001$).

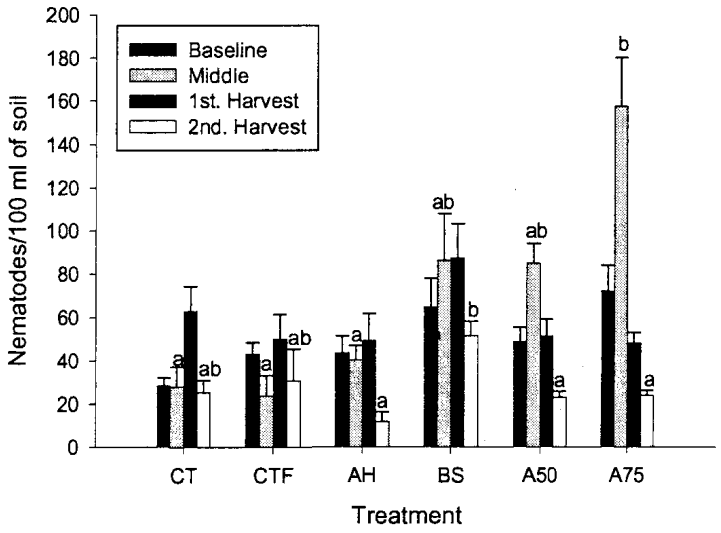


**Fig. 3.8 Bacterial feeding nematode populations in amendments (CT= soil; CTF= soil+fertilizer; AH= fly ash; BS= biosolids; A50= 50:50 mixture of biosolids and fly ash; A75= 75:25 mixture of biosolids and fly ash); (n=5, values +/- SE) at 4 sampling times (Baseline, Middle, 1st. Harvest, and 2nd. Harvest). Bars with the same letter are not significantly different (Tukey's range test, P<0.05). Summer 2007.**

### 3.2.2 Fungal Feeding Nematodes

At baseline, mean fungal feeding nematode (FFN) populations ranged from 11-24 FFN/100 ml of soil, with the highest found in the AH treatments (Fig. 3.9, "Baseline"). Midway through the growing season (Fig. 3.9, "Middle") mean FFN populations ranged from 11-33 FFN/100 ml of soil, with the highest found in the BS treatments. At first harvest (Fig. 3.9, "1st. Harvest") mean FFN populations ranged from 16-33 FFN/100 ml of soil, with the highest found in the A50 treatments. At second harvest (Fig. 3.9, "2nd. Harvest") mean FFN populations ranged from 13-27 FFN/100 ml of soil, with the highest found in the CTF treatments. Significant differences among groups were found only midway through the growing season ($F=3.5$, $df=5,18$, $P=0.023$)

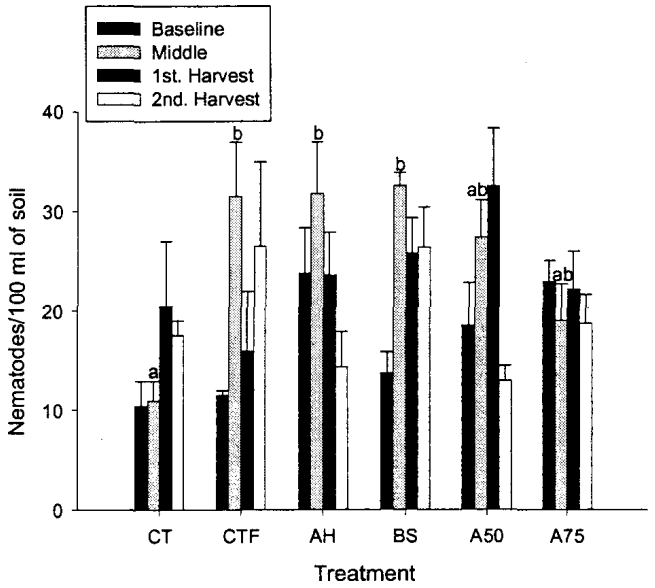


**Fig. 3.9 Fungal feeding nematode populations in amendments (CT= soil; CTF= soil+fertilizer; AH= fly ash; BS= biosolids; A50= 50:50 mixture of biosolids and fly ash; A75= 75:25 mixture of biosolids and fly ash); (n=5, values +/- SE) at 4 sampling times (Baseline, Middle, 1st. Harvest, and 2nd. Harvest). Bars with the same letter are not significantly different (Tukey's range test, P<0.05). Summer 2007.**

### 3.2.3 Plant Feeding Nematodes

At baseline, mean plant feeding nematode (PFN) populations ranged from 142-266 PFN/100 ml of soil, with the highest found in the CTF treatments (Fig. 3.10, "Baseline"). Midway through the growing season (Fig. 3.10, "Middle") mean PFN populations ranged from 186-245 PFN/100 ml of soil, with the highest found in the CT and A50 treatments. At first harvest (Fig. 3.10, "1st. Harvest") mean PFN populations ranged from 95-156 PFN/100 ml of soil, with the highest found in the CT treatments. At second harvest (Fig. 3.10, "2nd. Harvest") mean PFN populations ranged from 89-203 PFN/100 ml of soil, with the highest found in the BS treatments. No significant differences were found among groups in any of the sampling times.

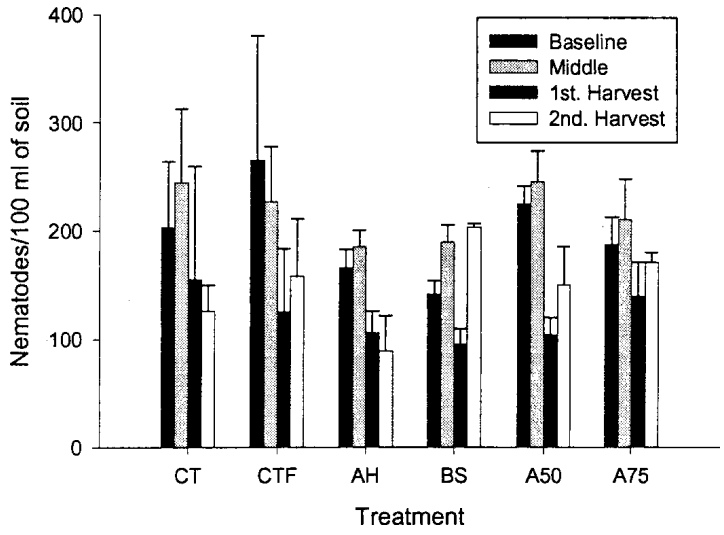


**Fig. 3.10 Plant feeding nematode populations in amendments (CT= soil; CTF= soil+fertilizer; AH= fly ash; BS= biosolids; A50= 50:50 mixture of biosolids and fly ash; A75= 75:25 mixture of biosolids and fly ash); (n=5, values +/- SE) at 4 sampling times (Baseline, Middle, 1st. Harvest, and 2nd. Harvest). Summer 2007.**

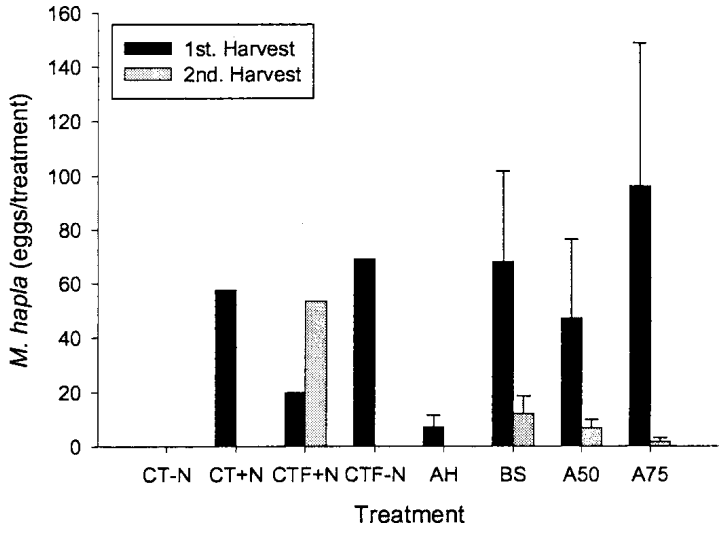


**Fig. 3.11 *Meloidogyne hapla* eggs in amendments (CT-N= soil without *M. hapla* inoculum; CT+N= soil with *M. hapla* inoculum; CTF-N= soil+fertilizer without *M. hapla* inoculum; CTF+N= soil+fertilizer with *M. hapla* inoculum AH= fly ash; BS= biosolids; A50= 50:50 mixture of biosolids and fly ash; A75= 75:25 mixture of biosolids and fly ash) at 2 sampling times (1st.Harvest and 2nd. Harvest); (n=1 for CT-N; CT+N; CTF-N, and CTF+N; n=5 for AH, BS, A50, and A75, values +/-SE). Summer 2007.**

### 3.2.4 *Meloidogyne hapla*

*Meloidogyne hapla* eggs recovered from secondary roots of carrots did not show a trend with amendment addition. At first harvest (Fig. 3.11, "1st. Harvest") mean number of eggs ranged from 0-96 eggs/treatment, with the highest number of eggs found in the A75 treatments. At second harvest (Fig. 3.11, "2nd. Harvest") mean number of eggs ranged from 0-53 eggs/treatment, with the highest found in the CTF+N treatments. Fewer eggs were recovered from the root samples from the second harvest because soil was not reinfected after the first harvest.

### 3.2.5 Electrical Conductivity

At baseline (Fig. 3.12, "Baseline") mean EC ranged from 200-1050 µS/cm, with the highest found in the CTF treatments. Midway through the growing season (Fig. 3.12, "Middle") mean EC ranged from 115-350 µS/cm, with the highest found in the AH treatments. At first harvest (Fig. 3.12, "1st. Harvest") mean EC

ranged from 120-471 µS/cm, with the highest found in the AH treatments. At second harvest (Fig. 3.12, "2nd. Harvest") mean EC ranged from 106-218 µS/cm, with the highest found in the A50 treatments. For the fertilized controls, EC was very high at baseline and declined very quickly throughout the experiment matching the levels of the controls without fertilizer. The amended treatments had high baseline EC values, declining throughout the experiment, but remained higher than the controls. Significant differences among groups were found only at baseline, ($F=3.5$, $df=5,18$, $P=0.021$).

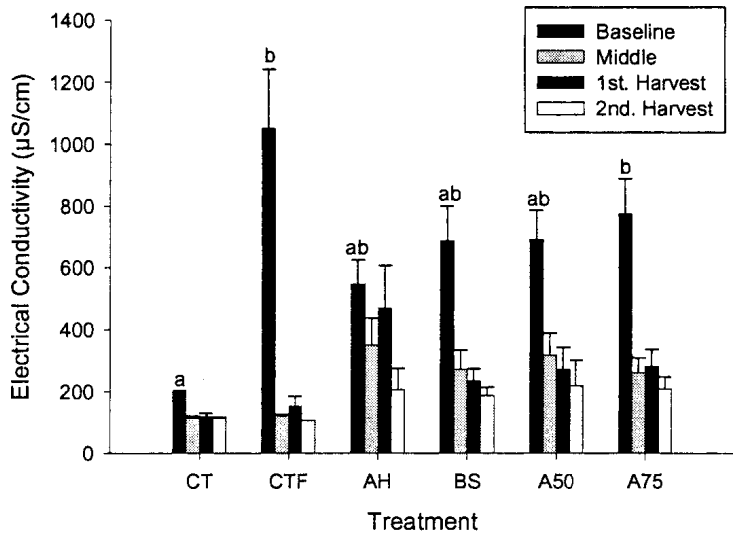


**Fig. 3.12 Electrical conductivity of amendments (CT= soil; CTF= soil+fertilizer; AH= fly ash; BS= biosolids; A50= 50:50 mixture of biosolids and fly ash; A75= 75:25 mixture of biosolids and fly ash); (n=5, values +/- SE) at 4 sampling times (Baseline, Middle, 1st. Harvest, and 2nd. Harvest). Bars with the same letter are not significantly different (Tukey's range test, P<0.05). Summer 2007.**

### 3.2.6 pH

At baseline, mean pH values ranged from 7.70-7.93 (Fig. 3.13, "Baseline") with the highest values found in the AH treatments. Midway through the growing season (Fig. 3.13, "Middle") mean pH ranged from 8.03-8.15, with the highest found in the CTF treatments. At first harvest (Fig. 3.13, "1st. Harvest") mean pH ranged from 7.96-8.23, with the highest found in the CT treatments. At second harvest (Fig. 3.13, "2nd. Harvest") mean pH ranged from 8.17-8.42, with the

highest found in the CTF treatments. With very few exceptions, pH values increased at every sampling time within each set of treatments. Significant differences among groups were found at baseline (F=4.5, df=5,18, *P*=0.008) and first harvest (F=4.4, df=5.18, *P*=0.009).

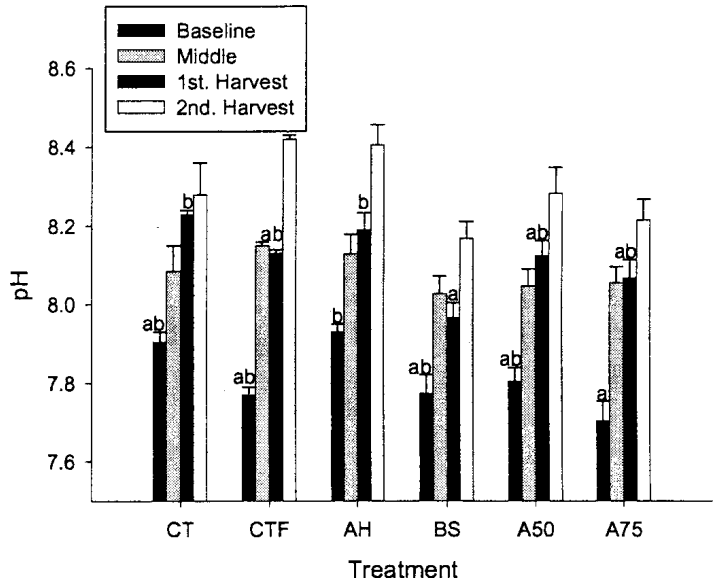


**Fig. 3.13 pH of amendments (CT= soil; CTF= soil+fertilizer; AH= fly ash; BS= biosolids; A50= 50:50 mixture of biosolids and fly ash; A75= 75:25 mixture of biosolids and fly ash); (n=5, values +/- SE) at 4sampling times (Baseline, Middle, 1st. Harvest, and 2nd. Harvest). Bars with the same letter are not significantly different (Tukey's range test, P<0.05). Summer 2007.**

### 3.2.7 Bacterial Colony Forming Units

At baseline, mean bacterial colony forming units (CFU) ranged from 1.1-2.2*$10^6$ CFU/g of soil (Fig. 3.14, "Baseline") with the highest values found in the A75 treatments. Midway through the growing season (Fig. 3.14, "Middle") mean bacterial CFU ranged from 0.8-2.5*$10^6$ CFU/g of soil, with the highest found in the A50 treatments. At first harvest (Fig. 3.14, "1st. Harvest") mean bacterial CFU ranged from 0.9-1.8*$10^6$ CFU/g of soil, with the highest found in the A50 treatments. At second harvest (Fig. 3.14, "2nd. Harvest") mean bacterial CFU ranged from 1.3-4.8*$10^6$ CFU/g of soil, with the highest found in the A75 treatments. Significant differences among groups were found at baseline (F=4.4,

df=5,18, *P*=0.009), midway through the growing season (F=3.8, df=5,18, *P*=0.015) and first harvest (F=5.1, df=5.18, *P*=0.004).

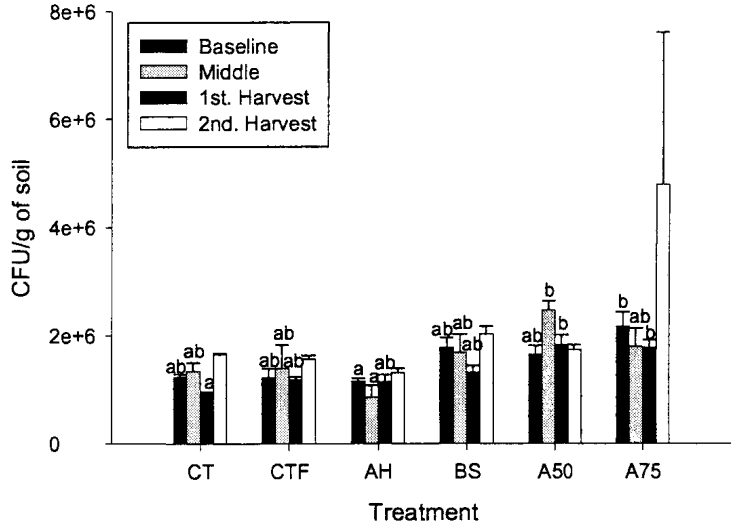


**Fig. 3.14 Bacterial colony forming units in amendments (CT= soil; CTF= soil+fertilizer; AH= fly ash; BS= biosolids; A50= 50:50 mixture of biosolids and fly ash; A75= 75:25 mixture of biosolids and fly ash); (n=5, values +/- SE) at 4 sampling times (Baseline, Middle, 1st. Harvest, and 2nd. Harvest). Bars with the same letter are not significantly different (Tukey's range test, P<0.05). Summer 2007.**

### 3.2.8 Fungal Colony Forming Units

At baseline, mean fungal colony forming units (CFU) ranged from 3.7-7.9*$10^4$ CFU/g of soil (Fig. 3.15, "Baseline") with the highest values found in the BS treatments. Midway through the growing season (Fig. 3.15, "Middle") mean fungal CFU ranged from 2.8-8.8*$10^4$ CFU/g of soil, with the highest found in the BS treatments. At first harvest (Fig. 3.15, "1st. Harvest") mean fungal CFU ranged from 2.7-6.3*$10^4$ CFU/g of soil, with the highest found in the BS treatments. At second harvest (Fig. 3.15, "2nd. Harvest") mean fungal CFU ranged from 3.3-8.4*$10^4$ CFU/g of soil, with the highest found in the BS treatments. Significant differences among groups were found at baseline (F=4.9, df=5,18, *P*=0.005), midway through the growing season (F=7.7, df=5,18,

*P*<0.001) and second harvest (F=6.3, df=5.18, *P*=0.002). BS treatments had the highest number of fungal CFU at all sampling times.

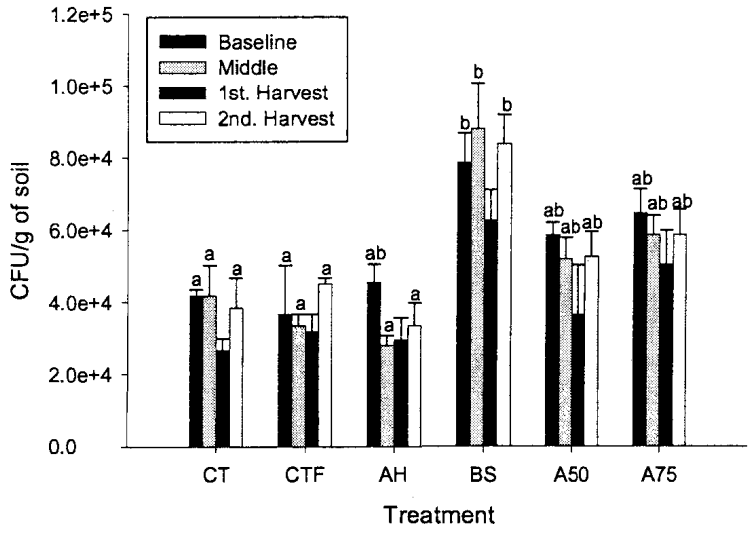


**Fig. 3.15 Fungal colony forming units in amendments (CT= soil; CTF= soil+fertilizer; AH= fly ash; BS= biosolids; A50= 50:50 mixture of biosolids and fly ash; A75= 75:25 mixture of biosolids and fly ash); (n=5, values +/- SE) at 4 sampling times (Baseline, Middle, 1st. Harvest, and 2nd. Harvest). Bars with the same letter are not significantly different (Tukey's range test, P<0.05). Summer 2007.**

### 3.2.9 Carrot Yield

There was a significant difference among treatment groups in carrot root yield for the first harvest (F=14.7, df = 5,18, *P*<0.001). Mean yield ranged from 63-204 g/treatment with the highest obtained from the A75 treatments (Fig. 3.16).

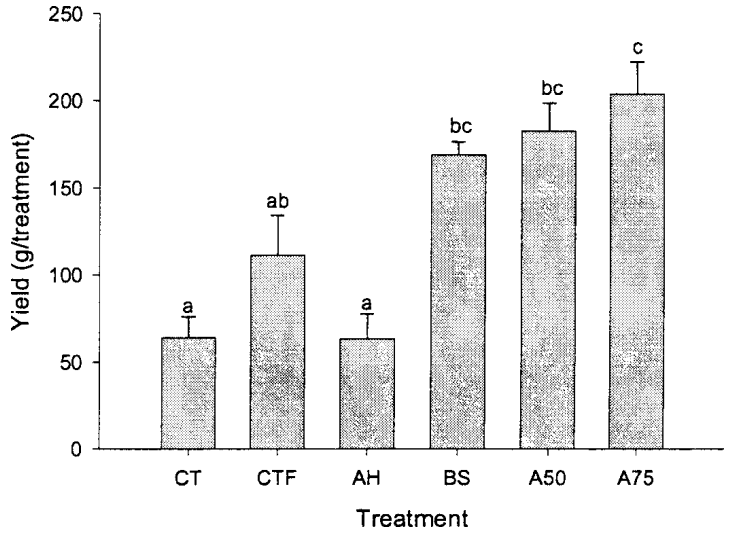


**Fig. 3.16 Carrot (primary tap root) yield in amendments (CT= soil; CTF= soil+fertilizer; AH= fly ash; BS= biosolids; A50= 50:50 mixture of biosolids and fly ash; A75= 75:25 mixture of biosolids and fly ash); (n=5, values +/- SE). Bars with the same letter are not significantly different (Tukey's range test, P<0.05). First harvest summer 2007.**

There was a significant difference among treatment groups in carrot root yield for the second harvest (F=5.1, df = 5,18, *P*=0.004). Mean yield ranged from 30-280 g/treatment with the highest obtained from the BS treatments (Fig. 3.17).

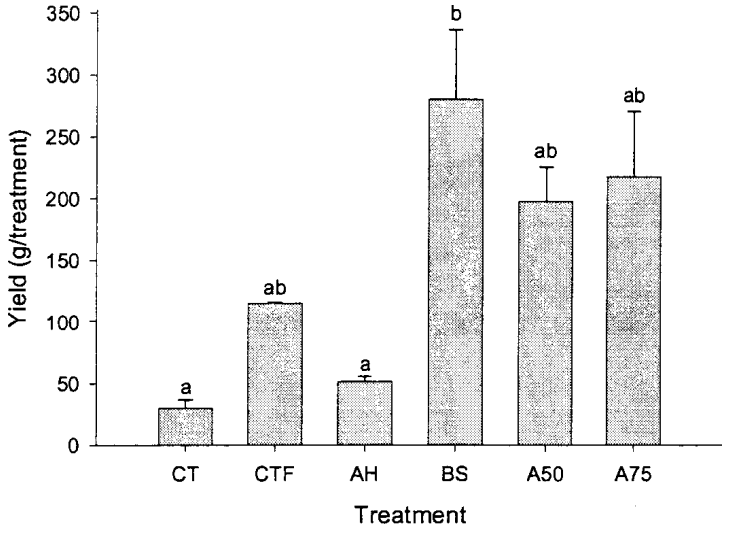


**Fig. 3.17 Carrot (primary tap root) yield in amendments (CT= soil; CTF= soil+fertilizer; AH= fly ash; BS= biosolids; A50= 50:50 mixture of biosolids and fly ash; A75= 75:25 mixture of biosolids and fly ash); (n=5, values +/- SE). Bars with the same letter are not significantly different (Tukey's range test, P<0.05). Second harvest summer 2007.**

## 3.3 Impact of Biosolids and Fly Ash on Natural Nematode Populations and *Meloidogyne hapla*, in the Absence of Plant Material (2007)

The impact of fly ash and biosolids on nematode populations in the absence of plant material was determined in the greenhouse for 2007. The data were inconclusive and are not included in the text of this thesis. The results and discussion are included in Appendix 5.

## 3.4 Impact of Biosolids and Fly Ash on *Meloidogyne hapla* and Tomato Yield (2008)

Data presented here are average values for replicates within each treatment to show overall effects of ratios of amendments; differences within/among replicates are not considered. Average data collected for the 2008 field season and from which figures in this section are made, are included in Table 3.6. Raw data are given in Appendix 8.

**Table 3.6 Average data collected for various treatments on *Meloidogyne hapla* (eggs/g of fresh root); bacterial and fungal colony forming units at baseline and harvest (CFU/g of dry soil); pH and electrical conductivity (µS/cm) at baseline and harvest; tomato yield (g/plant); above ground biomass (g); below ground biomass (g); and length of root (cm). (n=3 for all treatments, P values presented per treatment group). Baseline (July 8/08) and Harvest (Oct. 14/08).**

| | Baseline | | | | Harvest | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Treat.* | pH | EC | Bact. CFU $*10^6$ | Fung. CFU $*10^4$ | pH | EC | Bact. CFU $*10^6$ | Fung. CFU $*10^4$ | *M. hapla* eggs | Tomato yield | Above ground mass | Below ground mass |
| CT-N | 8.05 | 228 | 1.4 | 5.3 | 8.32 | 107 | 1.6 | 5.7 | 0 | 1700 | 216 | 23 |
| CT+N | 8.09 | 234 | 1.5 | 6.0 | 8.34 | 107 | 1.6 | 4.0 | 920 | 2075 | 170 | 27 |
| CTF-N | 8.00 | 329 | 1.6 | 5.6 | 8.23 | 114 | 1.9 | 5.8 | 0 | 2658 | 349 | 33 |
| CTF+N | 8.02 | 328 | 1.0 | 4.5 | 8.30 | 113 | 2.2 | 8.6 | 1408 | 2182 | 274 | 32 |
| CT P value | >.05 | <.001 | >.05 | >.05 | >.05 | >.05 | >.05 | >.05 | .024 | >.05 | >.05 | >.05 |
| BS-3 | 7.99 | 361 | 1.9 | 4.8 | 8.08 | 129 | 2.6 | 7.2 | 1089 | 3400 | 378 | 33 |
| BS-6 | 7.95 | 409 | 2.1 | 6.5 | 8.10 | 180 | 1.8 | 8.4 | 1189 | 3548 | 307 | 44 |
| BS-9 | 7.88 | 539 | 1.8 | 8.9 | 8.06 | 235 | 2.5 | 7.4 | 1709 | 2911 | 355 | 42 |
| BS-12 | 7.91 | 703 | 1.5 | 8.9 | 7.93 | 341 | 3.2 | 8.8 | 1617 | 2982 | 282 | 45 |
| BS-15 | 7.85 | 918 | 3.4 | 12.4 | 7.82 | 375 | 2.7 | 10.3 | 1599 | 3061 | 402 | 51 |
| BS P value | .019 | <.001 | .04 | <.001 | >.05 | <.001 | >.05 | >.05 | >.05 | >.05 | >.05 | >.05 |
| A50-3 | 8.07 | 298 | 1.9 | 6.6 | 8.33 | 119 | 2.1 | 8.8 | 1142 | 2732 | 268 | 34 |

Table 3.6 (cont.)

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A50-6 | 8.03 | 408 | 2.0 | 6.8 | 8.26 | 129 | 2.3 | 7.2 | 1348 | 2668 | 290 | 41 |
| A50-9 | 7.92 | 486 | 1.7 | 5.0 | 8.26 | 136 | 2.0 | 9.5 | 2844 | 3040 | 346 | 39 |
| A50-12 | 7.87 | 683 | 1.9 | 6.2 | 8.24 | 151 | 1.9 | 4.8 | 4606 | 3397 | 308 | 48 |
| A50-15 | 7.91 | 849 | 2.4 | 6.8 | 8.28 | 153 | 2.6 | 17.0 | 889 | 3008 | 285 | 38 |
| A50 P value | >.05 | <.001 | >.05 | >.05 | <.001 | >.05 | >.05 | >.05 | .034 | >.05 | >.05 | >.05 |
| A75-3 | 7.98 | 332 | 1.7 | 5.9 | 8.30 | 106 | 1.7 | 5.9 | 3025 | 2844 | 277 | 37 |
| A75-6 | 7.99 | 452 | 2.4 | 8.2 | 8.20 | 150 | 2.4 | 4.9 | 1322 | 2459 | 317 | 37 |
| A75-9 | 8.00 | 502 | 1.9 | 5.9 | 8.16 | 166 | 1.9 | 3.8 | 1946 | 3377 | 430 | 46 |
| A75-12 | 7.87 | 719 | 2.7 | 6.0 | 8.12 | 254 | 1.9 | 7.8 | 1132 | 2677 | 260 | 43 |
| A75-15 | 7.91 | 771 | 2.9 | 10.2 | 7.91 | 300 | 2.4 | 7.4 | 1534 | 3735 | 367 | 52 |
| A75 P value | >.05 | <.001 | >.05 | >.05 | <.001 | .013 | .013 | >.05 | >.05 | >.05 | >.05 | >.05 |

* Description of each treatment is given in Table 2.1

### 3.4.1 Electrical Conductivity and *M. hapla*

#### 3.4.1.1 Effect of Biosolids

Electrical conductivity increased with increasing biosolids application at baseline and harvest with baseline showing the highest values (Fig. 3.18). Average baseline EC increased from 360-918 µS/cm going from BS-3 to BS-15 (Fig. 3.18, "Baseline") and harvest samples (Fig. 3.18, "Harvest") ranged from 129-375 µS/cm. Significant differences among BS ratios were found at baseline ($F=69.3$, $df=4,10$, $P<0.001$) and harvest ($F=18.7$, $df=4,10$, $P<0.001$).

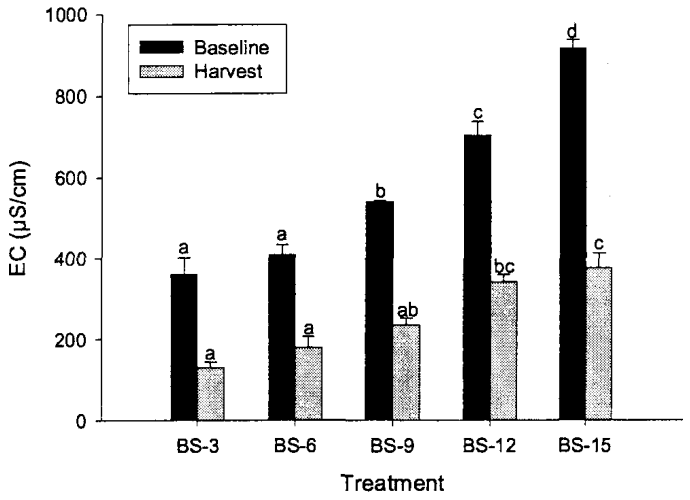


**Fig. 3.18 Electrical conductivity of amendments (BS-3= soil+3% biosolids; BS-6=soil+6% biosolids; BS-9=soil+9% biosolids; BS-12=soil+12% biosolids; BS-15=soil+15% biosolids); (n=3, values +/- SE) at 2 sampling times (Baseline and Harvest). Bars with the same letter are not significantly different (Tukey's range test, P<0.05). Summer 2008.**

Although large errors occurred among replicates of a given treatment, on average, *M. hapla* eggs increased with increasing biosolids application (Fig. 3.19). No significant differences among biosolids ratios were found for *M. hapla* eggs. However, it does appear that increased application of biosolids resulted in higher numbers of *M. hapla* eggs. This could be related to higher root biomass produced with increased biosolids application.

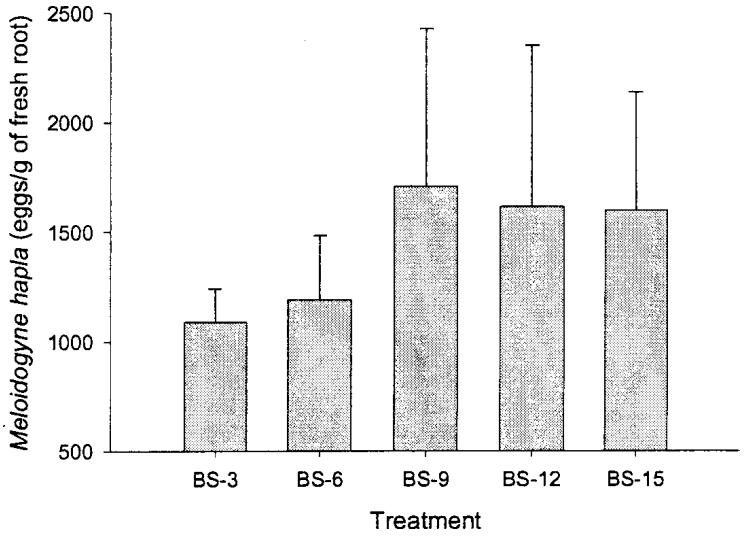


**Fig. 3.19 *Meloidogyne hapla* eggs at harvest in roots of tomato plants grown in amendments (BS-3= soil+3% biosolids; BS-6=soil+6% biosolids; BS-9=soil+9% biosolids; BS-12=soil+12% biosolids; BS-15=soil+15% biosolids), (n=3 values +/-SE). Summer 2008.**

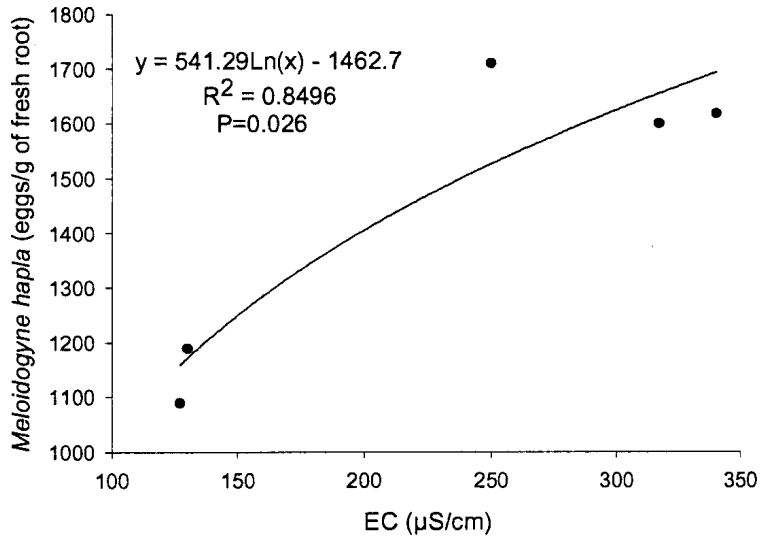


**Fig. 3.20 Correlation between electrical conductivity and *Meloidogyne hapla* eggs in tomato roots in treatments with soil and different ratios of biosolids at tomato harvest. (Each point represents the mean of 3 replicates) Summer 2008.**

There was a positive correlation between EC and *M. hapla* eggs for treatments with different ratios of biosolids at harvest (Fig. 3.20), ranging from 1089-1709 eggs/g of fresh root.

### 3.4.1.2 Effect of Biosolids and Fly Ash (BS50:AH50)

Electrical conductivity increased with increasing BS50:AH50 application at baseline and harvest with baseline showing the highest values (Fig. 3.21). Average baseline EC increased from 298-849 µS/cm going from A50-3 to A50-15 (Fig. 3.21, "Baseline") and harvest samples (Fig. 3.21, "Harvest") ranged from 119-153 µS/cm. Significant differences between BS50:AH50 ratios were found only at baseline ($F=25.1$, $df=4,10$, $P<0.001$).

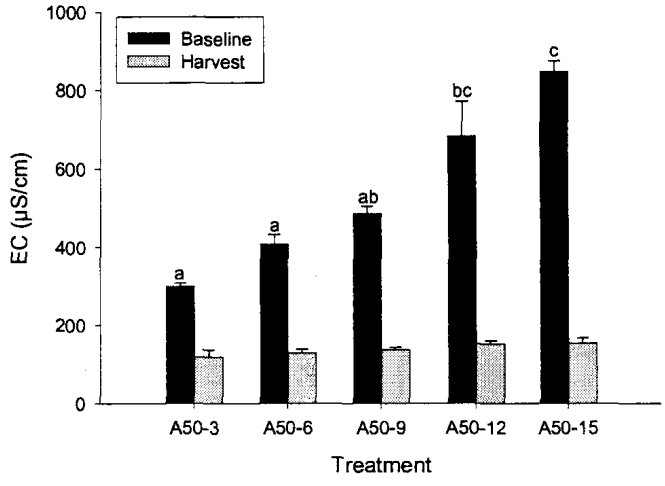


**Fig. 3.21 Electrical conductivity of amendments (A50-3= soil+3% of a 50% biosolids: 50% fly ash mix; A50-6=soil+6% of a 50% biosolids: 50% fly ash mix; A50-9=soil+9% of a 50% biosolids: 50% fly ash mix; A50-12=soil+12% of a 50% biosolids: 50% fly ash mix; A50-15=soil+15% of a 50% biosolids: 50% fly ash mix); (n=3, values +/- SE) at 2 sampling times (Baseline and Harvest). Bars with the same letter are not significantly different (Tukey's range test, P<0.05). Summer 2008.**

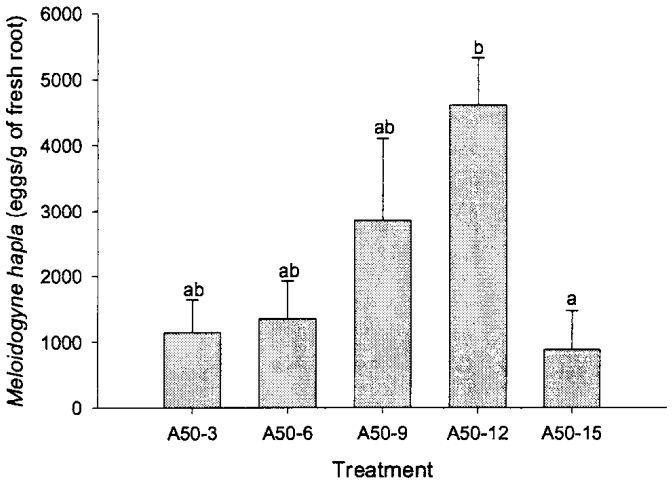


**Fig. 3.22 *Meloidogyne hapla* eggs at harvest in roots of tomato plants grown in amendments (A50-3= soil+3% of a 50% biosolids: 50% fly ash mix; A50-6=soil+6% of a 50% biosolids: 50% fly ash mix; A50-9=soil+9% of a 50% biosolids: 50% fly ash mix; A50-12=soil+12% of a 50% biosolids: 50% fly ash mix; A50-15=soil+15% of a 50% biosolids: 50% fly ash mix); (n=3 values +/-SE). Bars with the same letter are not significantly different (Tukey's range test, P<0.05) Summer 2008.**

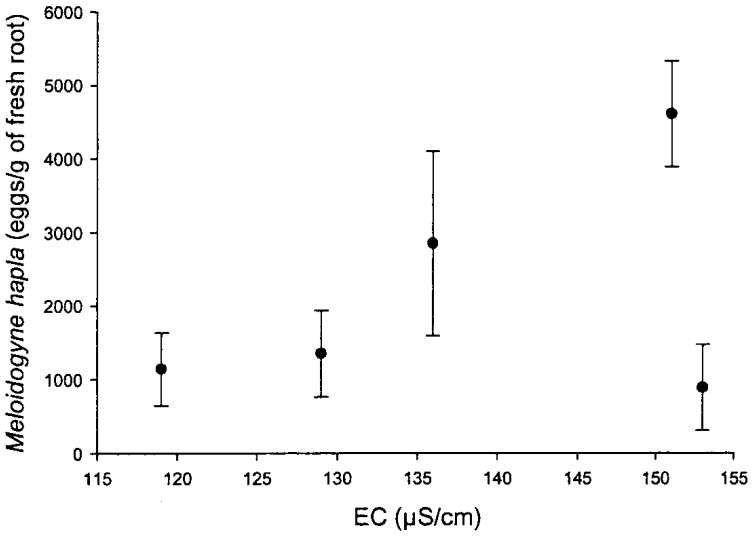


**Fig. 3.23 Relationship between electrical conductivity and *Meloidogyne hapla* eggs in tomato roots in treatments with soil and different ratios of a mixture of 50% biosolids and 50% fly ash at tomato harvest. (Each point represents the mean of 3 replicates) Summer 2008.**

With the exception of A50-15 (highest amendment application) *M. hapla* eggs increased with increasing amendment (Fig. 3.22), although large variability was noted among replicates. Significant differences among BS50:AH50 ratios for *M. hapla* eggs were found (F=4.0, df=4,10, *P*=0.034). Other than the same

exception noted previously for A50-15, there was a strong positive correlation between EC and *M. hapla* eggs at harvest (Fig. 3.23), ranging from 1142-4606 eggs/g of fresh root going from A50-3 to A50-12. The lowest number (889 eggs/g of fresh root) was found in the A50-15 treatments.

### 3.4.1.3 Effect of Biosolids and Fly Ash (BS75:AH25)

Electrical conductivity increased with increasing BS75:AH25 application at baseline and harvest with baseline showing the highest values (Fig. 3.24). Average baseline EC increased from 332-771 µS/cm going from A75-3 to A75-15 (Fig. 3.24, "Baseline") and harvest samples (Fig. 3.24, "Harvest") ranged from 106-300 µS/cm. Significant differences among BS75:AH25 ratios were found at baseline ($F=69.9$, df=4,10, $P<0.001$) and harvest ($F=5.5$, df=4,10, $P=0.013$).

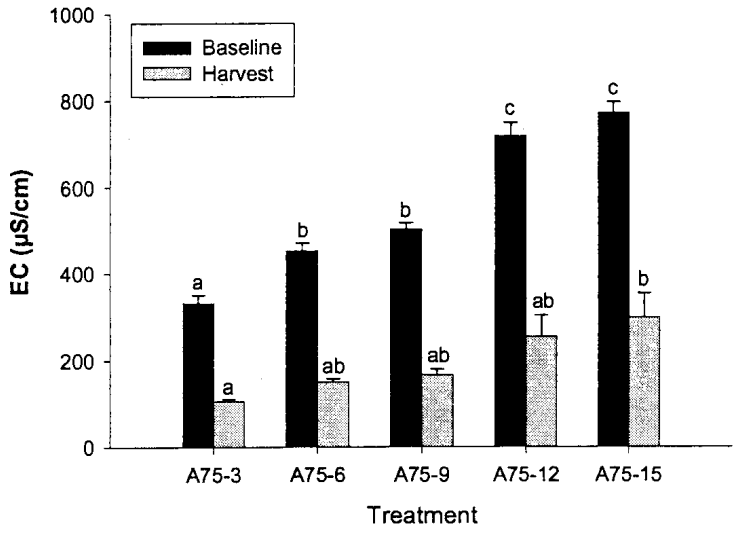


**Fig. 3.24 Electrical conductivity of amendments (A75-3= soil+3% of a 75% biosolids: 25% fly ash mix; A75-6=soil+6% of a 75% biosolids: 25% fly ash mix; A75-9=soil+9% of a 75% biosolids: 25% fly ash mix; A75-12=soil+12% of a 75% biosolids: 25% fly ash mix; A75-15=soil+15% of a 75% biosolids: 25% fly ash mix); (n=3, values +/- SE) at 2 sampling times (Baseline and Harvest). Bars with the same letter are not significantly different (Tukey's range test, P<0.05). Summer 2008.**

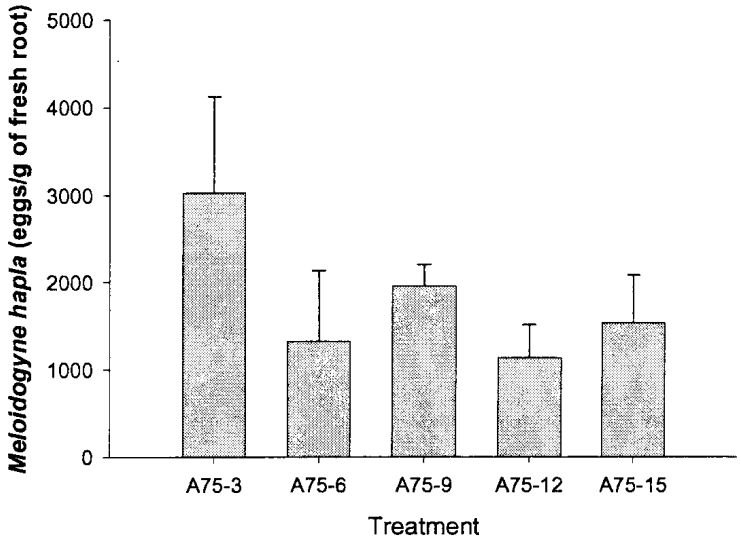


**Fig. 3.25 *Meloidogyne hapla* eggs at harvest in roots of tomato plants grown in amendments (A75-3= soil+3% of a 75% biosolids: 25% fly ash mix; A75-6=soil+6% of a 75% biosolids: 25% fly ash mix; A75-9=soil+9% of a 75% biosolids: 25% fly ash mix; A75-12=soil+12% of a 75% biosolids: 25% fly ash mix; A75-15=soil+15% of a 75% biosolids: 25% fly ash mix); (n=3 values +/-SE). Bars with the same letter are not significantly different (Tukey's range test, P<0.05) Summer 2008.**

The average number of *M. hapla* eggs decreased at amendments higher than A75-3, but no consistent trend was noted in the higher treatments (Fig. 3.25). Large variability was noted among replicates and no significant differences among BS75:AH25 ratios were found for *M. hapla* eggs.

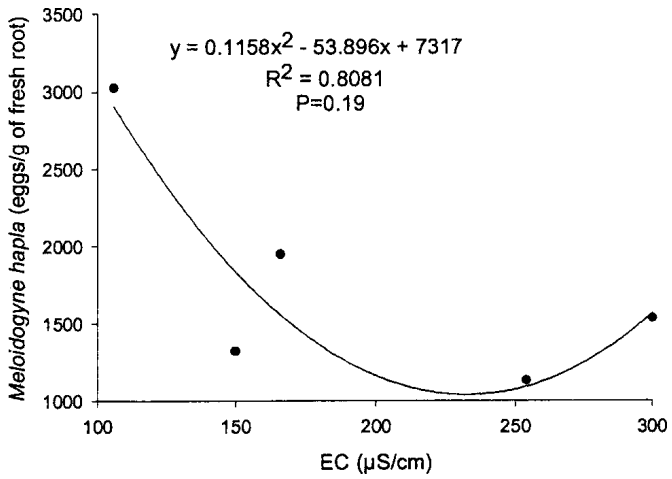


**Fig. 3.26 Relationship between electrical conductivity and *Meloidogyne hapla* eggs in tomato roots in treatments with soil and different ratios of a mixture of 75% biosolids and 25% fly ash at tomato harvest. (Each point represents the mean of 3 replicates) Summer 2008.**

No statistically significant correlation between EC and *M. hapla* eggs was found for treatments with different ratios of BS75:AH25 at harvest. However, fewer eggs were found in treatments with higher EC (Fig. 3.26). *Meloidogyne hapla* eggs ranged from 1132-3025 eggs/g of fresh root.

### 3.4.2 pH and *M. hapla*

#### 3.4.2.1 Effect of Biosolids

The pH decreases slightly with increasing application of biosolids at baseline and harvest with harvest showing marginally higher values (Fig. 3.27). Average baseline pH ranged from 7.85-7.99 going from BS-3 to BS-15 (Fig. 3.27, "Baseline") and harvest samples (Fig. 3.27, "Harvest") ranged from 7.82-8.10. Significant differences among BS ratios were found only at baseline ($F=4.9$, $df=4,10$, $P=0.019$).

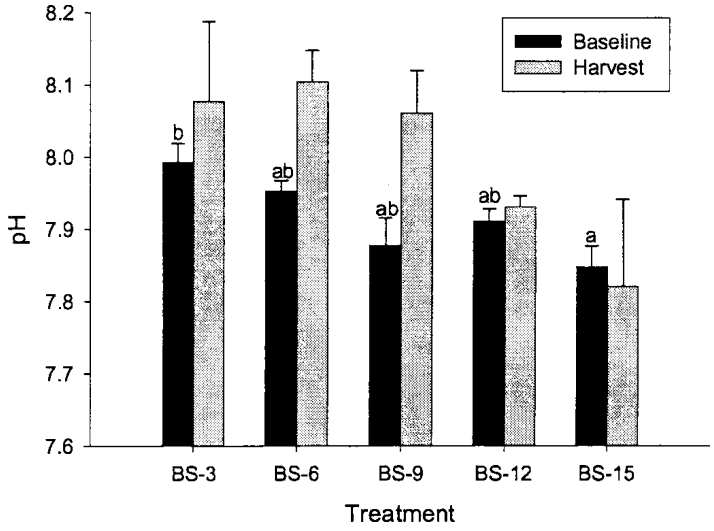


**Fig. 3.27 pH of amendments (BS-3= soil+3% biosolids; BS-6=soil+6% biosolids; BS-9=soil+9% biosolids; BS-12=soil+12% biosolids; BS-15=soil+15% biosolids); (n=3, values +/- SE) at 2 sampling times (Baseline and Harvest). Bars with the same letter are not significantly different (Tukey's range test, P<0.05). Summer 2008.**

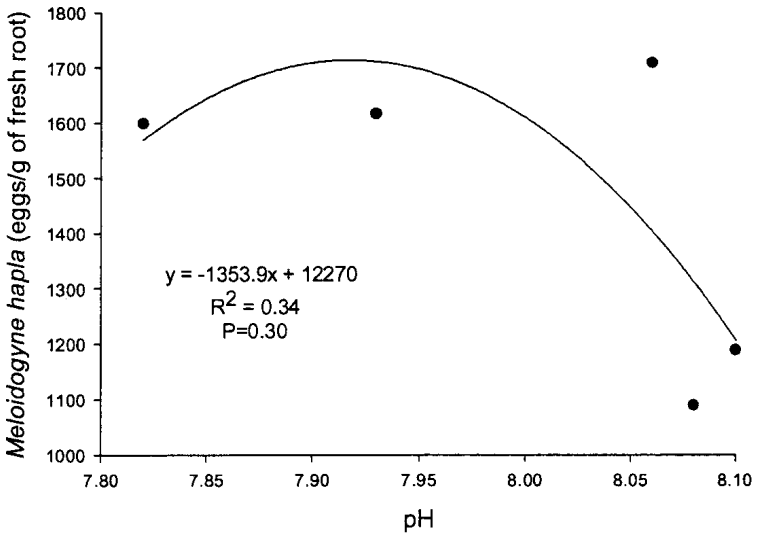


**Fig. 3.28 Relationship between pH and *Meloidogyne hapla* eggs in tomato roots in treatments with soil and different ratios of biosolids at tomato harvest. (Each point represents the mean of 3 replicates) Summer 2008.**

Even though no statistically significant correlation between pH and number of *M. hapla* eggs was found for treatments with different ratios of biosolids at harvest (Fig. 3.28); in general, a higher number of eggs was collected at lower pH, which coincided with the highest concentration of biosolids.

### 3.4.2.2 Effect of Biosolids and Fly Ash (BS50:AH50)

With the exception of A50-15 (highest amendment application), pH decreased with increasing BS50:AH50 application at baseline and harvest, with harvest showing the highest values (Fig. 3.29). Average baseline pH ranged from 7.87-8.07 (Fig. 3.29, "Baseline") and harvest samples (Fig. 3.29, "Harvest") ranged from 8.24-8.33. Significant differences among BS50:AH50 ratios were found only at harvest ($F=55.4$, $df=4,10$, $P<0.001$).

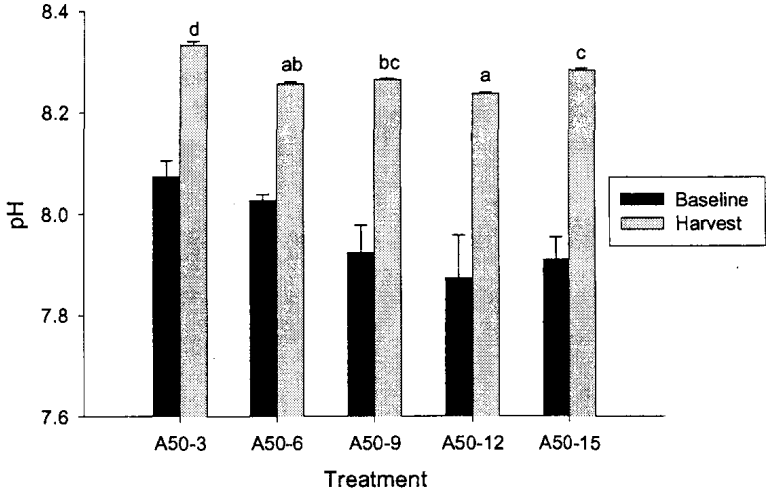


**Fig. 3.29 pH of amendments (A50-3= soil+3% of a 50% biosolids: 50% fly ash mix; A50-6=soil+6% of a 50% biosolids: 50% fly ash mix; A50-9=soil+9% of a 50% biosolids: 50% fly ash mix; A50-12=soil+12% of a 50% biosolids: 50% fly ash mix; A50-15=soil+15% of a 50% biosolids: 50% fly ash mix); (n=3, values +/- SE) at 2 sampling times (Baseline and Harvest). Bars with the same letter are not significantly different (Tukey's range test, P<0.05). Summer 2008.**

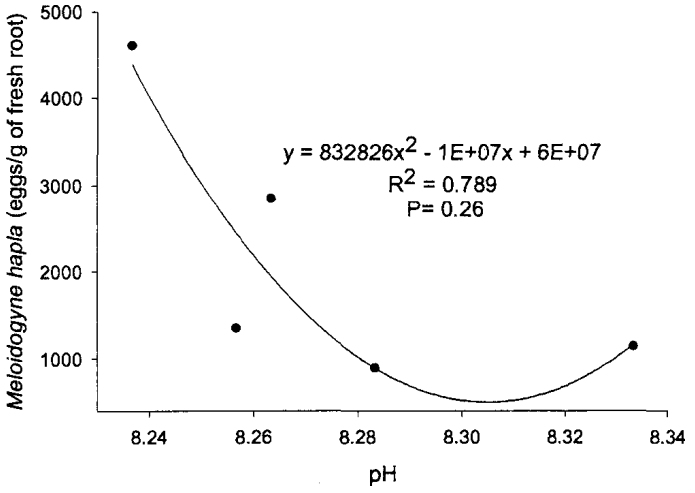


**Fig. 3.30 Relationship between pH and *Meloidogyne hapla* eggs in tomato roots in treatments with soil and different ratios of a mixture of 50% biosolids and 50% fly ash at tomato harvest. (Each point represents the mean of 3 replicates) Summer 2008.**

Even though no statistically significant correlation was found for the treatments with different ratios of BS50:AH50, fewer *M. hapla* eggs were found in treatments with higher pH at harvest (Fig. 3.30).

### 3.4.2.3 Effect of Biosolids and Fly Ash (BS75:AH25)

The pH decreased with increasing BS75:AH25 application at baseline and harvest with harvest showing the highest values (Fig. 3.31). Average baseline pH ranged from 7.87-8.00 (Fig. 3.31, "Baseline") and harvest samples (Fig. 3.31, "Harvest") ranged from 7.91-8.30. Significant differences among BS75:AH25 ratios were found at harvest ($F=35.7$, $df=4,10$, $P<0.001$).

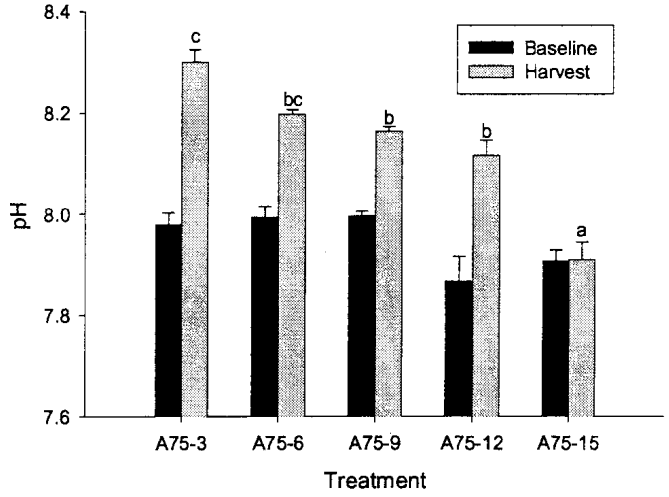


**Fig. 3.31 pH of amendments (A75-3= soil+3% of a 75% biosolids: 25% fly ash mix; A75-6=soil+6% of a 75% biosolids: 25% fly ash mix; A75-9=soil+9% of a 75% biosolids: 25% fly ash mix; A75-12=soil+12% of a 75% biosolids: 25% fly ash mix; A75-15=soil+15% of a 75% biosolids: 25% fly ash mix); (n=3, values +/- SE) at 2 sampling times (Baseline and Harvest). Bars with the same letter are not significantly different (Tukey's range test, P<0.05). Summer 2008.**

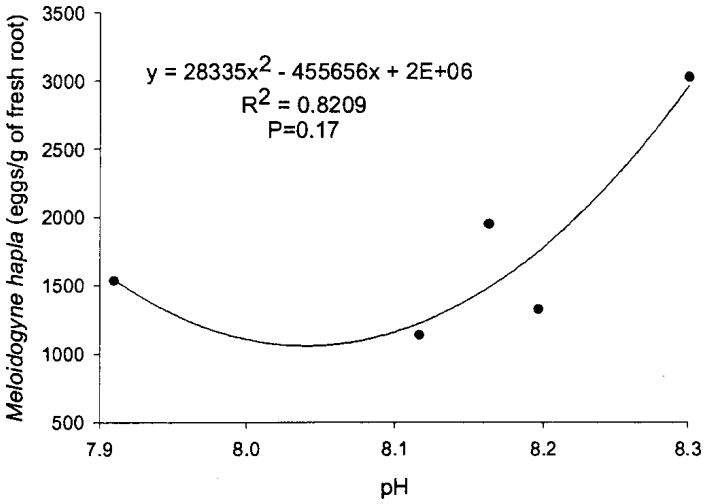


**Fig. 3.32 Relationship between pH and *Meloidogyne hapla* eggs in tomato roots in treatments with soil and different ratios of a mixture of 75% biosolids and 25% fly ash at tomato harvest. (Each point represents the mean of 3 replicates) Summer 2008.**

Even though no statistically significant correlation was found for the treatments with different ratios of BS75:AH25, a higher number of *M. hapla* eggs was found in treatments with higher pH at harvest (Fig. 3.32).

**Table 3.7 Correlation coefficients and significance ($R^2$/P) for each treatment group (BS, A50, A75) against variables shown (see text for description). Summer 2008.**

| | EC | | | PH | | | Bacterial CFU | Fungal CFU |
|---|---|---|---|---|---|---|---|---|
| Treat.* | Tomato yield | Above ground mass | Below ground mass | Tomato yield | Above ground mass | Below ground mass | *M. hapla* | *M. hapla* |
| BS | 0.75/0.06 | 0.16/0.84 | 0.45/0.19 | 0.31/0.33 | 0.07/0.66 | 0.56/0.14 | 0.31/0.33 | 0.14/0.87 |
| A50 | 0.62/0.11 | 0.07/0.65 | 0.48/0.54 | 0.37/0.27 | 0.42/0.23 | 0.77/0.05 | 0.84/0.16 | 0.53/0.16 |
| A75 | 0.37/0.64 | 0.06/0.94 | 0.67/0.08 | 0.58/0.42 | 0.13/0.55 | 0.83/0.03 | 0.79/0.21 | 0.35/0.65 |

### 3.4.3 Electrical Conductivity (EC) and Tomato Yield

Tomato yield ranged from 2911-3548 g/plant, generally decreasing with increasing EC from 127-340 µS/cm (Table 3.6). No statistically significant correlation was found between EC and tomato yield ($R^2$= 0.75; P=0.06) for treatments with different ratios of biosolids (Table 3.7).

Even though no statistically significant correlation ($R^2$= 0.62; P=0.11) was found between EC and tomato yield for treatments with different ratios of BS50:AH50 (Table 3.7), yield appeared to increase, ranging from 2668-3396 g/plant, with increasing EC from 119-153 µS/cm (Table 3.6).

Yield ranged from 2459-3735 g/plant and EC from 106-300 µS/cm (Table 3.6) in treatments with different ratios of a BS75:AH25 mixture. No statistically significant correlation ($R^2$=0.37; P=0.64) was found between EC and tomato yield (Table 3.7).

### 3.4.4 Electrical Conductivity and Above Ground Biomass

Above ground biomass ranged from 282-402 g/plant in treatments with different ratios of biosolids, with the highest obtained from BS-15 (Table 3.6). No statistically significant correlation ($R^2$=0.16; P=0.84) between EC and above ground biomass was found (Table 3.7).

Above ground biomass, for treatments with different ratios of BS50:AH50, ranged from 268-346 g/plant, with the highest obtained from A50-9 (Table 3.6). No statistically significant correlation ($R^2$=0.07; P=0.65) between EC and above ground biomass was found (Table 3.7).

No statistically significant correlation ($R^2$=0.06; P=0.94) between EC and above ground biomass was found for treatments with different ratios of BS75:AH25 (Table 3.7). Above ground biomass ranged from 260-430 g/plant, with the highest obtained from A75-9 (Table 3.6).

### 3.4.5 Electrical Conductivity and Below Ground Biomass

No statistically significant correlation ($R^2$=0.45; P=0.19) between EC and below ground biomass was found for treatments with different ratios of biosolids (Table 3.7). Below ground biomass weight ranged from 33-51 g/plant, with the highest obtained from BS-15 (Table 3.6).

No statistically significant correlation ($R^2$=0.48; P=0.54) between EC and below ground biomass was found for treatments with different ratios of BS50:AH50 (Table 3.7). Below ground biomass weight ranged from 34-48 g/plant, with the highest obtained from A50-12 (Table 3.6).

No statistically significant correlation ($R^2$=0.67; P=0.08) between EC and below ground biomass was found for treatments with different ratios BS75:AH25 (Table 3.7). Below ground biomass weight ranged from 37-52 g/plant, with the highest obtained from A75-15 (Table 3.6).

### 3.4.6 pH and Tomato Yield

Although no statistically significant correlation was found between pH and tomato yield for treatments with different ratios of biosolids ($R^2$=0.31; P=0.33),

BS50:AH50 ($R^2$=0.37; P=0.27), and BS75:AH25 ($R^2$=0.58; P=0.42), (Table 3.7), yield appears to increase with increasing pH for the biosolids treatments and yield appears to decrease with increasing pH for the BS50:AH50 and BS75:AH25 treatments (Table 3.6).

### 3.4.7 pH and Above Ground Biomass

No statistically significant correlation was found between pH and above ground biomass for treatments with different ratios of biosolids ($R^2$=0.07; P=0.66), BS50:AH50 ($R^2$=0.42; P=0.23), and BS75:AH25 ($R^2$=0.13; P=0.55), (Table. 3.8).

### 3.4.8 pH and Below Ground Biomass

Below ground biomass weight generally appeared to decrease with increasing pH in treatments with different ratios of biosolids (Table 3.6). No statistically significant correlation ($R^2$=0.56; P=0.14) was found between pH and below ground biomass (Table 3.7).

Even though no statistically significant correlation ($R^2$=0.77; P=0.05) between pH and below ground biomass was found for treatments with different ratios of BS50:AH50 (Table 3.7), below ground biomass weight appeared to decrease with increasing pH.

There was a negative correlation ($R^2$=0.83; P=0.03) between pH and below ground biomass for treatments with different ratios of BS75:AH25 (Table 3.7). Below ground biomass weight decreases as pH increases (Table 3.6).

### 3.4.9 Bacterial Colony Forming Units and *M. hapla* Eggs

No statistically significant correlation ($R^2$=0.31; P=0.33) between bacterial CFU and *M. hapla* eggs was found for the biosolids treatments (Table 3.7).

No statistically significant correlation ($R^2$=0.84; P=0.16) between bacterial CFU and *M. hapla* eggs was found for treatments with different ratios of BS50:AH50 (Table 3.7). However, fewer eggs were found in treatments with higher numbers of bacterial CFU (Table 3.6). It is possible that some form of biocontrol agents were favored by the addition of biosolids but these were not identified.

Even though no statistically significant correlation ($R^2$=0.79; P=0.21) was found between bacterial CFU and *M. hapla* eggs for treatments with different ratios of BS75:AH25 (Table 3.7), fewer eggs were found in treatments with higher numbers of bacterial CFU (Table 3.6).

### 3.4.10 Fungal Colony Forming Units and *M. hapla* Eggs

No statistically significant relationship ($R^2$=0.14; P=0.87) between fungal CFU and *M. hapla* eggs was found for the biosolids treatments (Table 3.7).

Even though no statistically significant relationship ($R^2$=0.53; P=0.16) between fungal CFU and *M. hapla* eggs was found for the BS50:AH50 treatments (Table 3.7), fewer eggs were collected from samples with higher fungal CFU (Table 3.6).

No statistically significant relationship ($R^2$=0.35; P=0.65) between fungal CFU and *M. hapla* eggs was found for the BS75:AH25 treatments (Table 3.7).

### 3.4.11 Tomato Yield

#### 3.4.11.1 Control Treatments

Average tomato yield for the control treatments ranged from 1700-2658 g/plant (Fig. 3.33). Control with fertilizer and no nematodes produced the highest yield. No significant differences in yield among control treatments were found. However, yield increased for controls without fertilizer in the presence of

nematodes and the opposite trend was noted for the controls with fertilizer when nematodes were added.

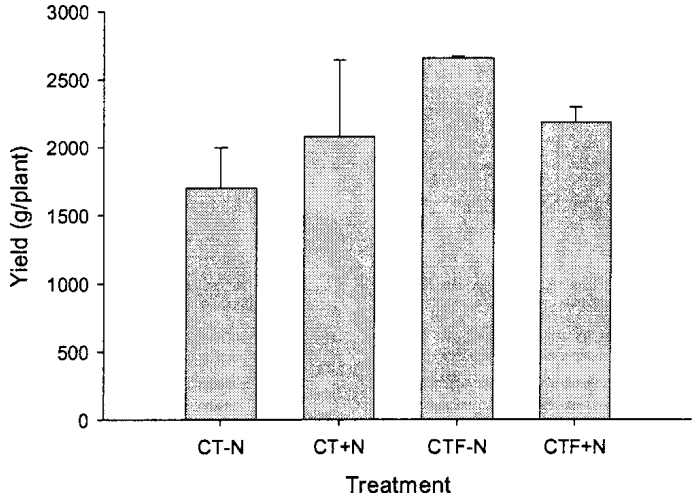


**Fig. 3.33 Tomato yield in control treatments (CT-N= Control without *M. hapla* inoculum; CT+N= Control with *M. hapla* inoculum; CTF-N= Fertilized control without *M. hapla* inoculum; CTF+N= Fertilized control with *M. hapla* inoculum); (n=3, values +/- SE). Summer 2008.**

### 3.4.11.2 Biosolids Treatments

Average tomato yield for the biosolids treatments ranged from 2911-3548 g/plant (Fig. 3.34). BS-6 produced the highest yield, representing a 33.5% increase in yield when compared to the highest control, which had a yield of 2658 g/plant. No significant differences in yield among BS treatments were found but there does appear to be a general decrease in yield with increasing application.

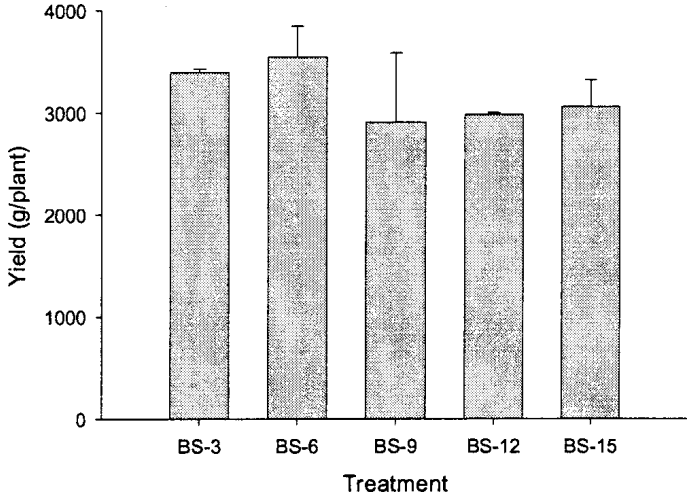


**Fig. 3.34 Tomato yield in amendments (BS-3= soil+3% biosolids; BS-6=soil+6% biosolids; BS-9=soil+9% biosolids; BS-12=soil+12% biosolids; BS-15=soil+15% biosolids); (n=3 values +/-SE). Summer 2008.**

### 3.4.11.3 BS50:AH50 Treatments

Average tomato yield for the BS50:AH50 treatments ranged from 2668-3397 g/plant (Fig. 3.35). Treatment A50-12 produced the highest yield, representing a 27.8% increase in yield when compared to the highest control, which had a yield of 2658 g/plant. No significant differences in yield among BS50:AH50 treatments were found but there is a general increase in yield with increasing amendment.

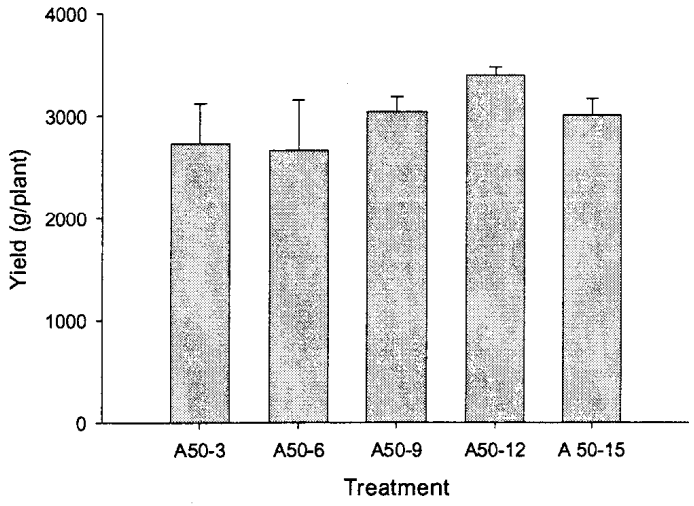


**Fig. 3.35 Tomato yield in amendments (A50-3= soil+3% of a 50% biosolids: 50% fly ash mix; A50-6=soil+6% of a 50% biosolids: 50% fly ash mix; A50-9=soil+9% of a 50% biosolids: 50% fly ash mix; A50-12=soil+12% of a 50% biosolids: 50% fly ash mix; A50-15=soil+15% of a 50% biosolids: 50% fly ash mix); (n=3, values +/- SE). Summer 2008.**

### 3.4.11.4 BS75:AH25 Treatments

Average tomato yield for the BS75:AH25 treatments ranged from 2459-3735 g/plant (Fig. 3.36). Treatment A75-15 produced the highest yield, representing a 40.5% increase in yield when compared to the highest control, which had a yield of 2658 g/plant. No significant differences in yield among BS75:AH25 treatments were found and no general trend was noticed.

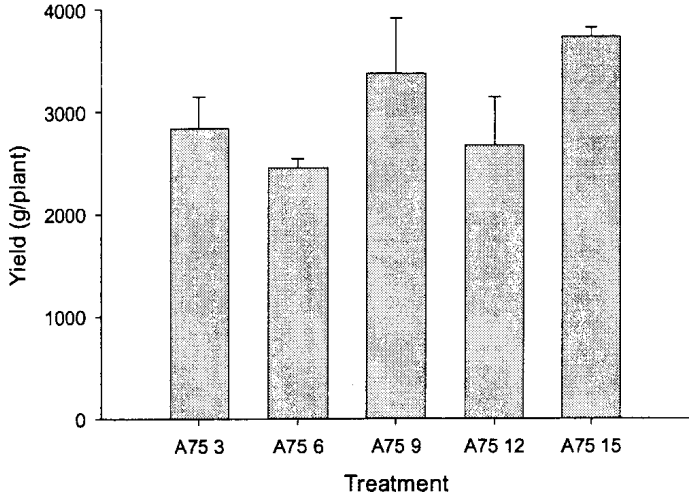


**Fig. 3.36 Tomato yield in amendments (A75-3= soil+3% of a 75% biosolids: 25% fly ash mix; A75-6=soil+6% of a 75% biosolids: 25% fly ash mix; A75-9=soil+9% of a 75% biosolids: 25% fly ash mix; A75-12=soil+12% of a 75% biosolids: 25% fly ash mix; A75-15=soil+15% of a 75% biosolids: 25% fly ash mix); (n=3, values +/- SE). Summer 2008.**

## 3.5 Impact of Biosolids and Fly Ash on *Meloidogyne hapla* and Tomato Plants Under Greenhouse Conditions (2008)

Average data for each amendment were used to determine the impact of the amendment on soils and nematodes. The composition of each amendment is given in Table 3.8. Average data collected for the 2008 greenhouse trials and from which figures in this section are made, are included in Table 3.9. Raw data are shown in Appendix 9.

**Table 3.8 Composition of amendments used in the 2008 greenhouse experiments.**

| Treatment | Composition |
|---|---|
| CT | Soil only |
| CTF | Soil + fertilizer |
| AH | Average of fly ash treatments for 2.5, 5.0, 7.5, 10.0, and 12.5% (w/w) fly ash added to soil |
| BS | Average of biosolids treatments for 2.5, 5.0, 7.5, 10.0, and 12.5% (w/w) biosolids added to soil |
| A50 | Average of 2.5, 5.0, 7.5, 10.0, and 12.5% (w/w) of an amendment made of 50% biosolids and 50% fly ash |
| A75 | Average of 2.5, 5.0, 7.5, 10.0, and 12.5% (w/w) of an amendment made of 75% biosolids and 25% fly ash |

**Table 3.9 Average data collected for various treatments on *Meloidogyne hapla* (eggs/g of fresh root); bacterial and fungal colony forming units (CFU/g of dry soil) at baseline and end; pH; electrical conductivity (µS/cm) at baseline and end; above ground biomass (g); below ground biomass (g). (n=15 for AH, BS, A50, and A75; n=12 for CT, P values for differences among treatments). Spring 2008.**

| | Baseline | | | | End | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Treat.* | pH | EC | Bacterial CFU*$10^6$ | Fungal CFU*$10^4$ | pH | EC | Bacterial CFU*$10^6$ | Fungal CFU*$10^4$ | *M. hapla* eggs | Above ground mass | Below ground mass |
| CT | 8.00 | 512 | 1.2 | 5.3 | 8.23 | 175 | 1.2 | 2.8 | 6184 | 18.5 | 5.3 |
| AH | 8.01 | 871 | 1.0 | 4.4 | 8.32 | 261 | 1.1 | 3.1 | 0 | 13.2 | 11.7 |
| BS | 8.09 | 1016 | 1.7 | 6.3 | 8.09 | 375 | 1.8 | 4.1 | 9818 | 29.6 | 14.4 |
| A50 | 7.87 | 457 | 1.9 | 4.8 | 8.33 | 331 | 1.9 | 3.4 | 26375 | 23.5 | 6.9 |
| A75 | 8.05 | 930 | 1.6 | 5.5 | 8.05 | 388 | 2.6 | 3.1 | 12461 | 28.1 | 10.8 |
| P value | <.001 | <.001 | <.001 | .014 | <.001 | <.001 | <.001 | >.05 | <.001 | <.001 | <.001 |

* Description of each treatment is given in Table 3.8

### 3.5.1 *Meloidogyne hapla*

Average number of *M. hapla* eggs ranged between 0-26375 eggs/g of fresh root. No eggs were recovered from fly ash treatments. The highest number of eggs was recovered from A50 treatments followed by A75, BS, and control (Fig. 3.37). Significant differences among treatments were found (F=13.8, df=4,71, *P*<0.001).

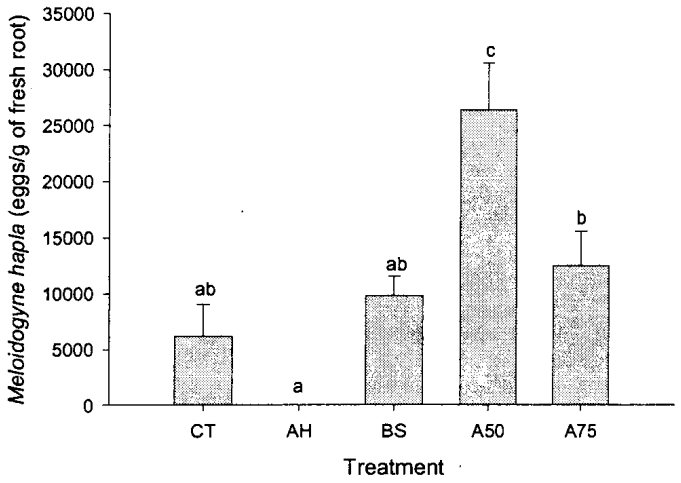


**Fig. 3.37 *Meloidogyne hapla* eggs in amendments (CT= soil; AH= fly ash; BS= biosolids; A50= 50: 50 mixture of biosolids and fly ash; A75= 75: 25 mixture of biosolids and fly ash) at the end of the experiment; (n=15 for AH, BS, A50, and A75; n=12 for CT, values +/-SE). Spring 2008.**

### 3.5.2 Electrical Conductivity

Electrical conductivity at baseline ranged between 457-1016 µS/cm with the highest found in the BS treatments (Fig. 3.38, "Baseline"). At the end of the experiment, EC ranged from 175-388 µS/cm, with the highest found in the A75 treatments (Fig. 3.38, "End"). Significant differences among treatments were found at baseline (F=32.4, df=4,71, $P$<0.001) and end (F=7.7, df=4,71, $P$<0.001).

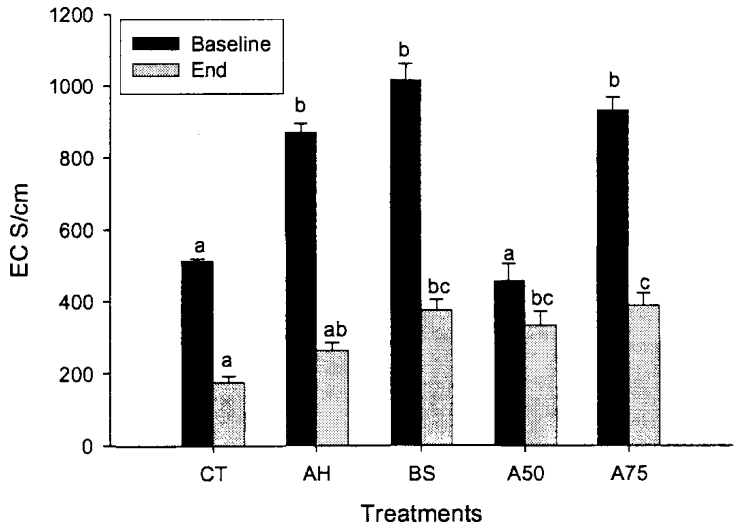


**Fig. 3.38 Electrical conductivity of amendments (CT= soil; CTF= soil+fertilizer; AH= fly ash; BS= biosolids; A50= 50: 50 mixture of biosolids and fly ash; A75= 75: 25 mixture of biosolids and fly ash); (n=15 for AH, BS, A50, and A75; n=12 for CT, values +/- SE) at 2 sampling times (Baseline and End). Spring 2008.**

### 3.5.3 pH

The pH values at baseline ranged between 7.87-8.01 and at harvest from 8.05-8.33. Generally, pH did not vary appreciably with amendment addition (Fig. 3.39). Significant differences among treatments were found at baseline (F=31.6, df=4,71, $P$<0.001) and end (F=24.7, df=4,71, $P$<0.001).

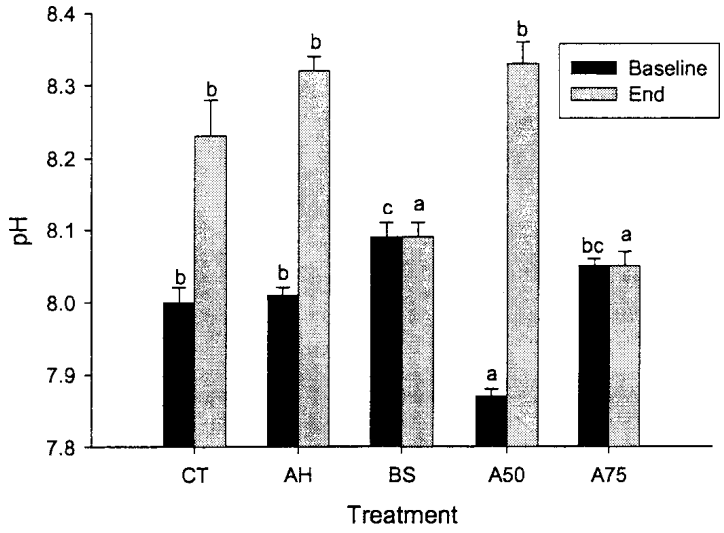


**Fig. 3.39 pH of amendments (CT= soil; CTF= soil+fertilizer; AH= fly ash; BS= biosolids; A50= 50: 50 mixture of biosolids and fly ash; A75= 75: 25 mixture of biosolids and fly ash); (n=15 for AH, BS, A50, and A75; n=12 for CT, values +/- SE) at 2 sampling times (Baseline and End). Spring 2008.**

### 3.5.4 Bacterial Colony Forming Units

Average numbers of bacterial colony forming units at baseline ranged between 1.0-1.9*$10^6$ CFU/g if dry soil. The highest number of CFU was found in the A50 treatments (Fig. 3.40, "Baseline"). At the end of the experiment, bacterial CFU ranged 1.1-2.6*$10^6$ CFU/g of dry soil, with the highest number of CFU found in the A75 treatments (Fig. 3.40, "End"). Significant differences among treatments were found at baseline ($F=16.2$, df=4,71, $P<0.001$) and end ($F=13.3$, df=4,71, $P<0.001$).

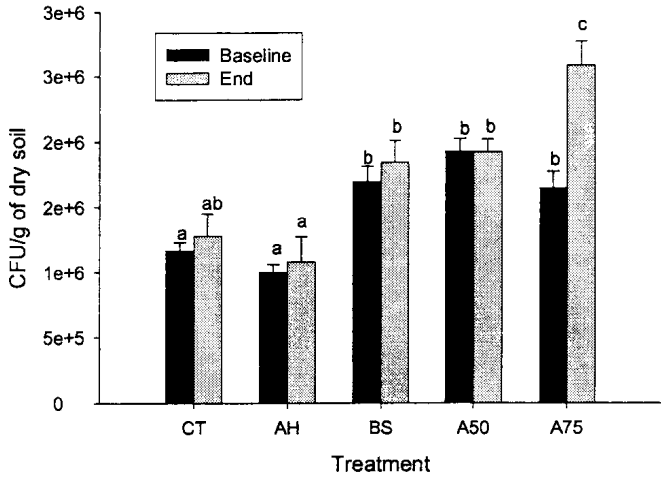


**Fig. 3.40 Bacterial colony forming units in amendments (CT= soil; CTF= soil+fertilizer; AH= fly ash; BS= biosolids; A50= 50: 50 mixture of biosolids and fly ash; A75= 75: 25 mixture of biosolids and fly ash); (n=15 for AH, BS, A50, and A75; n=12 for CT, values +/- SE) at 2 sampling times (Baseline and End). Spring 2008.**

### 3.5.5 Fungal Colony Forming Units

Fungal colony forming units at baseline ranged between 4.4-6.3*$10^4$ CFU/g of dry soil. At the end of the experiments, fungal CFU ranged between 2.8-4.1*$10^4$ CFU/g of dry soil. The highest number of fungal CFU was found in the BS treatments at both sampling times (Fig. 3.41). Significant differences among treatments were found at only at baseline ($F=3.4$, $df=4,71$, $P=0.014$).

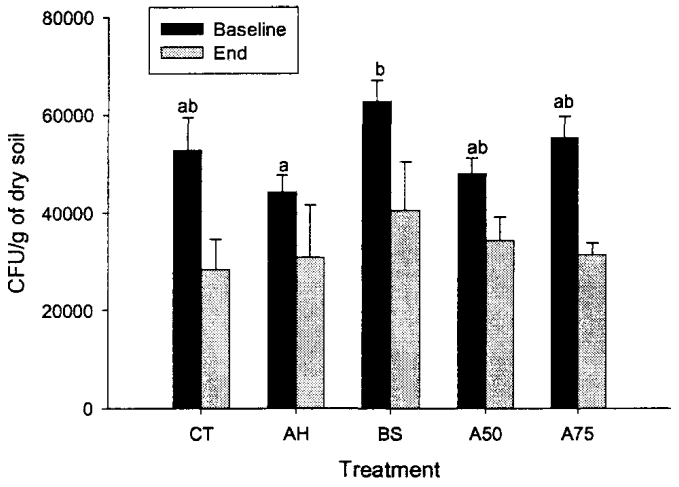


**Fig. 3.41. Fungal colony forming units in amendments (CT= soil; CTF= soil+fertilizer; AH= fly ash; BS= biosolids; A50= 50: 50 mixture of biosolids and fly ash; A75= 75: 25 mixture of biosolids and fly ash); (n=15 for AH, BS, A50, and A75; n=12 for CT, values +/- SE) at 2 sampling times (Baseline and End). Spring 2008.**

### 3.5.6 Above and Below Ground Biomass

Weight of above ground biomass ranged between 13-30 g and below ground biomass weight ranged between 5-14 g. The highest weights for both variables were found in the BS treatments. All treatments, except for AH, outperformed CT. Significant differences among treatments were found for above ($F=12.1$, $df=4,71$, $P<0.001$) and below ground biomass ($F=13.1$, $df=4,71$, $P<0.001$).

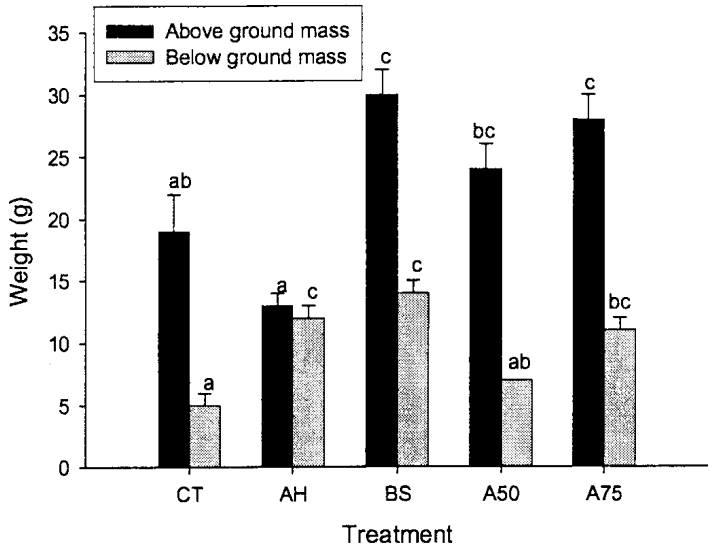


**Fig. 3.42. Above and below ground biomass of tomato plants grown in amendments (CT= soil; CTF= soil+fertilizer; AH= fly ash; BS= biosolids; A50= 50: 50 mixture of biosolids and fly ash; A75= 75: 25 mixture of biosolids and fly ash); (n=15 for AH, BS, A50, and A75; n=12 for CT, values +/- SE). Spring 2008.**

# 4 DISCUSSION

## 4.1 Impact of Biosolids and Fly Ash on Natural Nematode Populations, *Meloidogyne hapla,* and Carrot Yield (2006 and 2007)

It should be noted that results from the experiments had large variability and this is due in part to the lack of replication that the treatments had. Any work related with nematode research should have fewer treatments and more replication in order to compare results among treatment ratios properly.

### 4.1.1 Bacterial Feeding Nematodes

In the 2006 field season, no significant differences among treatments for BFN populations were found when all treatments were included in the statistical analyses. However, higher BFN populations were found in the middle of the growing season for the AH, A50, and A75 treatments when compared to the BS and control treatments (Fig. 3.1, "Middle"). This finding suggests that higher BFN populations might be related to increases in the addition of ash rather than biosolids for the period of this study; results might vary for longer study periods. BFN populations for the CTF treatments reaching similar levels as AH, A50, A75 cannot be explained in the same way. This might be accounted for by the influx of nutrients from chemical fertilizers affecting the microbial biomass in these treatments, causing BFN to increase. At the end of this experiment, similar BFN populations found for the BS, A50, A75, and CTF treatments strongly suggests that concentrations of amendments were too low to cause significant changes in BFN populations (Fig. 3.1, "Harvest"). In the present study, ash or biosolids treatments contained the highest levels of the metals in question (Table 3.3) relative to control soils. However, the increased concentrations due to the application of ash and biosolids were significantly lower than those reported by other authors to impact nematode populations.

Bacterial feeding nematode populations for the 2007 field season were significantly different in the middle of the experiment and at second harvest. The increases in BFN populations in the middle of the growing season for the BS, A50, and A75 treatments might be more closely related to the addition of biosolids rather than ash, contradicting the results obtained in the 2006 season (Fig. 3.8, "Middle"). Weiss and Larink (1991) suggested that the increase in BFN populations found in the sewage sludge-amended treatments was due to an increased nutrient content that led to an increase in microbial biomass. This is in agreement with the results obtained by Dmowska and Kozlowska (1988) and coincides with the results from the 2007 field season. During the first harvest, BFN populations in all treatments showed an overall decline with no significant differences among treatments. Significant differences among populations of BFN were found at second harvest with AH, A50, and A75 having similar levels, while populations of BFN for the BS treatments were significantly higher (Fig. 3.8, "2nd. Harvest"). As seen in the 2006 field season, BFN populations for the 2007 season decreased dramatically in the ash treatments toward the end of the experiment. Similar trends were found in a greenhouse experiment where Mitchell et al. (1978) found higher BFN populations at the beginning of the test, declining toward the end. Higher populations were associated with anaerobically digested sludges with heavy metals in the range between 250-2600 mg $kg^{-1}$ Zn, 150-500 mg $kg^{-1}$ Cu, 3-43 mg $kg^{-1}$ Cd, 60-500 mg $kg^{-1}$ Cr, 200-1400 mg $kg^{-1}$ Pb, and 28-201 mg $kg^{-1}$ Ni. The same considerations regarding heavy metal concentrations reported for the 2006 field season can be applied to the 2007 season, where concentrations of heavy metals in the mixtures might not have been high enough to influence important changes in BFN populations.

Biosolids and ash used for amendment preparation were the same for all experiments (2006, 2007, 2008). Changes in microbial communities from year-to-year due to changes in the biosolids and ash might explain the variation in nematode taxa for the 2006 and 2007 field seasons. Soil used for all experiments was collected around the same time of the year from the same plot at the ESW

field station. Since baseline BFN populations found in the control treatments for the 2006 and 2007 experiments were similar, it is likely that the amendments caused the change in their numbers. It should be noted that little variation would be expected in the control treatments but since new media were produced in 2006 and 2007, differences in their composition may have caused differences in BFN populations.
Metals may be directly toxic to nematodes or may indirectly affect their populations by reducing plant growth.

Some of the important elements that affect nematode populations include As, Cd, Cr, Cu, Ni, Pb, and Zn. Bardgett et al. (1994) found that microbial respiration decreased and bacterial feeding nematode (BFN) populations increased as Cu, Cr, and As increased for a stony silt loam at pH 5.7. For a loamy, moderately fine sandy soil, Korthals et al. (1996a) found that at pH of 4-4.7 and 125 mg $kg^{-1}$ Cu, total nematode populations decreased; additionally, lower pH and higher levels of Cu caused BFN populations to decrease. In the same study, they postulated that Cu and Cd enter the nematode through the cuticle due to increased osmotic pressure causing BFN to die. Another study by Korthals et al. (1996b) found that concentrations of 1600 mg $kg^{-1}$ Cu applied to a sandy soil resulted in increases of BFN relative to other species. The difference between the last two experiments is that the first looked at the long-term effects and the latter at short-term effects, suggesting that exposure time to the contaminant caused BFN to react differently. Differences between these two experiments cannot be related to soil texture because they were carried out in the same field area. Weiss and Larink (1991) reported that BFN populations increased dramatically with the addition of sewage sludge and/or sewage sludge with metals added for a loamy sandy soil at pH 6.4; where total nematode populations and bacterial feeding nematodes increased from 1729-7216 and 318-2950/100 g of soil, respectively. In another experiment (Georgieva et al., 2002) that used sludge with metals added (Ni, Cu, and Zn), total BFN populations were 16% and 21% higher in treatments to which Ni and Zn had been added, respectively. In the latter study, it

was suggested that the increase in BFN populations might be due to the wide range of life strategies exhibited by these nematodes, allowing them to adapt to polluted environments. Several studies have found that different concentrations of Cd, Cr, Se, and Zn reduce BFN populations because the bacteria they feed on adsorbed enough metals during their life cycle to make them undesirable as food (Bisessar, 1981; Doelman et al., 1984; Bouwman et al., 2005; Smit et al., 2002; Korthals et al., 1998; Bakonyi et al., 2003). In a lab assay, Doelman et al. (1984) showed that the addition of 2-20 mg $kg^{-1}$ Cd and 20-1000 mg $kg^{-1}$ Pb to growth media lead to the adsorption of the metals onto bacteria, which resulted in a reduction of BFN. In an experiment involving sludge application to a gravelly loam at pH of 7.2-7.6, Mannion et al. (1994) reported very little variation in BFN populations, suggesting that at mildly basic pH levels, the influence of sludge to BFN is insignificant.

Georgieva et al. (2002) found that soils that have received heavy metals go through changes that might alter the microbial community, favoring a few species and reducing the positive interactions among them.

### 4.1.2 Fungal Feeding Nematodes

In the 2006 field season, the overall population of FFN appeared to be lower as a result of the addition of fly ash and biosolids alone (Fig. 3.2). The application of combined fly ash and biosolids caused FFN populations to reach levels comparable to that of the control treatments. Significant differences in FFN populations were found at harvest where treatments with BS only had the lowest populations, while AH, A50, and A75 had similar population levels, and control treatments had the highest populations (Fig. 3.2, "Harvest"). It would appear that FFN experienced changes associated with application of ash and sludge alone and in combination. These changes can be attributed to the increased nutrient availability resulting from amendment application, which in turn caused fungal populations to change, ultimately affecting FFN. The pH levels in the present

study were slightly basic, resulting in different interactions among microorganisms and promoting the increase of FFN in the fly ash-biosolids and control treatments. However, higher levels of FFN found in the control treatments suggest that this taxon would benefit over time in environments that have had mild or no disturbances. Overall, FFN populations did not change dramatically with the addition of the amendments, suggesting that the levels in this study were not large enough to cause changes in FFN populations. In this study, the highest levels of As, Cd, Cr, Cu, Ni, Pb, and Zn were 0.9, 0.13, 0.35, 2.4, 0.5, 2.2, and 3.3 mg $kg^{-1}$, respectively (Table 3.3), being significantly lower than the levels reported in other studies.

In the 2007 field season the FFN populations remained quite stable among treatments within sampling times. Significant differences found only in the middle of the growing season show higher populations of FFN in the fertilized control, AH, and BS treatments, while the combination of ash and biosolids had lower populations (Fig. 3.9, "Middle"). The same consideration regarding biosolids and ash used for mixture preparation (Section 4.1.1) can be applied to baseline levels of naturally occurring FFN populations for the 2007 season. These populations might have behaved differently from the populations found in the 2006 field season because the biosolids used in the mixtures were one year older. Stored biosolids experience organic matter decay with time, and this may influence the mobility and the availability of elements (McBride et al., 1997). The changes in the stored biosolids might have caused changes in nutrient availability, which in turn caused microbial populations to differ from year to year, ultimately affecting nematode populations.

Similarly to BFN populations, fungal feeding nematode (FFN) populations are controlled by direct and indirect effects. In the experiment by Korthals et al. (1996a) higher FFN populations were found under the same soil conditions described previously (Section 4.1.1). In another experiment using the same soil, Korthals et al. (1998) found that at 400 mg $kg^{-1}$ each of Cu and Zn, there was an

increase in FFN. The authors suggest that higher concentrations of Cu and Zn and lower pH cause bacterial biomass to decrease, favoring fungal growth. This shift in microbial communities related to pH change was shown in the experiment conducted by Rousk et al. (2009). Additionally, there is reduced food competition and predation for FFN, causing an increase in their populations. Smit et al. (2002) and Nagy (1999) found that at 1800 mg $kg^{-1}$ Zn and 270 mg $kg^{-1}$ Cu, few species of FFN increased. Contrasting with the results from Nagy (1999), a long-term study by Bakonyi et al. (2003) showed a reduction in FFN at a total of 270 mg $kg^{-1}$ Cd, Cr, and Zn in different proportions. Georgieva et al. (2002) showed that low levels of Ni (19 mg $kg^{-1}$), Zn+Ni (97 and 16.5 mg $kg^{-1}$, respectively), and Zn +Cu (108.9 and 67.6 mg $kg^{-1}$, respectively) resulted in an increase in FFN. In a short-term study discussed previously (Section 4.1.1), the results reported by Korthals et al. (1996b) showed a reduction in FFN relative to other species. Bisessar (1981) (Section 4.11) reported that concentrations of Pb, As, Cd, and Cu of 3564, 163, 26, and 333 mg $kg^{-1}$, respectively, decreased FFN populations. In the lab assay by Doelman et al. (1984) described in section 4.1.1, levels of Cd and Pb ranged between 1-25 mg $kg^{-1}$ and 10-250 mg $kg^{-1}$, respectively. The latter authors have suggested that the reduction in FFN is due to the absorption of heavy metals by fungal hyphae, which make this food source toxic, causing the nematodes to stop feeding. Furthermore, high heavy metal concentration in the soil reduces fungal biomass, reducing food availability. These studies were carried out in the pH range between 4.5-6.4, suggesting that extreme pH levels enhance heavy metal effects. In the study by Mannion et al. (1994), described above (Section 4.1.1) FFN populations remained stable.

### 4.1.3 Plant Feeding Nematodes

In the 2006 field season of this study, populations of PFN remained stable from the beginning to the end of the experiment with significant differences among treatments found only in the middle of the growing season (Fig. 3.3, "Middle"). At this sampling period, the highest PFN populations found in the CT and A75

treatments are probably not related to increased plant biomass produced in the A75 treatments when compared to the control. Even though significant differences were not found at the end of the experiment, PFN populations appear to decrease in the treatments that yielded higher plant biomass.

No significant differences among treatments were found for PFN populations for the 2007 field season at any of the sampling times (Fig. 3.10). However, the apparent decrease found in PFN populations seems to be related to amendment addition. At every sampling time, the highest populations of PFN were found in the control treatments. This is somewhat similar to the results found in the 2006 season, where PFN populations might have reached the population balance discussed previously.

Plant feeding nematode (PFN) populations seem to be more affected by indirect rather than direct effects of the amendments. Weiss and Larink (1991) reported higher PFN populations in plots that had higher plant biomass when sludge and heavy metals were added to a loamy sandy soil at pH 6.4. Similar results were found by Bouwman et al. (2005) and Georgieva et al. (2002), where higher PFN populations were associated with higher levels of Cd and Zn. Bouwman et al. (2005) suggested that PFN kept feeding on the roots of plants with high levels of heavy metals because metal content was lower in the root than the shoot. Additionally higher Zn contents, which reduced plant biomass, might have also reduced PFN antagonists including root nodule bacteria, mycorrhizal fungi, and predatory nematodes. Bakonyi et al. (2003, see Table 3 for list) found higher PFN populations associated with improved plant growth in plots that received 270 mg $kg^{-1}$ Zn. It has also been shown that intermediate levels of Zn (50-200 mg $kg^{-1}$) applied to a sandy loam at pH of 4.1 caused PFN populations to increase, suggesting that higher root leakage diminished the plant's defense mechanism (Korthals et al., 1998). In a calcareous loamy chernozem with a pH of 7.4, Nagy (1999) found that plots that received a total of 270 mg $kg^{-1}$ Ni and Zn at different ratios had higher wheat yield and higher PFN populations. In the same study,

228 mg $kg^{-1}$ Cd and 10 mg $kg^{-1}$ Cr proved to be phytotoxic, reducing availability of wheat as a food source for PFN. These results suggest that PFN populations are more closely related to food supply and not directly to concentrations of heavy metals. Under experimental conditions described previously (Section 4.1.1), Mannion et al. (1994) found that low applications of sludge (8-24% w/w) did not have an effect on PFN populations.

### 4.1.4 *Meloidogyne hapla*

Inoculum levels for the 2007 field season were 105 nematodes/100 $cm^3$, being well within the range of lowered carrot weight and length as shown by Vrain (1982). The highest numbers of nematode eggs were recovered in the first harvest from secondary roots of BS, A50, and A75 treatments (Fig. 3.11). These are the treatments that had the highest yields, relating higher *M. hapla* infection levels with treatments with higher plant biomass. Even though inoculum levels used in the 2007 field season were in the range proposed by Vrain (1982), he used infested roots and soil as inoculum directly, while the inoculum for the present study was obtained with 1% NaOCl solution (Hussey and Barker, 1973). The inoculum for the 2007 field season included both eggs and live (as determined in the laboratory) juveniles (12600 and 2400/pot respectively) improving the chances of carrot infection. It is possible that exposure of eggs and juveniles to NaOCl reduced their hatchability and infectivity even though positive infection in secondary roots of carrots was confirmed with egg extraction after the growing season.

The severity of the damage caused by *M. hapla* might depend on some soil parameters as well as tolerance to nematode attack and crop cycle. As discussed earlier, certain levels of heavy metals can affect plant feeding nematode populations by making their food supply more scarce or abundant, which in turn causes population changes of other nematode taxa.

Bisessar et al. (1983) found that celery plants grown in soils with 7500 mg $kg^{-1}$ Ni and 800 mg $kg^{-1}$ Cu and inoculated with *M. hapla* juveniles had the lowest shoot weight and height when compared to control soils with no heavy metal addition or nematode inoculation. These celery plants showed the highest level of galled roots and the authors suggested that high levels of Ni enhance infestation levels of *M. hapla* in these muck soils. Levels of Ni (0.5 mg $kg^{-1}$) and Cu (2.4 mg $kg^{-1}$) for the 2007 field season (Table 3.3) were extremely low when compared to those reported by Bisessar et al. (1983), suggesting that infestation of carrots by *M. hapla* in the 2007 season was not influenced by the two heavy metals in question. In an *in vitro* hatching assay, Parveen and Alam (1999) found that Pb and Cd inhibited the hatching of *M. incognita* eggs at concentrations of 60 mg $kg^{-1}$. Levels of Pb (2.2 mg $kg^{-1}$) and Cd (0.1 mg $kg^{-1}$) for the 2007 field season (Table 3.3) were substantially lower than those reported by those authors, therefore they cannot be compared to that study.

Because *M. hapla* is a sedentary endoparasite, it relies exclusively on its plant host for nutrition. Initial parasite densities might cause the crop to respond differently to infection based on its threshold. Vrain (1982) suggests that for carrots, a level as low as 40 nematodes/100 $cm^3$ of soil is enough to cause loses associated with carrot quality. The author experimented with different *M. hapla* inoculation levels on carrots under field conditions, and found that root length and weight decreased as inoculum level increased. The inoculum levels used by Vrain (1982) ranged between 20-240 nematodes/100 $cm^3$ of soil.

### 4.1.5 Electrical Conductivity

Soil electrical conductivity can be an important predictor of soil biological activity, affecting important soil processes related to physical and chemical interactions. Such interactions can influence nematode activity in soils in relationship with plant development and microbial communities. In a report by Vellidis et al. (2006), soil electrical conductivity was used in combination with nematode

sampling to correlate areas of specific EC with root-knot nematode abundance. The authors reported that lower populations of nematodes (4-115 nematodes/unit of soil) were found in areas with EC in the range of 27.5-100.0 µS/cm. Nkem et al. (2006) reported no nematode survival in soil samples with EC >4100 µS/cm, while at 1945 µS/cm they reported 80-97% survival rates. These researchers suggest that different species of nematodes can tolerate different levels of salinity by entering into a state of osmobiosis. Electrical conductivity levels from the present study (2006 field season) ranged between 165-656 µS/cm (Fig. 3.4), which is within the range for optimal nematode activity as determined by other authors. Electrical conductivity levels found for the 2007 field season ranged between 106-1050 µS/cm (Fig. 3.12), which is also within the optimal range.

### 4.1.6 pH

Several studies (Korthals et al., 1996a; Korthals et al., 1996b; Korthals et al., 1998; Korthals et al., 2000; Bardgett et al., 1994; Bouwman et al., 2005; Burns, 1970) have demonstrated that pH values in the range of 4.1-6.0 might enhance the direct and indirect effects that influence changes in nematode community structure. The combination of direct and indirect effects might favor one or more nematode feeding groups based on increased availability of food or reduced predation from omnivorous or predatory nematodes, which appear to be highly sensitive to heavy metals. Other effects from heavy metals, enhanced by extreme pH levels, might be higher bioavailability of metals that cause a reduction in plant, bacterial, or fungal biomass, influencing shifts in nematode populations from different feeding groups. In the experiment by Weiss and Larink (1991) described in Section 4.1.1, the authors found increased nematode populations from all feeding groups. Populations of bacterial, fungal, and plant feeding nematodes remained stable throughout the experiment by Mannion et al. (1994) described in Section 4.1.1. Levels of pH from the 2006 season soil samples range between 7.7-8.1 (Fig. 3.5), which is close to those reported by the

latter authors. Furthermore, pH did not change enough in this study to be able to relate changes to differences in nematode populations.

Levels of pH from the 2007 field season soil samples ranged between 7.7-8.4 (Fig. 3. 13), which is comparable to levels found in the 2006 field season. Even though significant differences were found among treatments at baseline and first harvest, the pH values among treatments are so close together, that there is probably no clear interaction between nematodes populations and pH. It is important to note that the highest pH levels were found in the control treatments, which was expected since calcareous soils in Ontario usually have pH levels higher than 8. The experiments conducted in 2006 and 2007 included treatments with biosolids and ash mixtures that had pH values above 7.5 and still yielded marketable carrots that typically grow best at pH close to 6.5.

### 4.1.7 Soil Bacteria and Fungi

It appears that bacterial colony forming units for the present study were benefited by the combination of fly ash and biosolids addition as evidenced by the higher bacterial CFU found for the A50 and A75 treatments (Fig. 3.14). Fungal colony forming units were benefited by biosolids addition as evidenced by the higher fungal CFU found in the BS treatments at all sampling times (Fig. 3.15). Levels of heavy metals in the present study were adequate for an improved microbial community. This is in agreement with the literature where addition of organic matter to soils will benefit bacterial and fungal populations improving nutrient cycling and enhancing soil health (Riegel and Noe, 2000; Litterick et al., 2004).

As discussed previously (Sections 4.1.1, 4.1.2. and 4.1.3), application of biosolids containing heavy metals has an effect on soil microbial communities. Bacterial and fungal colony forming units are also affected by the same parameters that affect nematodes, favoring or inhibiting their growth and their role as decomposers. In a soil medium that received anaerobically digested

sewage sludge with different concentrations of Cd (0.1-111 mg $kg^{-1}$), Cu (11-556 mg $kg^{-1}$), and Cr (1-556 mg $kg^{-1}$), Zibilske and Wagner (1982) reported an initial positive response in bacterial and fungal activity that declined over time and even exhibited inhibition at the end of the incubation period. Microbial populations exhibited these changes at different incubation times depending on the metal added (2 weeks for Cd and Cr, and 1 week for Cu). Additionally, the authors suggest that the higher tested levels of Cd and Cr affect fungal sporulation favoring some species and inhibiting others. The experimental levels of the selected heavy metals from that study are similar to those found in some sludges, presenting a realistic approach to show reduction in microbial activity fundamental to sludge decomposition. A study by Schutter and Fuhrmann (2001) showed that application of 25% of fly ash to soils might benefit fungi and some bacteria. They determined this by measuring whole-soil fatty acid content, which indicated that these populations were enhanced in a soil system that had improved plant growth and nutrient content as a result of fly ash application. The heavy metal levels in the soil amended with fly ash used by the latter authors were 10.00 mg $kg^{-1}$ As, 5.20 mg $kg^{-1}$ B, 0.03 mg $kg^{-1}$ Cd, 1.00 mg $kg^{-1}$ Cr, 1.20 mg $kg^{-1}$ Cu, 0.70 mg $kg^{-1}$ Ni, and 0.50 mg $kg^{-1}$ Pb, which in some cases is higher and in some cases lower than those used in the 2007 field season from the present study (Table 3.3).

### 4.1.8 Carrot Yield

Carrots harvested in the 2006 field season had a yield increase of 120% for BS, 51% for A50, and 100% for A75 treatments, respectively, when compared to the fertilized controls (CTF). The yield for the AH and CT treatments was 56% and 27% lower than the fertilized controls (Fig 3.6). Above ground biomass followed the same trend as carrot tap root (Fig. 3.7). Addition of biosolids, and to a lesser extent fly ash, resulted in improved soil texture and nutrient availability, which had a positive effect in carrot yield.

Carrots harvested in the 2007 field season did not show visible nematode attack even though plots were inoculated with the northern root-knot nematode (*M. hapla*) and the variety used is not known to be nematode resistant. Carrot infestation was previously discussed in Section 4.1.4.

As in the 2006 field season, the addition of biosolids and fly ash + biosolids amendments resulted in improved yields when compared to the fertilized controls. For the first harvest of the 2007 field season, there was an increase in yield of 52% for BS, 64% for A50, and 84% for A75 when compared to the fertilized controls. Fly ash (AH) and CT controls had 43% and 42% lower yields, respectively, when compared to the fertilized controls (Fig. 3.16). For the second harvest there was an increase of 144% for BS, 72% for A50, and 90% for A75 treatments when compared to the fertilized controls. Fly ash (AH) and CT controls had 55% and 74% lower yields than the fertilized controls (Fig. 3.17). Yield results for the 2007 field season are similar to the results from the 2006 season. Improved yield was obtained as a result of biosolids addition.

Recommendations by Fritz et al. (2006) for carrot cultivation include the selection of well-drained soils with a sandy loam texture in the pH range 5.5-7.0. Soil texture for the present study resembles a sandy loam in which drainage and organic matter were improved with increased biosolids application. Sterrett et al. (1982) reported phytotoxicity to several cropped plants at >500 mg $kg^{-1}$ Zn, >500 mg $kg^{-1}$ Mn, >25 mg $kg^{-1}$ Cu, and > 50 mg $kg^{-1}$ Ni.

Levels of these heavy metals in the present study were: 3.3 mg $kg^{-1}$ Zn, 72 mg $kg^{-1}$ Mn, 2.4 $kg^{-1}$ Cu, and 0.5 mg $kg^{-1}$ Ni, being much lower that those reported by the latter author. None of the carrots harvested in the 2006 field season showed visible nematode attack. *Meloidogyne hapla* was not found occurring naturally in the soil and probably the species of plant feeding nematodes that were present did not visibly affect the carrot roots. Additionally, the variety of carrot sown has a very short production cycle (63 days) and even though a typical nematode life

cycle is approximately 30 days under summer conditions, the carrots had no visible nematode damage.

## 4.2 Impact of Biosolids and Fly Ash on *Meloidogyne hapla* and Tomato Yield (2008)

Similarly to the considerations for the 2006 and 2007 field seasons, the 2008 field season should have included fewer treatments and more replication to reduce the variability.

### 4.2.1 Electrical Conductivity

Replicate data for *M. hapla* eggs and EC in the BS, BS50:AH50, and BS75:AH25 treatments for the 2008 field season can be found in Section 3.4. The average EC for each group of treatments ranged from 113-252 µS/cm, which is well below reported levels that will affect nematode activity. This would explain why average treatment results show little correlation between *M. hapla* eggs and EC for all treatments. However, there are subtle differences among replicates within each treatment group. A strong correlation between EC an M. hapla eggs occurs for the BS50:AH50 group except for the highest application rate (A50-15); no correlation occurred in the BS75:AH25 group, and a weak correlation occurs for the biosolids group. It should be noted that average number of *M. hapla* recovered from all groups was very small, ranging from 1440 for the biosolids to 2165 for BS50:AH50.

Soil electrical conductivity relates plant nutrition to availability of anions and cations in the soil matrix and can have both direct and indirect effects on nematode populations (Edongali et al., 1982). Electrical conductivity levels in this study (group treatment averages ranging from 137 in BS50:AH50 to 252 in biosolids) were much lower than reported in other studies and significantly lower when their potential impact on bacteria, fungi, tomato yield, and biomass are taken into account.

As discussed in Section 4.1.5, EC values that promote nematode activity are generally in the range 1945-4100 µS/cm. In a greenhouse experiment, Edongali et al. (1982) used EC values from 1500-5000 µS/cm to determine the infectivity of *M. incognita* to resistant and susceptible tomato cultivars. Their results show that at the higher EC level, *M. incognita* juveniles were less infective but this was closely related to host susceptibility.

Group average tomato yield for the 2008 field season was not significantly different for any group ranging from 2969 g/plant in BS50:AH50 to 3180 g/plant in BS. It should also be noted that there was little or no correlation among replicates within any treatment group. Other studies with higher levels of EC have shown the effect of EC on tomato yield. In a soil-less greenhouse experiment Li et al. (2001) showed that elevated EC levels reduced tomato yield due to smaller fruit production. The EC levels in the nutrient solution used in their experiment ranged between 6500-9000 µS/cm, with the most detrimental effect to tomato yield found with the highest EC levels. Sonneveld (1988) suggested an EC threshold value of 2500 µS/cm for tomato production. Campos et al. (2006) conducted a field experiment with an industrial tomato cultivar that received irrigation water with EC in the range between 1000-5000 µS/cm. They reported that the highest yield (3191g/plant) was obtained from tomato plants grown in the 1000 µS/cm treatments and the lowest yield (1538g/plant) obtained from tomato plants grown in the 5000 µS/cm treatments. It should be noted that for each of the treatment groups, the highest yield was obtained at a narrow range of EC; BS, EC of 180, yield of 3548 g/plant; A50, EC 150, yield 3397 g/plant; A75, EC 166, yield 3377 g/plant. These data are consistent with the work conducted by Campos et al. (2006).

### 4.2.2. pH

As discussed in Section 4.1.6, calcareous soils in Ontario are naturally basic. There was no significant difference in pH measured among replicates or

treatment groups during the 2008 field season. Furthermore, the range of pH noted (7.9-8.3) is not wide enough to have influenced changes for bacteria, fungi, *M. hapla* eggs, tomato yield, and biomass as shown by other authors.

Different species of plant-parasitic nematodes have optimum pH levels where they reach maximum infectivity. For *Pratylenchus penetrans* the range is between 5.2-6.2 (Wills, 1972); for *Meloidogyne incognita* it is 6.5 (Loewenberg et al., 1960); *Heterodera glycines* reproduced better at pH values of 6.5 and 7.5 (Anand et al., 1995). It is possible that *M. hapla* populations have adapted to different soil environments and different pH regimes. Melakerberhan et al. (2007) proposed this idea when they tested low application rates of N-Viro soil applied to different soil types that had received chemical inputs to improve fertility and reduce *M. hapla* populations. Their results suggest that there is an optimal pH level that maximizes nematode reproduction, and it varies depending on how long the nematode population has been exposed to that environment. As noted in these studies, nematodes favor pH below 6.5, which is much lower than measured in any of the samples in this study.

Rogovska et al. (2009) reported that higher *Heterodera glycines* populations were found in calcareous soils with higher pH values. They suggested that plants growing in calcareous soils at high pH and under nutritional deficiencies might be more susceptible to nematode attack. In the experiment by Zasada et al. (2007), described previously, the addition of amendments caused pH levels to increase dramatically (up to 9.2) with increasing amendment application, resulting in lower soybean yields. The authors suggested that such high pH levels achieved with amendment application are not ideal for plant growth. Numerous authors have reported that application of alkaline-stabilized biosolids raises the pH of the soil (above 8.5) resulting in high ammonia concentrations that have been shown to be detrimental to plant-parasitic nematodes (Zasada, 2005; Zasada and Tenuta, 2004; Oka and Pivonia, 2002; Castagnone-Sereno and Kermarrec, 1991). In the

present study, the yield or any other parameter were not significantly affected by the small range of pH change.

### 4.2.3. Metals

As discussed in Section 4.1.3 and 4.1.4, plant-parasitic nematode populations are more affected by indirect effects from amendments with high concentrations of heavy metals. Numerous studies show that levels of metals toxic to nematodes are also toxic to the plants which host them (Bisessar et al., 1983; Parveen and Alam, 1999). Parveen (2004) conducted a greenhouse experiment where tomato plants were grown with Cd concentrations of 7.5-60 mg $kg^{-1}$. Treatments included inoculated and uninoculated pots with *M. incognita* juveniles. Even though fewer nematodes penetrated the tomato roots at higher Cd concentrations, the weight of plants was highly affected as well. Levels of Cd for the present study ranged between 0.1-0.2 mg $kg^{-1}$, being significantly lower than the levels that caused a reduction in nematode infestation and reduced plant weight in the experiment by Parveen (2004). Other indirect effects that might be enhanced by the addition of low concentrations of heavy metals include the increase of root nodule bacteria, which can act as nematode antagonists (Georgieva et al., 2002). Shaukat and Siddiqui (2003) tested how addition of 0.9 mg $kg^{-1}$ of Zn in combination with two strains of root nodule bacteria would affect root-knot nematode attack to tomato. Their results show that the addition of Zn resulted in increased plant weights and reduced infection by *M. javanica*. The latter authors suggest that application of Zn at the reported concentration favored root colonization by the bacteria, which produced nematicidal compounds reflecting on the reduction in root galls. Concentrations of Zn in the present study ranged between 1.2-5.5 mg $kg^{-1}$, being close to the range at which Shaukat and Siddiqui (2003) reported reduction in nematode infection, and well below the levels reported in Section 4.1.3, which favored plant feeding nematode populations. Levels of heavy metals in the present study (Appendix 12) are well below the levels that can have an effect on nematode populations as well as

toxic effects to plants. The levels used in the present study were below permissible and recommended levels of heavy metals in soils after sludge application (Appendix 10).

### 4.2.4. Soil Bacteria and Fungi

Addition of organic matter to soils has been shown to have an impact on soil microbial communities (Akhtar and Alam, 1993). Several species of fungi feed on nematodes and bacteria parasitize them. In addition, soil bacteria and fungi produce toxins, which can indirectly affect plant-parasitic nematodes (Jatala, 1986; Litterick et al., 2004). In a microplot experiment Riegel at al. (1996) found increasing rates of chicken litter decreased juveniles of *M. incognita* due to increases in bacteria and fungi. Similar numbers of bacterial CFU were found in this study when compared to the results obtained by Riegel et al. (1996). However, Riegel et al. (1996) used significantly lower addition of organic matter (0.25-1%) as chicken litter when compared to the 3-15% organic matter used in the present study. What cannot be compared between the two studies is whether the biocontrol potential of the bacteria and fungi was the same since the raw materials between the two studies are different. It should be noted that the number of bacterial CFU as group averages in this study ranged narrowly between 2.1-2.5x$10^6$ CFU/g dry soil and the number of fungal CFU ranged between 6.0-9.4x$10^4$ CFU/g dry soil and therefore there was very little difference in either, relative to treatment groups.

Other studies have been done to show the impact of tomatoes grown with different microbes and organic matter. In a greenhouse experiment, Siddiqui (2004) grew tomato plants with 3 different bacterial species in combination with 4 different composted animal manures (cow, goat, horse, and poultry). The author reported significant improvement in plant growth when bacterial strains were used in combination with the organic manures in the absence of *M. incognita*. When *M. incognita* eggs were inoculated into the treatments, the best growth

response from tomato plants was obtained in the poultry manure + *Pseudomonas fluorescens* treatments. The author suggests that reduced galling of tomato roots caused by nematodes was due to the rapid invasion of *P. fluorescens* into tomato roots. Results obtained in the present study show no relationship between bacterial or fungal colony forming units and tomato yield. It is possible that plant growth is more related to microbial populations and not necessarily to its yield; however, a "healthier" soil has the ability to sustain more biological activity, improving nutrient cycles that in turn will increase productivity (Litterick et al., 2004).

### 4.2.5 Potential Impact of Application Rates

Barbosa et al. (2004), found a reduction in root galls, egg masses, and eggs of *M. javanica* in tomato roots when sewage sludge compost was applied at rates between 50-100%. The inoculation level used by these authors was 133 eggs/100 $cm^3$ of soil, while the present study received two inoculations of 94 and 24100 eggs/100 $cm^3$ of soil respectively. The former study was done in a greenhouse as compared to this study done in the field. Such high levels of sewage sludge application used by the former authors would not be feasible under field conditions.

Bryan and Lance (1991) conducted an experiment under field conditions and reported enhanced growth in tomato plants that received 0.5-1.1% of heat-treated biosolids, however, application rates of 1.7% resulted in smaller tomato plants. Zasada et al. (2007) tested the effect of alkaline-stabilized biosolids (N-Viro [Logan and Harrison, 1995]) and different *M. incognita* inoculation rates in microplot experiments using susceptible and resistant soybean cultivars. The application rates used by Zasada et al. (2007) ranged between 1.25-5% (wt.). These authors found that the only factor that had a significant effect in reducing nematode populations was the use of a resistant soybean cultivar and not the amendment rates of N-Viro application. The present study used realistic

application rates of 3-15% (wt.), and even though the number of *M. hapla* eggs inoculated were much higher, the tomato plants did not show visible signs of nematode infection. Nematode threshold levels in the 2008 field season might not have breached levels in which tomato plants start to show nematode damage.

It should also be noted that application rates of fly ash in soils above 10% (wt.) and as high as 25% (wt.) have been shown to decrease microbial respiration and other microbiological activity. The maximum level of fly ash used in this study was 7.5% (wt.) and therefore should not be expected to have influenced microbial activity (Schutter and Fuhrmann, 2001). However, it was out of the scope of this study to measure all aspects of microbial activity.

### 4.3 Impact of Biosolids and Fly Ash on *Meloidogyne hapla* and Tomato Plants Under Greenhouse Conditions (2008)

Discussion regarding the effects of biosolids and fly ash for the 2008 greenhouse experiments on the various parameters are similar to those discussed in Section 4.2.

An attempt was made to replicate the 2008 tomato field study in the greenhouse during the same period as the field experiment. The experimental design in the greenhouse was slightly different than for the field (See Tables 3.1 and 3.8). Raw data for the 2008 greenhouse experiment are given in Appendix 9.

Two weeks after *M. hapla* inoculation, it was noted that the tomato plants were affected by undetermined reasons. Abnormal plant growth might have affected many of the soil parameters.

In general, as with the 2008 field season few differences among replicates or treatment groups were found relative to published information. The results of the 2008 greenhouse and field experiments will be compared in this section.

### 4.3.1 *Meloidogyne hapla*

Numbers of *M. hapla* in the greenhouse study ranged from 0-26375 eggs/g of fresh root. Greenhouse data ranged from 4-10 times higher than their field counterparts. Tomato plants grown in the greenhouse were unhealthy at the beginning of the experiment, which might have made them more susceptible to nematode infection as evidenced by the higher number of nematode eggs recovered from their roots. It should be noted that no *M. hapla* eggs were found in any of the fly ash replicates. Results from this study show that low pH (Zasada, 2005; Zasada and Tenuta, 2004; Oka and Pivonia, 2002; Castagnone-Sereno and Kermarrec, 1991), low EC (Edongali et al., 1982), and reduced below ground biomass found in the A50 treatments, enabled higher infection rates by M. hapla, as evidenced by the highest number of eggs recovered from that treatment group.

### 4.3.2 Electrical Conductivity

Electrical conductivity for the greenhouse treatment groups was higher than the field but not significantly and was seldom more than twice that found in the field. It is likely that climatic exposure in the field study resulted in higher leaching and lower total dissolved solids than in the greenhouse where plants were watered a set amount as required but no fertilizer was applied to any of the treatments. In addition, it is possible that higher transpiration rates in the field allowed the plants to tolerate EC better (Li et al., 2001).

### 4.3.3 pH

There is virtually no difference in pH among replicates or any of the treatment groups for either of the studies. Field results range from 7.9-8.3 and those for the greenhouse ranged from 8.1-8.3.

### 4.3.4 Soil Bacteria and Fungi

Bacterial CFU ranged from 1 million in the greenhouse AH group to 2.6 million in the A75 group. These data are more or less within plus or minus 50% for the same groups in the field study. This finding would be consistent with the fact that bacteria would thrive similarly under either greenhouse or field conditions.

There was a significant difference in fungal CFU between the greenhouse (unsterilized soil) and field experiments with the field data being 2 to 3 times higher than for the greenhouse. It is likely that the use of electronic fans to keep humidity and temperature down in the greenhouse resulted in lower numbers than for the field.

### 4.3.5 Plant Biomass

As would be expected above and below ground biomass was much less in the greenhouse experiments relative to the field. These differences can easily be explained by pot size and natural against greenhouse climatic conditions.

# 5 CONCLUSIONS AND FUTURE WORK

Plant-parasitic nematodes have been regularly managed by using chemical pesticides, and even though the problem has been partially controlled, losses attributed to nematode damage remain an important concern in agriculture worldwide. In most tropical regions of the world, environmental conditions enable nematodes such as *Meloidogyne incognita* to affect crops that are grown year round. The use of chemical pesticides is becoming more restricted as we discover the detrimental effect that these compounds have on the environment. It is critical that integrated nematode management practices be developed by considering the soil-plant-microorganism system as a whole.

The type and application rates of ash and biosolids used in this study did not alter soil parameters such as metals, EC, pH, or microbes enough to affect significantly the nematode numbers. Even though some of the data for selected treatment groups as either biosolids or ash or mixtures of the two did result in altered physical and chemical properties of the soil, the change was not significant enough to be able to recommend any given treatment as a nematode suppressant. It could be recommended that application rates should increase, based on the composition of the raw materials, which will increase productivity and inhibit nematode growth. The major contribution of this work is that it shows that application of the specific ash and biosolids used, up to a total of 600 tons/ha, while positively affecting yield would not negatively affect the soil parameters measured.

Addition of biosolids and fly ash alone or in combination may improve multiple soil properties including physical, chemical, and biological activity, all of which contribute to soil health. A healthier soil has the ability to supply enough nutrients for plants and microorganisms without relying solely on chemical inputs. One of the major questions to be answered when using biosolids or ash in soils is whether or not national or international guidelines are breached regarding heavy

metal content in soils. The amendments as applied in this study attained a maximum for either BS or AH of 15% and in most cases were not greater than 7.5% of either, which are well below the levels of international guidelines. While these amounts were sufficient to cause improved yields, it is unlikely that they affected the plants enough to create more nematode resistant conditions.

In addition, the relatively low concentration of potentially toxic elements to nematodes and plants in the amendments used, at the rates in which they were applied, were not high enough to impact either flora or fauna (Appendix 12).

There was a direct response to carrot yield with increasing amendment application. However, nematode populations did not correlate with increased yield. Inspection of the roots did not show the presence of galls in the main root nor was branching of the carrots seen. It is likely that the amount of inoculation in the carrot trials was too small to produce significant nematode numbers. It should also be noted that the carrot crop cycle was short, thus not allowing nematodes to dramatically damage the carrots.

As with carrots, the yield of tomato plants was improved with the addition of the amendments. Even though large changes in populations of *Meloidogyne hapla* were not seen, the tomato plants were able to resist infestation and produce better than fertilized controls. It is possible that although tomato plants were infected, nematode threshold levels were not breached and plants were able to have improved yields related to amendment application.

Extracting, growing, and inoculating nematodes are laborious processes that result in extremely variable results. Several different methods of extracting and counting nematodes were used at different stages of this study, and it is likely that these changes in methods produced some of the large variability seen in results. In addition, extraction of roots from the soil was not quantitative and there

is no guarantee that the root sampled were evenly infected, which would have created differences in nematode numbers.

Any future work on the impact of ash, biosolids or combinations of the two should take into account the limits for the variables that have the potential to control nematodes in soils while considering the health of the plant.

The following aspects of the impact of amendments on nematode populations should be taken into account:

- Preliminary studies on different sources of ash and sludge should be conducted in order to identify those raw materials whose physical and chemical parameters bracket conditions for nematode survival or death.
- Ratios and application rates of different sources of raw materials should be determined in order to optimize the impact on nematode viability.
- Biocontrol agents that affect nematode populations and that flourish in the amendments should be identified rather than simply using colony forming units.
- Multiple soil and climatic conditions should be selected in order to recommend ideal application rates for individual geographic areas.
- The experimental design in this thesis (specially number of replicates) resulted in pooled results and any future work should include more replicates so that the results from each treatment can be compared to each other more critically.

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# 7 APPENDICES

## Appendix 1.

Precipitation Recorded at the ESW Field Station During the Summers of 2006, 2007, and 2008.

| Year | Month | Precipitation (mm) |
|---|---|---|
| 2006 | May | 88.0 |
| 2006 | June | 65.0 |
| 2006 | July | 201.5 |
| 2006 | August | 92.0 |
| 2006 | September | 134.5 |
| 2006 | October | 282.0 |
| 2007 | May | 73.0 |
| 2007 | June | 79.0 |
| 2007 | July | 28.5 |
| 2007 | August | 86.0 |
| 2007 | September | 50.0 |
| 2007 | October | 74.0 |
| 2008 | May | 115.5 |
| 2008 | June | 100.0 |
| 2008 | July | 62.0 |
| 2008 | August | 63.0 |
| 2008 | September | 145.0 |
| 2008 | October | 109.0 |

## Appendix 2.

## Microplot Distribution, Summer 2006, 2007.

| | | |
|---|---|---|
| CT-Na | A75 15b | A50 12a |
| AH 6a | BS 3a | CT+Na |
| BS 9a | A75 12a | BS 6a |
| A75 9a | AH 12c | AH 9a |
| A50 3a | A75 6a | A50 9c |
| CTF-Na | CTF+Na | BS 12a |
| A75 12c | A75 6c | A50 6c |
| AH 15a | BS 15a | A75 9c |
| A50 12c | AH 12b | A50 15c |
| A50 9b | AH 9b | A75 3c |
| AH 12a | AH 3a | AH 15c |
| A75 15c | CT-Nb | A50 3b |
| A50 6b | BS 9b | CTF-Nc |
| A50 15b | AH 6b | BS 6b |
| CT+Nb | BS 3b | BS 15c |
| A75 3a | CT+Nc | CTF+Nb |
| AH 3b | A50 12b | BS 12b |
| A75 3b | A75 9b | A50 15a |
| AH 9c | A75 6b | BS 9c |
| BS 12c | CTF-Nb | A50 6a |
| CTF+Nc | A50 9a | A75 12b |
| BS 6c | A50 3c | AH 3c |
| AH 15b | BS 15b | BS 3c |
| A75 15a | AH 6c | CT-Nc |

## Appendix 3.

## Microplot Distribution, Summer 2008.

| | | |
|---|---|---|
| A75 12b | BS 3c | CT-Nc |
| CT-Na | A75 15b | A50 12a |
| BS 9a | BS 3a | CT+Na |
| A75 9a | A75 12a | BS 6a |
| A50 3a | A75 6a | A50 9c |
| CTF-Na | CTF+Na | BS 12a |
| A75 12c | A75 6c | A50 6c |
| A50 12c | BS 15a | A75 9c |
| A50 9b | CT-Nb | A50 15c |
| A75 15c | BS 9b | A75 3c |
| A50 6b | BS 3b | A50 3b |
| A50 15b | CT+Nc | CTF-Nc |
| CT+Nb | A50 12b | BS 6b |
| A75 3a | A75 9b | BS 15c |
| A75 3b | A75 6b | CTF+Nb |
| BS 12c | CTF-Nb | BS 12b |
| CTF+Nc | A50 9a | A50 15a |
| BS 6c | A50 3c | BS 9c |
| A75 15a | BS 15b | A50 6a |

## Appendix 4.

**pH for *Meloidogyne hapla* cultures.**

| Pot number/sample | pH | Comments |
|---|---|---|
| Pot # 5 | 7.95 | Nematode culture (*M. hapla*) |
| Pot # 7 | 7.94 | Nematode culture (*M. hapla*) |
| Pot # 14 | 7.89 | Nematode culture (*M. hapla*) |
| Pot # 17 | 7.92 | Nematode culture (*M. hapla*) |
| Mother culture | 8.04 | Nematode culture (*M. hapla*) Original culture from Quebec |
| Secondary mother culture | 7.61 | Nematode culture (*M. hapla*) Original culture from Quebec in sandy soil |
| Promix | 5.89 | Promix from UWO greenhouse |
| Sandy soil + Promix | 7.49 | Leftover mixture prepared for nematode cultures |
| Sand | 8.12 | Sand used for soil mixtures |

## Appendix 5.

## Impact of Biosolids and Fly Ash on Natural Nematode Populations and *Meloidogyne hapla*, in the Absence of Plant Material (2007).

Average data for each amendment were used to determine the impact of the amendment on soils and nematodes. The composition of each amendment is given in Table 2.2.

### A.5.1 Bacterial feeding nematodes

No bacterial feeding nematodes (BFN) were found in the X50:50 treatment in any of the sampling times. At baseline, BFN populations ranged from 2-19 BFN/100 ml of soil, with the highest found in the CT treatment (Fig. 5.18, "Baseline"). Midway through the experiment (Fig. 5.18, "Middle") BFN populations ranged from 13-84 BFN/100 ml of soil, with the highest found in the CT treatment. At the end of the experiment (Fig. 5.18, "End") BFN populations ranged from 26-64 BFN/100 ml of soil, with the highest found in the CT treatment. Significant differences among groups were found at baseline ($F=4.7$, $df=2,12$, $P=0.032$) and in the middle of the experiment ($F=5.9$, $df=2,12$, $P=0.017$). CT and X50:50 were not included in the statistical analysis due to a lack of replicates.

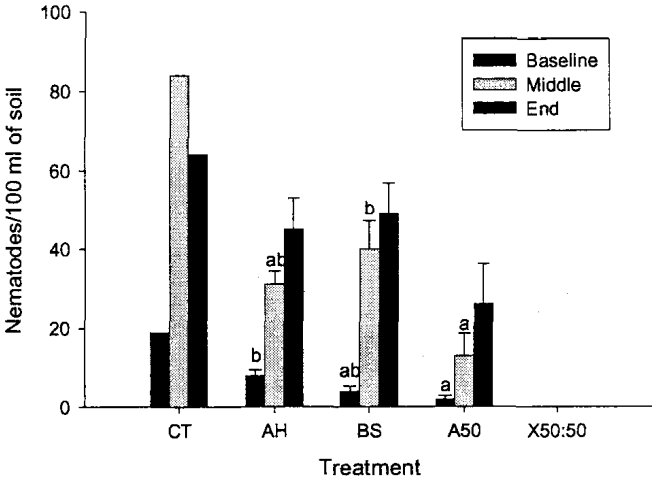


**Fig. A5.1 Bacterial feeding nematode populations in amendments (CT= soil; AH= fly ash with soil; BS= biosolids with soil; A50= 50:50 mixture of biosolids and fly ash with soil; X50: 50= 50:50 mixture of biosolids and fly ash without soil); (n=1 for CT and X50: 50); (n=3 for AH, BS, and A50, values +/- SE) at 3 sampling times (Baseline, Middle, and End). Winter 2007.**

## A.5.2 Fungal feeding nematodes

No fungal feeding nematodes (FFN) were found in the X50: 50 treatment in any of the sampling times. At baseline, FFN populations ranged from 7-10 FFN/100 ml of soil, with the highest found in the AH treatments (Fig. 5.19, "Baseline"). Midway through the experiment (Fig. 5.19, "Middle") FFN populations ranged from 3-15 FFN/100 ml of soil, with the highest found in the CT treatment. At the end of the experiment (Fig. 5.19, "End") FFN populations ranged from 6-10 FFN/100 ml of soil, with the highest found in the CT and AH treatments. No significant differences were found among groups in any of the sampling times. CT and X50: 50 were not included in the statistical analysis due to a lack of replicates.

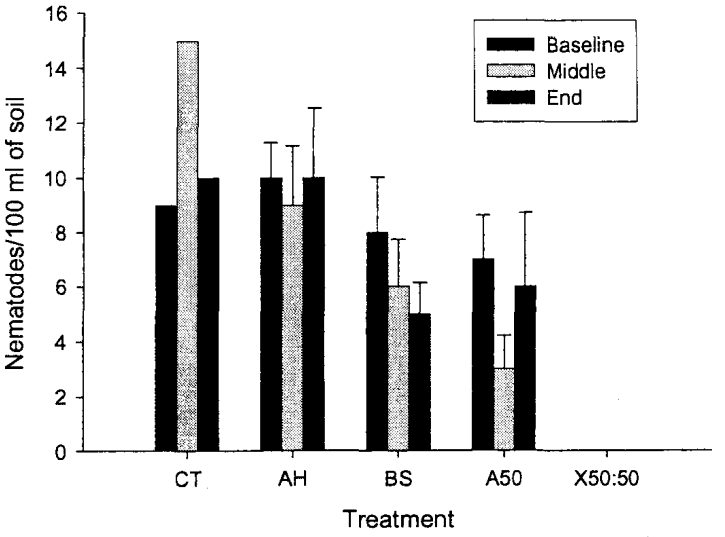


**Fig. A5.2 Fungal feeding nematode populations in amendments (CT= soil; AH= fly ash with soil; BS= biosolids with soil; A50= 50:50 mixture of biosolids and fly ash with soil; X50: 50= 50:50 mixture of biosolids and fly ash without soil); (n=1 for CT and X50: 50); (n=3 for AH, BS, and A50, values +/- SE) at 3 sampling times (Baseline, Middle, and End). Winter 2007.**

### A.5.3 Plant feeding nematodes

No plant feeding nematodes (PFN) were found in the X50:50 treatment in any of the sampling times. At baseline, PFN populations ranged from 5-21 PFN/100 ml of soil, with the highest found in the CT treatment (Fig. 5.20, "Baseline"). Midway through the experiment (Fig. 5.20, "Middle") PFN populations ranged from 17-64 PFN/100 ml of soil, with the highest found in the CT treatment. At the end of the experiment (Fig. 5.20, "End") PFN populations ranged from 29-52 PFN/100 ml of soil, with the highest found in the BS treatments. Significant differences among groups were found only in the middle of the experiment ($F=8.1$, $df=2,12$, $P=0.006$). CT and X50:50 were not included in the statistical analysis due to a lack of replicates.

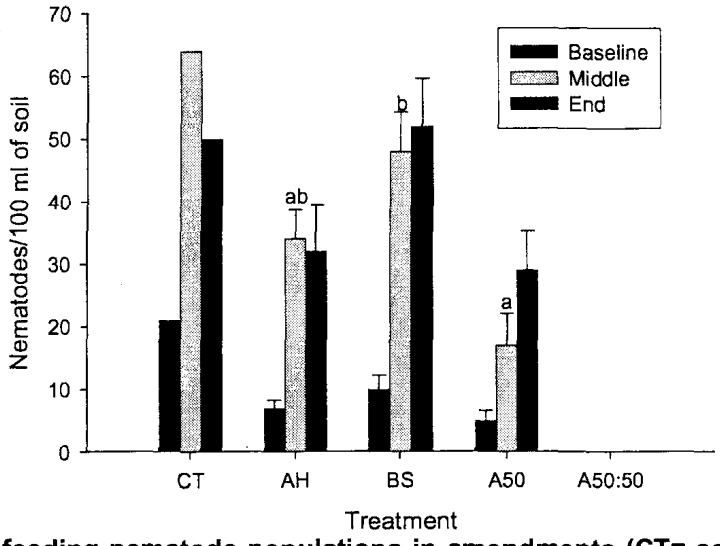


**Fig. A5.3 Plant feeding nematode populations in amendments (CT= soil; AH= fly ash with soil; BS= biosolids with soil; A50= 50:50 mixture of biosolids and fly ash with soil; X50: 50= 50:50 mixture of biosolids and fly ash without soil); (n=1 for CT and X50: 50); (n=3 for AH, BS, and A50, values +/- SE) at 3 sampling times (Baseline, Middle, and End). Winter 2007.**

## A.5.4 Electrical conductivity

Electrical conductivity increased with increasing amendment application. At baseline, EC ranged from 208-1677 µS/cm with the highest found in the A50: 50 treatment (Fig. 5.21, "Baseline"). Midway through the experiment (Fig. 5.21, "Middle") EC ranged from 253-1939 µS/cm, with the highest found in the A50: 50 treatment. At the end of the experiment (Fig. 5.21, "End") EC ranged from 283-1601 µS/cm, with the highest found in the X50: 50 treatment. Significant differences among groups were found only at baseline ($F=4.0$, $df=2,12$, $P=0.046$). CT and X50: 50 were not included in the statistical analysis due to a lack of replicates.

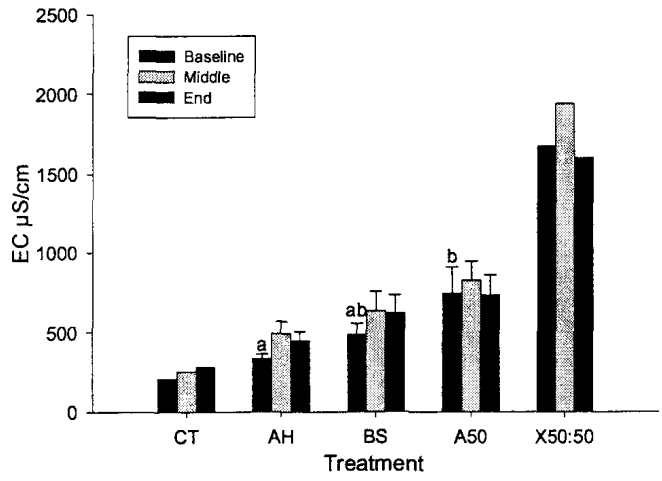


**Fig. A5.4 Electrical conductivity in amendments (CT= soil; AH= fly ash with soil; BS= biosolids with soil; A50= 50:50 mixture of biosolids and fly ash with soil; X50: 50= 50:50 *mixture of biosolids and fly ash without soil*); (*n*=1 *for* CT *and* X50: 50); (n=3 for AH, BS, and A50, values +/- SE) at 3 sampling times (Baseline, Middle, and End). Winter 2007.**

## A.5.5 pH

At baseline, pH ranged from 7.72-7.90, with the highest found in the AH treatments (Fig. 5.22, "Baseline"). Midway through the experiment (Fig. 5.22, "Middle") pH ranged from 7.61-7.90, with the highest found in the AH treatments. At the end of the experiment (Fig. 5.22, "End") pH ranged from 7.70-7.99, with the highest found in the AH treatments. The highest pH values were found in the AH treatments at all sampling times. No significant differences were found between groups in any of the sampling times. CT and A50: 50 were not included in the statistical analysis due to a lack of replicates.

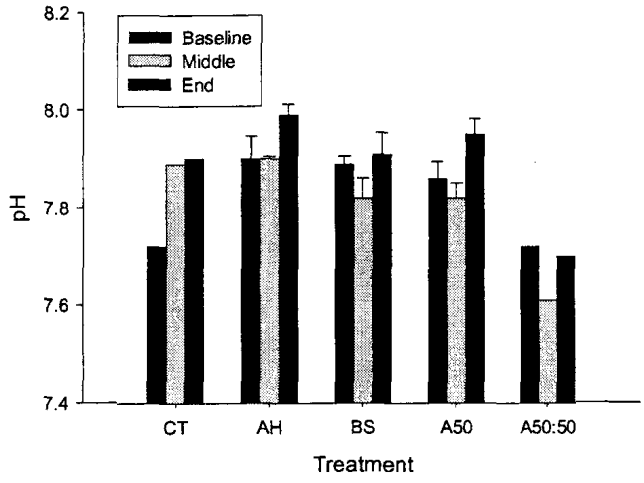


**Fig. A5.5 pH in amendments (CT= soil; AH= fly ash with soil; BS= biosolids with soil; A50= 50:50 mixture of biosolids and fly ash with soil; X50: 50= 50:50 mixture of biosolids and fly ash without soil); (n=1 for CT and X50: 50); (n=3 for AH, BS, and A50, values +/- SE) at 3 sampling times (Baseline, Middle, and End). Winter 2007.**

### A.5.6 Discussion on the Impact of Biosolids and Fly Ash on Natural Nematode Populations and *Meloidogyne hapla* in the Absence of Plant Material (2007)

Parameters that affect nematode populations have been discussed in Section 4.1. Results obtained in the present study show that in the absence of plant material, natural populations of bacterial, fungal, and plant feeding nematodes were higher in the control treatments (Fig. A1; A2; A3). Zasada and Tenuta (2004) experimented with a mixture of digested municipal biosolids and fly ash that were mixed with sand, inoculated with plant-parasitic nematodes (*Meloidogyne incognita* and *Heterodera glycines*) and incubated for 24 hours. Their experiment did not have any plant material so nematodes would be directly exposed to the mixtures. They found that juveniles were the most sensitive life stage and that eggs were able to resist against the exposure to the mixtures. In another experiment using the same mixtures, Zasada (2005) found that only the mixtures that increased pH levels to 10 and higher suppressed the inoculated *H. glycines*. In the present study, *M. hapla* eggs were inoculated to Dixie® cups without plant material, and after the incubation period, no juveniles were recovered. This might be due to direct chemical exposure to the mixtures and

also because the concentration of NaOCl that was used to process the inoculum, might have reduced egg viability.

The pH values for the present study (Fig. A5) were much lower that those reported by Zasada (2005) so the absence of eggs or juveniles in the present experiment cannot be attributed to extreme pH levels. Electrical conductivity levels increased with increased amendments (Fig. A4) and reached the highest levels in the treatments that did not have soil (X50: 50). Electrical conductivity levels are within the levels discussed in Section 4.1.5; therefore absence of *M. hapla* in the experiment is not attributed to EC. Treatments without soil (X50: 50) had no nematodes during any part of the experiment; this is because no nematodes were found in the biosolids or fly ash as raw materials.

## Appendix 6.

**Data collected for various treatments on nematode populations (nematodes/100 ml of soil; BF=bacterial feeding nematodes; FF= fungal feeding nematodes; PF= plant feeding nematodes); pH; EC=electrical conductivity (µS/cm); Top=weight of above ground biomass per treatment (g); Root=weight of carrot roots per treatment (g). Summer 2006.**

| | Baseline | | | | | Middle | | | | | Harvest | | | | | CARROT YIELD | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Treat.* | BF | FF | PF | pH | EC | BF | FF | PF | pH | EC | BF | FF | PF | pH | EC | Top | Root |
| CT | 60 | 0 | 120 | 7.70 | 250 | 30 | 30 | 110 | 7.89 | 180 | 0 | 60 | 70 | 8.06 | 170 | 62.5 | 164 |
| CT | 0 | 30 | 120 | 7.75 | 250 | 40 | 20 | 130 | 7.93 | 180 | 0 | 80 | 120 | 8.06 | 160 | 42.6 | 110 |
| CTF | 0 | 0 | 60 | 7.73 | 260 | 110 | 20 | 60 | 7.96 | 170 | 30 | 120 | 140 | 8.06 | 170 | 48.7 | 181 |
| CTF | 0 | 60 | 60 | 7.8 | 270 | 70 | 0 | 60 | 7.95 | 250 | 40 | 20 | 50 | 8.10 | 170 | 63.0 | 196 |
| AH-3 | 0 | 0 | 60 | 7.81 | 420 | 170 | 10 | 80 | 8.16 | 140 | 0 | 20 | 70 | 8.06 | 200 | 55.4 | 150 |
| AH-6 | 0 | 0 | 60 | 7.81 | 510 | 110 | 30 | 70 | 8.18 | 140 | 0 | 40 | 70 | 8.14 | 180 | 53.5 | 117 |
| AH-9 | 0 | 0 | 60 | 7.82 | 640 | 60 | 0 | 40 | 8.15 | 210 | 10 | 20 | 130 | 8.17 | 210 | 39.3 | 71.5 |
| AH-12 | 0 | 0 | 90 | 7.83 | 770 | 40 | 60 | 80 | 8.14 | 210 | 20 | 40 | 120 | 8.04 | 350 | 31.7 | 35.8 |
| AH-15 | 0 | 30 | 90 | 7.83 | 900 | 0 | 30 | 40 | 8.07 | 320 | 10 | 30 | 50 | 8.02 | 490 | 28.9 | 41.9 |
| BS-3 | 30 | 30 | 30 | 7.77 | 420 | 50 | 30 | 80 | 7.98 | 160 | 40 | 30 | 130 | 8.05 | 170 | 96.3 | 355 |
| BS-6 | 0 | 30 | 90 | 7.73 | 480 | 30 | 30 | 80 | 7.94 | 180 | 0 | 0 | 40 | 8.01 | 180 | 98.6 | 388 |
| BS-9 | 0 | 0 | 180 | 7.71 | 630 | 120 | 20 | 70 | 7.88 | 190 | 40 | 20 | 60 | 8.00 | 200 | 101.6 | 359 |
| BS-12 | 30 | 60 | 120 | 7.66 | 730 | 70 | 0 | 110 | 7.83 | 230 | 30 | 10 | 40 | 7.97 | 220 | 104.8 | 455 |
| BS-15 | 90 | 0 | 240 | 7.64 | 840 | 0 | 20 | 100 | 7.89 | 250 | 60 | 0 | 110 | 7.93 | 260 | 113.1 | 517 |
| A50-3 | 60 | 0 | 180 | 7.86 | 370 | 80 | 60 | 70 | 8 | 150 | 20 | 40 | 140 | 8.06 | 170 | 68.4 | 253 |
| A50-6 | 60 | 0 | 120 | 7.80 | 510 | 70 | 40 | 80 | 7.99 | 170 | 50 | 40 | 60 | 8.05 | 180 | 75.7 | 273 |
| A50-9 | 60 | 0 | 30 | 7.79 | 640 | 200 | 80 | 70 | 7.95 | 180 | 30 | 60 | 40 | 8.07 | 190 | 88.8 | 266 |
| A50-12 | 30 | 0 | 90 | 7.76 | 740 | 110 | 60 | 90 | 7.85 | 180 | 70 | 70 | 70 | 8.04 | 230 | 80.9 | 298 |
| A50-15 | 0 | 0 | 60 | 7.70 | 860 | 120 | 50 | 110 | 7.79 | 250 | 50 | 30 | 70 | 7.95 | 370 | 96.8 | 337 |
| A75-3 | 30 | 30 | 210 | 7.87 | 390 | 90 | 30 | 80 | 8.04 | 150 | 10 | 70 | 40 | 8.03 | 170 | 77.8 | 283 |
| A75-6 | 30 | 0 | 90 | 7.81 | 520 | 100 | 10 | 130 | 8.02 | 170 | 30 | 10 | 70 | 8.04 | 180 | 95.0 | 373 |
| A75-9 | 60 | 60 | 60 | 7.74 | 680 | 100 | 0 | 120 | 7.91 | 190 | 70 | 40 | 60 | 8.02 | 190 | 117.3 | 405 |
| A75-12 | 30 | 30 | 30 | 7.71 | 800 | 260 | 130 | 90 | 7.9 | 200 | 30 | 30 | 50 | 7.89 | 310 | 107.3 | 408 |
| A75-15 | 30 | 30 | 120 | 7.67 | 890 | 110 | 120 | 140 | 7.9 | 200 | 60 | 60 | 40 | 7.92 | 260 | 69.8 | 421 |

* Description of each treatment is given in Table 2.1

## Appendix 7.

**A7.A Data collected for various treatments on nematode populations (nematodes /100 ml of soil; BF=bacterial feeding nematodes; FF= fungal feeding nematodes; PF= plant feeding nematodes); pH; EC= electrical conductivity (µS/cm) at Baseline (June 11/07), Middle (July 9/07), 1st. Harvest (July 23/07), and 2nd. Harvest (Oct. 8/07). Carrot yield for 1st. and 2nd. Harvests (g). 1st. Harvest (July 23/07), and 2nd. Harvest (Oct. 8/07).**

| | Baseline | | | | | Middle | | | | | 1st. Harvest | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Treatment* | BF | FF | PF | pH | EC | BF | FF | PF | pH | EC | BF | FF | PF | pH | EC |
| CT-N | 25 | 8 | 142 | 7.88 | 196 | 19 | 9 | 176 | 8.02 | 122 | 74 | 14 | 260 | 8.24 | 109 |
| CT+N | 32 | 13 | 264 | 7.93 | 203 | 37 | 13 | 313 | 8.15 | 108 | 51 | 27 | 51 | 8.22 | 130 |
| CTF+N | 48 | 12 | 151 | 7.75 | 1240 | 14 | 26 | 176 | 8.16 | 126 | 61 | 22 | 66 | 8.12 | 120 |
| CTF-N | 38 | 11 | 380 | 7.79 | 860 | 33 | 37 | 278 | 8.14 | 118 | 39 | 10 | 184 | 8.14 | 184 |
| AH-3 | 51 | 30 | 209 | 8 | 307 | 36 | 24 | 205 | 8.29 | 107 | 80 | 22 | 164 | 8.3 | 124 |
| AH-6 | 26 | 21 | 194 | 7.93 | 485 | 39 | 25 | 204 | 8.18 | 198 | 62 | 37 | 110 | 8.26 | 211 |
| AH-9 | 41 | 21 | 130 | 7.93 | 546 | 64 | 52 | 195 | 8.1 | 391 | 45 | 25 | 94 | 8.18 | 476 |
| AH-12 | 70 | 37 | 122 | 7.89 | 652 | 39 | 30 | 199 | 8.01 | 463 | 6 | 24 | 41 | 8.15 | 803 |
| AH-15 | 30 | 10 | 175 | 7.9 | 752 | 23 | 28 | 125 | 8.06 | 590 | 54 | 10 | 121 | 8.06 | 740 |
| BS-3 | 27 | 14 | 133 | 7.93 | 352 | 47 | 30 | 210 | 8.15 | 131 | 68 | 20 | 72 | 8.09 | 144 |
| BS-6 | 59 | 19 | 190 | 7.82 | 551 | 38 | 35 | 131 | 8.1 | 184 | 46 | 17 | 78 | 8.01 | 157 |
| BS-9 | 50 | 7 | 141 | 7.76 | 701 | 74 | 35 | 211 | 8.02 | 225 | 76 | 24 | 86 | 7.89 | 249 |
| BS-12 | 102 | 12 | 127 | 7.7 | 861 | 121 | 29 | 216 | 7.96 | 328 | 116 | 33 | 87 | 7.95 | 262 |
| BS-15 | 86 | 17 | 117 | 7.66 | 976 | 151 | 34 | 180 | 7.9 | 488 | 131 | 35 | 152 | 7.88 | 360 |
| A50-3 | 68 | 11 | 277 | 7.9 | 432 | 68 | 35 | 290 | 8.2 | 140 | 66 | 39 | 160 | 8.2 | 142 |
| A50- 6 | 29 | 11 | 233 | 7.86 | 535 | 66 | 28 | 256 | 8.06 | 198 | 48 | 51 | 87 | 8.18 | 143 |
| A50-9 | 52 | 16 | 233 | 7.8 | 709 | 80 | 34 | 319 | 8.03 | 305 | 32 | 19 | 67 | 8.17 | 217 |
| A50-12 | 56 | 21 | 191 | 7.76 | 808 | 113 | 14 | 194 | 7.99 | 380 | 71 | 23 | 117 | 8.08 | 316 |
| A50-15 | 38 | 34 | 190 | 7.71 | 970 | 98 | 26 | 168 | 7.95 | 560 | 40 | 31 | 88 | 7.98 | 533 |
| A75-3 | 54 | 28 | 266 | 7.86 | 445 | 84 | 24 | 319 | 8.19 | 146 | 64 | 24 | 255 | 8.16 | 142 |
| A75-6 | 60 | 23 | 224 | 7.77 | 584 | 156 | 29 | 276 | 8.06 | 200 | 52 | 33 | 72 | 8.2 | 167 |
| A75-9 | 46 | 17 | 127 | 7.66 | 841 | 141 | 8 | 115 | 8.05 | 241 | 44 | 25 | 129 | 8 | 295 |
| A75-12 | 90 | 27 | 173 | 7.64 | 921 | 193 | 14 | 156 | 8.04 | 282 | 37 | 19 | 146 | 7.98 | 388 |
| A75-15 | 110 | 20 | 146 | 7.59 | 1074 | 213 | 20 | 184 | 7.93 | 433 | 44 | 10 | 97 | 7.99 | 411 |

* Description of each treatment is given in Table 2.1

**A7.A (cont.)**

| | 2nd. Harvest | | | | | Carrot yield | |
|---|---|---|---|---|---|---|---|
| Treatment | BF | FF | PF | pH | EC | 1st. | 2nd. |
| CT-N | 31 | 16 | 150 | 8.2 | 117 | 52 | 23.6 |
| CT+N | 19 | 19 | 101 | 8.36 | 112 | 76 | 36.41 |
| CTF+N | 45 | 35 | 211 | 8.41 | 106 | 88 | 113.6 |
| CTF-N | 16 | 18 | 106 | 8.43 | 106 | 134 | 116 |
| AH-3 | 25 | 27 | 192 | 8.49 | 89 | 94 | 46.7 |
| AH-6 | 6 | 16 | 141 | 8.5 | 87 | 84 | 49.7 |
| AH-9 | 20 | 13 | 41 | 8.46 | 136 | 66 | 67.7 |
| AH-12 | 8 | 7 | 32 | 8.35 | 268 | 59 | 50.9 |
| AH-15 | 0 | 9 | 37 | 8.23 | 448 | 12 | 41.5 |
| BS-3 | 44 | 34 | 195 | 8.3 | 122 | 188 | 223.2 |
| BS-6 | 34 | 21 | 206 | 8.22 | 138 | 176 | 232.9 |
| BS-9 | 58 | 21 | 194 | 8.13 | 217 | 142 | 407.4 |
| BS-12 | 48 | 18 | 214 | 8.15 | 178 | 165 | 124.9 |
| BS-15 | 73 | 38 | 206 | 8.04 | 277 | 173 | 411.6 |
| A50-3 | 23 | 14 | 228 | 8.41 | 106 | 180 | 119.8 |
| A50-6 | 20 | 15 | 245 | 8.36 | 116 | 234 | 165.4 |
| A50-9 | 14 | 15 | 105 | 8.35 | 124 | 178 | 283.2 |
| A50-12 | 28 | 7 | 93 | 8.26 | 209 | 187 | 192.4 |
| A50-15 | 30 | 14 | 78 | 8.03 | 536 | 132 | 228.1 |
| A75-3 | 21 | 13 | 166 | 8.39 | 123 | 153 | 58.2 |
| A75-6 | 31 | 27 | 169 | 8.23 | 167 | 249 | 185.3 |
| A75-9 | 20 | 24 | 181 | 8.25 | 138 | 236 | 252 |
| A75-12 | 28 | 13 | 197 | 8.11 | 273 | 210 | 207.7 |
| A75-15 | 20 | 17 | 141 | 8.09 | 333 | 171 | 385.7 |

* Description of each treatment is given in Table 2.1

**A7.B Data collected for various treatments on bacterial and fungal colony forming units (CFU) per gram of soil at Baseline (June 11/07), Middle (July 9/07), 1st. Harvest (July 23/07), and 2nd. Harvest (Oct. 8/07).**

| | Baseline | | Middle | | 1st. Harvest | | 2nd. Harvest | |
|---|---|---|---|---|---|---|---|---|
| Treatment* | Bacterial CFU*$10^6$ | Fungal CFU*$10^4$ | Bacterial CFU*$10^6$ | Fungal CFU*$10^4$ | Bacterial CFU*$10^6$ | Fungal CFU*$10^4$ | Bacterial CFU*$10^6$ | Fungal CFU*$10^4$ |
| CT-N | 1.2 | 4.3 | 1.2 | 5.0 | 0.9 | 3.0 | 1.7 | 4.7 |
| CT+N | 1.3 | 4.0 | 1.5 | 3.3 | 0.9 | 2.3 | 1.6 | 3.0 |
| CTF+N | 1.1 | 2.3 | 1.8 | 3.0 | 1.2 | 2.7 | 1.6 | 4.7 |
| CTF-N | 1.4 | 5.0 | 0.9 | 3.7 | 1.1 | 3.7 | 1.5 | 4.3 |
| AH-3 | 1.1 | 4.7 | 0.9 | 2.7 | 0.8 | 4.3 | 1.3 | 1.7 |
| AH-6 | 1.1 | 6.0 | 1.6 | 3.0 | 0.9 | 4.0 | 1.6 | 4.0 |
| AH-9 | 1.4 | 3.0 | 0.9 | 2.0 | 1.2 | 3.3 | 1.3 | 5.3 |
| AH-12 | 1.0 | 5.0 | 0.5 | 2.7 | 1.6 | 1.0 | 1.1 | 3.3 |
| AH-15 | 1.0 | 4.0 | 0.4 | 3.7 | 1.2 | 2.0 | 1.2 | 2.3 |
| BS-3 | 1.3 | 6.0 | 0.9 | 4.7 | 1.2 | 5.0 | 1.9 | 6.3 |
| BS-6 | 1.5 | 7.7 | 1.7 | 9.3 | 1.2 | 8.3 | 2.1 | 8.3 |
| BS-9 | 1.9 | 6.7 | 2.1 | 7.3 | 1.0 | 5.3 | 1.9 | 9.7 |
| BS-12 | 1.7 | 10.7 | 2.7 | 11.7 | 1.5 | 4.3 | 1.7 | 7.0 |
| BS-15 | 2.4 | 8.3 | 0.9 | 11.0 | 1.7 | 8.3 | 2.5 | 10.7 |
| A50- 3 | 1.2 | 5.0 | 2.3 | 3.0 | 1.6 | 1.7 | 1.4 | 3.0 |
| A50-6 | 1.5 | 7.0 | 2.0 | 5.0 | 2.4 | 4.7 | 1.6 | 4.3 |
| A50-9 | 1.6 | 5.3 | 2.4 | 6.0 | 1.5 | 1.7 | 1.9 | 6.3 |
| A50-12 | 2.1 | 6.3 | 2.6 | 6.3 | 1.5 | 1.7 | 1.9 | 6.7 |
| A50-15 | 1.9 | 5.7 | 3.1 | 5.7 | 2.1 | 8.7 | 1.7 | 6.0 |
| A75-3 | 1.7 | 4.7 | 1.1 | 5.0 | 1.4 | 4.0 | 1.7 | 6.7 |
| A75-6 | 1.9 | 5.0 | 2.3 | 6.0 | 2.2 | 3.3 | 16.0 | 6.0 |
| A75-9 | 2.2 | 7.7 | 0.9 | 4.3 | 1.8 | 4.7 | 2.2 | 8.0 |
| A75-12 | 1.8 | 7.7 | 2.5 | 7.3 | 1.7 | 4.7 | 1.9 | 4.3 |
| A75-15 | 3.1 | 7.3 | 2.2 | 6.7 | 1.7 | 8.7 | 2.0 | 4.3 |

* Description of each treatment is given in Table 2.1

## Appendix 8.

**Data collected for various treatments on *Meloidogyne hapla* (eggs/g of fresh root); bacterial and fungal colony forming units at baseline and harvest (CFU/g of dry soil); pH and electrical conductivity (µS/cm) at baseline and harvest; tomato yield (g/plant); above ground biomass (g); below ground biomass (g). Summer 2008.**

| | | Baseline | | | | Harvest | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Treat.* | Rep. | pH | EC | Bact. CFU* $10^6$ | Fung. CFU* $10^4$ | pH | EC | Bact. CFU* $10^6$ | Fung. CFU* $10^4$ | *M. hapla* eggs | Tomato yield | Above ground mass | Below ground mass |
| CT-N | 1 | 8.10 | 225 | 1.0 | 5.4 | 8.34 | 106 | 1.7 | 5.6 | 0 | 2085 | 356 | 27 |
| CT-N | 2 | 7.98 | 222 | 1.3 | 6.4 | 8.38 | 90 | 1.3 | 4.9 | 0 | 1904 | 206 | 22 |
| CT-N | 3 | 8.07 | 236 | 1.8 | 4.2 | 8.25 | 124 | 1.9 | 6.7 | 0 | 1111 | 86 | 19 |
| CT+N | 1 | 8.08 | 242 | 2.1 | 6.7 | 8.38 | 86 | 1.5 | 5.2 | 417 | 1028 | 87 | 21 |
| CT+N | 2 | 8.09 | 238 | 1.4 | 6.2 | 8.35 | 112 | 1.9 | 4.0 | 374 | 2980 | 292 | 33 |
| CT+N | 3 | 8.10 | 222 | 1.0 | 5.1 | 8.29 | 124 | 1.4 | 2.9 | 1968 | 2218 | 131 | 27 |
| CTF-N | 1 | 8.01 | 313 | 1.3 | 4.7 | 8.24 | 113 | 2.4 | 4.4 | 0 | 2663 | 565 | 40 |
| CTF-N | 2 | 7.92 | 357 | 1.8 | 4.0 | 8.26 | 86 | 1.4 | 5.0 | 0 | 2679 | 283 | 31 |
| CTF-N | 3 | 8.08 | 317 | 1.6 | 8.1 | 8.20 | 144 | 1.9 | 8.0 | 0 | 2631 | 198 | 29 |
| CTF+N | 1 | 8.02 | 312 | 1.4 | 6.7 | 8.29 | 122 | 2.0 | 6.8 | 269 | 1994 | 307 | 31 |
| CTF+N | 2 | 8.02 | 320 | 0.9 | 4.4 | 8.42 | 82 | 2.2 | 7.8 | 2758 | 2377 | 331 | 35 |
| CTF+N | 3 | 8.03 | 352 | 0.8 | 2.3 | 8.20 | 136 | 2.4 | 11.1 | 1196 | 2176 | 185 | 31 |
| BS-3 | 1 | 7.99 | 335 | 1.6 | 5.7 | 7.86 | 127 | 3.1 | 7.5 | 1179 | 3428 | 468 | 31 |
| BS-3 | 2 | 7.95 | 440 | 1.9 | 4.1 | 8.15 | 103 | 2.2 | 7.2 | 796 | 3428 | 482 | 41 |
| BS-3 | 3 | 8.04 | 307 | 2.2 | 4.6 | 8.22 | 157 | 2.4 | 6.9 | 1293 | 3344 | 185 | 28 |
| BS-6 | 1 | 7.98 | 450 | 1.0 | 5.3 | 8.07 | 130 | 1.9 | 7.8 | 1531 | 3004 | 347 | 37 |
| BS-6 | 2 | 7.95 | 365 | 3.0 | 7.6 | 8.05 | 222 | 2.0 | 12.3 | 1435 | 3578 | 282 | 44 |
| BS-6 | 3 | 7.93 | 411 | 2.4 | 6.6 | 8.19 | 187 | 1.7 | 5.1 | 602 | 4062 | 291 | 52 |
| BS-9 | 1 | 7.80 | 533 | 1.7 | 9.9 | 8.15 | 250 | 2.3 | 7.7 | 1532 | 3045 | 552 | 41 |
| BS-9 | 2 | 7.92 | 537 | 1.2 | 8.8 | 8.08 | 199 | 2.4 | 4.7 | 564 | 4005 | 357 | 48 |
| BS-9 | 3 | 7.91 | 547 | 2.7 | 8.0 | 7.95 | 255 | 2.8 | 9.7 | 3031 | 1682 | 156 | 36 |
| BS-12 | 1 | 7.94 | 673 | 1.0 | 10.2 | 7.92 | 340 | 5.5 | 17.3 | 3036 | 2968 | 347 | 50 |
| BS-12 | 2 | 7.88 | 769 | 2.1 | 8.1 | 7.96 | 371 | 2.3 | 3.5 | 1232 | 2963 | 269 | 49 |
| BS-12 | 3 | 7.91 | 668 | 1.6 | 8.4 | 7.91 | 313 | 1.7 | 5.6 | 583 | 3014 | 229 | 37 |
| BS-15 | 1 | 7.84 | 936 | 3.7 | 10.5 | 7.87 | 317 | 2.2 | 10.7 | 2516 | 3177 | 689 | 67 |
| BS-15 | 2 | 7.90 | 877 | 3.0 | 12.0 | 7.59 | 443 | 2.9 | 10.8 | 1627 | 3444 | 332 | 51 |
| BS-15 | 3 | 7.80 | 941 | 3.6 | 14.8 | 8.00 | 364 | 2.9 | 9.3 | 656 | 2561 | 186 | 35 |
| A50-3 | 1 | 8.07 | 300 | 2.4 | 6.4 | 8.35 | 85 | 2.1 | 6.9 | 1010 | 3475 | 396 | 33 |
| A50-3 | 2 | 8.02 | 313 | 1.3 | 6.9 | 8.32 | 129 | 2.1 | 4.1 | 2057 | 2573 | 230 | 37 |
| A50-3 | 3 | 8.13 | 282 | 2.3 | 6.5 | 8.33 | 142 | 1.9 | 15.3 | 360 | 2149 | 178 | 32 |
| A50-6 | 1 | 8.01 | 458 | 1.4 | 6.7 | 8.25 | 112 | 2.2 | 6.5 | 637 | 2238 | 522 | 48 |
| A50-6 | 2 | 8.02 | 379 | 2.1 | 7.9 | 8.26 | 132 | 2.9 | 7.5 | 899 | 3639 | 230 | 46 |
| A50-6 | 3 | 8.05 | 386 | 2.5 | 5.8 | 8.26 | 142 | 1.7 | 7.6 | 2509 | 2127 | 119 | 30 |
| A50-9 | 1 | 8.02 | 450 | 1.1 | 4.9 | 8.26 | 130 | 2.1 | 8.6 | 2100 | 2857 | 465 | 44 |
| A50-9 | 2 | 7.92 | 502 | 2.2 | 5.3 | 8.27 | 127 | 2.4 | 9.9 | 1132 | 3328 | 413 | 44 |
| A50-9 | 3 | 7.83 | 505 | 1.8 | 4.8 | 8.26 | 150 | 1.6 | 9.9 | 5301 | 2936 | 161 | 31 |

A.8 (cont.)

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A50-12 | 1 | 7.89 | 619 | 2.4 | 7.0 | 8.24 | 165 | 1.5 | 2.5 | 6033 | 3301 | 351 | 40 |
| A50-12 | 2 | 7.72 | 860 | 0.9 | 6.3 | 8.23 | 153 | 2.2 | 6.6 | 3758 | 3548 | 319 | 57 |
| A50-12 | 3 | 8.01 | 571 | 2.6 | 5.4 | 8.24 | 136 | 2.0 | 5.3 | 4027 | 3342 | 253 | 48 |
| A50-15 | 1 | 7.99 | 802 | 2.9 | 6.8 | 8.28 | 153 | 1.9 | 4.6 | 1990 | 2826 | 437 | 37 |
| A50-15 | 2 | 7.90 | 853 | 2.1 | 9.6 | 8.28 | 130 | 3.7 | 39.1 | 0 | 3320 | 218 | 35 |
| A50-15 | 3 | 7.84 | 892 | 2.3 | 3.9 | 8.29 | 177 | 2.1 | 7.5 | 677 | 2879 | 200 | 42 |
| A75-3 | 1 | 8.02 | 352 | 1.9 | 5.6 | 8.32 | 98 | 1.9 | 4.1 | 3139 | 3236 | 343 | 36 |
| A75-3 | 2 | 7.94 | 296 | 2.2 | 7.7 | 8.25 | 116 | 1.7 | 8.4 | 4877 | 3053 | 278 | 42 |
| A75-3 | 3 | 7.98 | 348 | 1.0 | 4.2 | 8.33 | 104 | 1.6 | 5.3 | 1058 | 2243 | 210 | 33 |
| A75-6 | 1 | 7.95 | 470 | 2.4 | 12.1 | 8.18 | 138 | 2.9 | 6.7 | 2878 | 2537 | 583 | 49 |
| A75-6 | 2 | 8.01 | 472 | 2.5 | 6.1 | 8.20 | 150 | 2.1 | 2.8 | 937 | 2554 | 239 | 31 |
| A75-6 | 3 | 8.02 | 415 | 2.2 | 6.4 | 8.21 | 162 | 2.1 | 5.4 | 152 | 2287 | 130 | 31 |
| A75-9 | 1 | 7.98 | 473 | 0.9 | 5.3 | 8.16 | 161 | 2.0 | 3.1 | 1508 | 3990 | 654 | 52 |
| A75-9 | 2 | 8.01 | 527 | 2.0 | 4.6 | 8.15 | 146 | 1.8 | 5.5 | 1951 | 3836 | 401 | 45 |
| A75-9 | 3 | 8.00 | 507 | 3.0 | 7.7 | 8.18 | 192 | 2.1 | 2.8 | 2379 | 2304 | 236 | 43 |
| A75-12 | 1 | 7.93 | 767 | 2.7 | 6.5 | 8.06 | 350 | 2.0 | 4.6 | 1887 | 1852 | 253 | 32 |
| A75-12 | 2 | 7.90 | 665 | 2.8 | 6.3 | 8.13 | 229 | 1.9 | 8.4 | 764 | 3475 | 252 | 45 |
| A75-12 | 3 | 7.77 | 726 | 2.5 | 5.3 | 8.16 | 184 | 2.0 | 10.4 | 746 | 2704 | 274 | 52 |
| A75-15 | 1 | 7.88 | 821 | 3.4 | 6.9 | 7.84 | 286 | 2.3 | 5.6 | 2612 | 3696 | 535 | 65 |
| A75-15 | 2 | 7.95 | 737 | 2.3 | 8.2 | 7.94 | 213 | 2.6 | 8.3 | 1131 | 3609 | 324 | 43 |
| A75-15 | 3 | 7.89 | 756 | 3.0 | 15.5 | 7.95 | 401 | 2.4 | 8.4 | 858 | 3899 | 241 | 48 |

* Description of each treatment is given in Table 2.1

## Appendix 9.

**Data collected for various treatments on *Meloidogyne hapla* (eggs/g of fresh root); bacterial and fungal colony forming units at baseline and end (CFU/g of dry soil); pH and electrical conductivity (µS/cm) at baseline and end; above ground biomass (g); below ground biomass (g). Spring 2008.**

| | | Baseline | | | | End | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Treat.* | Rep. | pH | EC | Bacterial CFU*$10^6$ | Fungal CFU*$10_4$ | pH | EC | Bacterial CFU*$10^6$ | Fungal CFU*$10_4$ | *M. hapla* Eggs | Above ground mass | Below ground mass |
| CTAH | 1 | 7.95 | 661 | 1.4 | 5.4 | 8.21 | 173 | 0.5 | 4.3 | 20 | 10.4 | 6.5 |
| CTAH | 2 | 7.92 | 655 | 1.0 | 6.7 | 8.24 | 283 | 0.6 | 3.1 | 25 | 5.4 | 1.7 |
| CTAH | 3 | 7.96 | 650 | 1.2 | 5.7 | 8.33 | 271 | 0.6 | 3.7 | 7 | 5.9 | 4.6 |
| AH-2.5 | 1 | 7.96 | 720 | 0.9 | 3.5 | 8.47 | 105 | 0.7 | 2.5 | 2 | 10.5 | 6.9 |
| AH-2.5 | 2 | 7.97 | 722 | 0.8 | 5.1 | 8.40 | 140 | 0.7 | 1.8 | 1 | 9.9 | 8.2 |
| AH-2.5 | 3 | 8.02 | 723 | 0.9 | 4.4 | 8.33 | 281 | 0.8 | 2.8 | 0 | 9.4 | 8.9 |
| AH-5.0 | 1 | 7.99 | 838 | 1.4 | 3.2 | 8.32 | 201 | 0.7 | 2.2 | 0 | 10.7 | 8.2 |
| AH-5.0 | 2 | 8.00 | 819 | 1.1 | 5.1 | 8.43 | 196 | 0.6 | 2.3 | 0 | 12.2 | 6.7 |
| AH-5.0 | 3 | 8.01 | 809 | 1.4 | 6.7 | 8.47 | 123 | 0.8 | 2.7 | 0 | 13.5 | 9.8 |
| AH-7.5 | 1 | 7.94 | 935 | 1.5 | 5.1 | 8.22 | 361 | 3.1 | 1.2 | 0 | 18.3 | 18.9 |
| AH-7.5 | 2 | 8.00 | 879 | 0.9 | 6.0 | 8.27 | 330 | 2.0 | 1.4 | 0 | 14.6 | 12.5 |
| AH-7.5 | 3 | 7.99 | 911 | 0.8 | 3.2 | 8.36 | 221 | 2.2 | 1.5 | 0 | 15.2 | 12.4 |
| AH-10.0 | 1 | 8.01 | 941 | 0.9 | 3.2 | 8.31 | 278 | 0.6 | 4.0 | 0 | 13.0 | 12.9 |
| AH-10.0 | 2 | 8.02 | 918 | 0.9 | 4.4 | 8.25 | 295 | 0.6 | 1.2 | 1 | 14.2 | 17.1 |
| AH-10.0 | 3 | 8.04 | 956 | 0.7 | 5.7 | 8.26 | 355 | 0.6 | 1.9 | 1 | 18.2 | 12.6 |
| AH-12.5 | 1 | 7.97 | 995 | 0.9 | 2.9 | 8.26 | 330 | 1.1 | 2.1 | 0 | 11.8 | 13.3 |
| AH-12.5 | 2 | 8.11 | 938 | 0.9 | 5.4 | 8.25 | 359 | 0.8 | 0.6 | 0 | 14.4 | 12.7 |
| AH-12.5 | 3 | 8.05 | 965 | 0.9 | 2.6 | 8.22 | 336 | 0.9 | 17.9 | 0 | 12.8 | 14.7 |
| CTBS | 1 | 8.00 | 669 | 1.2 | 5.1 | 8.25 | 132 | 1.9 | 2.6 | 14666 | 18.1 | 6.8 |
| CTBS | 2 | 8.16 | 650 | 1.2 | 3.9 | 8.22 | 199 | 0.9 | 1.6 | 1673 | 14.4 | 6.5 |
| CTBS | 3 | 8.11 | 641 | 1.1 | 3.9 | 8.24 | 147 | 1.1 | 2.1 | 3155 | 13.5 | 6.4 |
| BS-2.5 | 1 | 8.12 | 760 | 1.2 | 6.0 | 8.17 | 246 | 0.9 | 3.3 | 12028 | 21.0 | 9.4 |
| BS-2.5 | 2 | 8.19 | 733 | 1.3 | 4.7 | 8.24 | 177 | 1.0 | 3.5 | 23528 | 28.5 | 16.8 |
| BS-2.5 | 3 | 8.20 | 742 | 1.2 | 5.1 | 8.22 | 193 | 1.9 | 2.7 | 10403 | 15.9 | 5.6 |
| BS-5.0 | 1 | 8.06 | 976 | 1.4 | 6.2 | 8.08 | 401 | 1.3 | 0.5 | 2320 | 26.8 | 12.7 |
| BS-5.0 | 2 | 7.98 | 968 | 1.4 | 4.7 | 8.07 | 366 | 1.4 | 1.3 | 12949 | 27.5 | 12.5 |
| BS-5.0 | 3 | 7.96 | 960 | 1.5 | 5.3 | 8.15 | 326 | 2.4 | 1.4 | 3887 | 28.7 | 18.7 |
| BS-7.5 | 1 | 8.13 | 1022 | 1.5 | 5.9 | 8.07 | 310 | 2.3 | 3.2 | 9244 | 30.5 | 14.8 |
| BS-7.5 | 2 | 8.03 | 1035 | 1.4 | 7.1 | 8.14 | 360 | 1.6 | 2.2 | 3016 | 29.2 | 16.9 |
| BS-7.5 | 3 | 8.06 | 1028 | 1.5 | 3.8 | 7.97 | 588 | 1.8 | 2.8 | 10577 | 20.2 | 6.8 |
| BS-10.0 | 1 | 8.12 | 1077 | 2.0 | 5.4 | 8.00 | 513 | 2.0 | 8.1 | 25147 | 22.2 | 12.3 |
| BS-10.0 | 2 | 8.12 | 1097 | 1.9 | 9.1 | 7.98 | 415 | 3.1 | 3.6 | 7122 | 26.4 | 13.0 |
| BS-10.0 | 3 | 8.15 | 1054 | 2.0 | 6.8 | 8.08 | 407 | 1.2 | 5.8 | 8297 | 41.5 | 16.1 |
| BS-12.5 | 1 | 8.12 | 1242 | 2.5 | 6.4 | 8.05 | 421 | 1.7 | 2.3 | 2628 | 39.5 | 17.4 |
| BS-12.5 | 2 | 8.12 | 1269 | 2.5 | 9.5 | 8.05 | 466 | 2.6 | 3.8 | 5748 | 38.2 | 17.9 |

A.9 (cont.)

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BS-12.5 | 3 | 7.98 | 1278 | 2,2 | 8.2 | 8.03 | 436 | 2.4 | 16.2 | 10368 | 47.6 | 25.6 |
| CTA50 | 1 | 7.97 | 119 | 1.4 | 5.9 | 8.16 | 160 | 1.8 | 3.1 | 8570 | 28.2 | 4.9 |
| CTA50 | 2 | 7.97 | 122 | 1.5 | 3.1 | 8.30 | 155 | 1.8 | 5.0 | 4899 | 23.8 | 5.0 |
| CTA50 | 3 | 8.00 | 115 | 1.4 | 3.4 | 8.51 | 142 | 1.0 | 1.2 | 9363 | 21.5 | 5.0 |
| A50-2.5 | 1 | 7.91 | 246 | 1.6 | 4.0 | 8.48 | 150 | 1.6 | 1.9 | 18810 | 21.5 | 5.4 |
| A50-2.5 | 2 | 7.95 | 206 | 1.9 | 4.9 | 8.49 | 181 | 2.1 | 6.0 | 7906 | 16.0 | 3.9 |
| A50-2.5 | 3 | 7.93 | 237 | 2.0 | 3.1 | 8.51 | 186 | 2.1 | 1.2 | 9799 | 28.5 | 6.1 |
| A50-5.0 | 1 | 7.89 | 337 | 2.0 | 2.8 | 8.37 | 247 | 1.9 | 4.4 | 19455 | 20.3 | 6.4 |
| A50-5.0 | 2 | 7.93 | 320 | 1.9 | 4.0 | 8.50 | 184 | 2.4 | 1.9 | 19146 | 17.3 | 8.0 |
| A50-5.0 | 3 | 7.93 | 338 | 1.9 | 3.7 | 8.49 | 160 | 2.1 | 6.3 | 20021 | 27.3 | 7.1 |
| A50-7.5 | 1 | 7.87 | 415 | 1.9 | 5.8 | 8.21 | 335 | 1.6 | 1.9 | 25157 | 22.5 | 7.0 |
| A50-7.5 | 2 | 7.87 | 437 | 1.5 | 4.0 | 8.29 | 298 | 1.8 | 4.6 | 13582 | 15.8 | 6.1 |
| A50-7.5 | 3 | 7.88 | 428 | 1.6 | 5.8 | 8.35 | 257 | 1.9 | 2.2 | 21598 | 22.7 | 5.5 |
| A50-10.0 | 1 | 7.83 | 570 | 1.6 | 4.8 | 8.23 | 438 | 1.5 | 5.4 | 29179 | 24.8 | 7.1 |
| A50-10.0 | 2 | 7.81 | 601 | 1.8 | 6.7 | 8.33 | 373 | 1.6 | 1.3 | 37124 | 29.0 | 7.5 |
| A50-10.0 | 3 | 7.85 | 554 | 1.8 | 6.1 | 8.20 | 444 | 2.2 | 6.3 | 24392 | 31.3 | 7.9 |
| A50-12.5 | 1 | 7.78 | 764 | 2.7 | 4.5 | 8.16 | 540 | 2.8 | 3.2 | 42542 | 25.9 | 11.0 |
| A50-12.5 | 2 | 7.80 | 681 | 2.6 | 6.9 | 8.19 | 599 | 1.6 | 2.5 | 73587 | 14.6 | 7.0 |
| A50-12.5 | 3 | 7.81 | 725 | 2.3 | 4.8 | 8.21 | 574 | 1.7 | 2.5 | 33332 | 35.5 | 8.0 |
| CTA75 | 1 | 7.98 | 623 | 0.7 | 8.7 | 8.01 | 162 | 1.7 | 3.8 | 11006 | 22.4 | 4.0 |
| CTA75 | 2 | 7.99 | 616 | 0.8 | 5.6 | 8.13 | 138 | 1.8 | 1.6 | 0 | 43.0 | 8.5 |
| CTA75 | 3 | 8.03 | 618 | 1.0 | 6.2 | 8.20 | 135 | 1.5 | 1.9 | 20820 | 15.8 | 3.5 |
| A75-2.5 | 1 | 8.06 | 731 | 0.9 | 2.5 | 8.02 | 195 | 1.7 | 2.5 | 21342 | 20.2 | 4.5 |
| A75-2.5 | 2 | 8.08 | 738 | 1.0 | 6.2 | 8.19 | 174 | 1.9 | 4.1 | 29578 | 15.0 | 5.1 |
| A75-2.5 | 3 | 8.05 | 740 | 1.5 | 2.8 | 8.20 | 223 | 2.1 | 3.1 | 12466 | 31.3 | 6.9 |
| A75-5.0 | 1 | 8.08 | 832 | 1.5 | 8.4 | 8.07 | 311 | 2.1 | 2.8 | 0 | 35.0 | 8.1 |
| A75-5.0 | 2 | 8.00 | 848 | 1.4 | 5.6 | 8.05 | 317 | 2.2 | 5.1 | 15676 | 25.4 | 8.6 |
| A75-5.0 | 3 | 8.10 | 809 | 1.5 | 5.8 | 8.19 | 257 | 2.4 | 3.1 | 0 | 12.1 | 2.8 |
| A75-7.5 | 1 | 8.02 | 944 | 1.6 | 4.3 | 8.05 | 402 | 2.5 | 3.1 | 13296 | 29.8 | 15.3 |
| A75-7.5 | 2 | 8.09 | 941 | 1.8 | 6.4 | 8.00 | 479 | 2.2 | 2.2 | 27199 | 26.6 | 5.8 |
| A75-7.5 | 3 | 8.10 | 923 | 1.5 | 6.5 | 8.03 | 442 | 2.2 | 2.9 | 10311 | 34.5 | 16.4 |
| A75-10.0 | 1 | 8.04 | 997 | 1.7 | 6.2 | 8.05 | 492 | 2.9 | 4.1 | 0 | 23.3 | 14.1 |
| A75-10.0 | 2 | 8.03 | 1021 | 1.3 | 5.1 | 7.97 | 484 | 2.4 | 2.6 | 0 | 27.9 | 16.1 |
| A75-10.0 | 3 | 7.99 | 1064 | 1.8 | 4.0 | 8.05 | 466 | 2.4 | 3.2 | 31112 | 35.7 | 15.4 |
| A75-12.5 | 1 | 8.00 | 1167 | 2.4 | 6.6 | 7.99 | 460 | 3.7 | 1.9 | 0 | 30.0 | 11.3 |
| A75-12.5 | 2 | 8.00 | 1098 | 2.6 | 4.7 | 7.97 | 517 | 3.9 | 1.9 | 0 | 38.5 | 18.3 |
| A75-12.5 | 3 | 8.05 | 1100 | 2.3 | 8.0 | 7.97 | 604 | 4.0 | 4.4 | 25931 | 36.8 | 13.4 |

* Description of each treatment is given in Table 2.3

## Appendix 10.

**Recommended upper limits and critical concentrations of potentially toxic metals in soils after receiving sludge and waste materials. Concentration in mg $kg^{-1}$. (Data from Alloway, 1995; Kabata-Pendias, 2001; Pais and Jones, 1997).**

| Element | Rec. upper limit after sludge application* | Critical soil total conc.** |
|---|---|---|
| As | 20 | |
| Cd | 3 | |
| Cr | 600 | 75-100 |
| Cu | 135 | 60-125 |
| Ni | 75 | 100 |
| Pb | 250 | 100-400 |
| Zn | 300 | 70-400 |

* Recommended upper limits for potentially toxic metals in the upper 15 cm of soil after sludge application.

** Critical soil concentrations of metals allowed in soils receiving waste materials.

## Appendix 11.

### Mehlich III extraction procedure (Mehlich, 1984)

Reagents:

Mehlich 3 (M3) is composed of 0.2M $CH_3COOH$, 0.25M $NH_4N0_3$, 0.015M $NH_4F$, 0.013M $HN0_3$, and 0.001M EDTA

Apparatus:

1. Precision balance
2. Reciprocating shaker
3. 125 ml Erlenmeyer flasks
4. Filter funnels
5. Filter paper (Whatman No. 42)
6. 50 ml glass vials
7. Disposable plastic tubes

Procedure:

1. Weigh 3g of < 2 mm soil from the soil sample into a 125 ml Erlenmeyer flask.
2. Add 30 ml of Mehlich III extraction solution
3. Shake immediately on the reciprocating shaker for 7 minutes
4. Filter through No.42 Whatman filter paper and save the filtrate in glass vials.
5. Pour approximately 10 ml of the filtrate into labeled plastic tubes.
6. Immediately analyze solution with ICP-AES.

## Appendix 12

**Concentration of elements in various treatments used in the 2006, 2007 and 2008 field seasons. Concentration in mg $kg^{-1}$.**

| | Element concentration (mg $kg^{-1}$) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Treatment* | As | B | Ca | Cd | Cr | Cu | Fe | K | Mg | Mn | Ni | P | Pb | Zn |
| CT-N | 0.1 | 1.3 | 3781 | 0.1 | 0.1 | 1.3 | 72 | 40 | 119 | 72 | 0.5 | 11.3 | 1.8 | 1.3 |
| CT+N | 0.0 | 1.4 | 3692 | 0.1 | 0.1 | 1.3 | 72 | 40 | 120 | 72 | 0.5 | 11.7 | 1.8 | 1.3 |
| CTF-N | 0.1 | 1.2 | 3380 | 0.1 | 0.2 | 1.2 | 73 | 37 | 111 | 69 | 0.4 | 13.0 | 1.7 | 1.2 |
| CTF+N | 0.2 | 1.5 | 3313 | 0.1 | 0.2 | 1.2 | 72 | 36 | 110 | 67 | 0.4 | 13.1 | 1.6 | 1.2 |
| BS-3 | 0.2 | 2.1 | 4054 | 0.1 | 0.2 | 1.8 | 81 | 50 | 145 | 74 | 0.4 | 26.9 | 2.0 | 2.0 |
| BS-6 | 0.1 | 1.7 | 4003 | 0.1 | 0.1 | 1.9 | 77 | 47 | 148 | 66 | 0.3 | 37.2 | 2.0 | 2.3 |
| BS-9 | 0.4 | 1.2 | 4023 | 0.1 | 0.2 | 2.4 | 87 | 49 | 160 | 63 | 0.3 | 62.8 | 2.2 | 3.4 |
| BS-12 | 0.4 | 0.8 | 4224 | 0.1 | 0.2 | 2.7 | 88 | 57 | 181 | 79 | 0.3 | 61.7 | 2.3 | 3.7 |
| BS-15 | 0.3 | 0.8 | 5062 | 0.1 | 0.2 | 3.2 | 91 | 65 | 208 | 64 | 0.3 | 80.7 | 2.6 | 5.0 |
| A50-3 | 0.5 | 2.2 | 2988 | 0.1 | 0.3 | 1.1 | 80 | 32 | 105 | 50 | 0.3 | 24.7 | 1.4 | 1.8 |
| A50-6 | 0.3 | 2.7 | 3707 | 0.1 | 0.3 | 1.5 | 82 | 46 | 132 | 61 | 0.3 | 27.9 | 1.7 | 2.5 |
| A50-9 | 0.6 | 4.5 | 3836 | 0.1 | 0.3 | 1.9 | 93 | 50 | 148 | 60 | 0.4 | 48.7 | 2.0 | 2.5 |
| A50-12 | 0.8 | 5.8 | 4570 | 0.1 | 0.3 | 2.3 | 98 | 57 | 172 | 67 | 0.4 | 52.2 | 2.3 | 3.1 |
| A50-15 | 0.6 | 6.1 | 4216 | 0.1 | 0.3 | 2.5 | 109 | 58 | 170 | 60 | 0.4 | 66.9 | 2.1 | 3.4 |
| A75-3 | 0.3 | 1.8 | 3608 | 0.1 | 0.1 | 1.4 | 79 | 43 | 127 | 65 | 0.4 | 25.5 | 1.9 | 1.8 |
| A75-6 | 0.3 | 2.6 | 4185 | 0.2 | 0.2 | 2.0 | 89 | 50 | 153 | 69 | 0.4 | 42.0 | 2.3 | 2.5 |
| A75-9 | 0.7 | 3.1 | 3864 | 0.1 | 0.3 | 2.2 | 95 | 49 | 156 | 60 | 0.4 | 62.6 | 2.1 | 3.0 |
| A75-12 | 0.5 | 3.5 | 4009 | 0.1 | 0.3 | 2.3 | 92 | 53 | 165 | 58 | 0.3 | 61.1 | 2.1 | 3.4 |
| A75-15 | 0.5 | 4.6 | 5530 | 0.1 | 0.3 | 3.6 | 103 | 72 | 223 | 60 | 0.4 | 90.8 | 2.7 | 5.5 |

* Description of each treatment is given in Table 2.1

## Appendix 13

**Historical data for biosolids and fly ash. All elements were measured using the Mehlich III soil extractant. Concentration of elements in mg $kg^{-1}$. Organic matter content (OM) as percentage.**

| | Biosolids* (n=20) | Fly ash** (n=6) |
|---|---|---|
| pH | 7.87 | 8.32 |
| % OM | 9.5 | Nd |
| $NH_4$ | 114 | Nd |
| $NO_3$ | 38.491 | Nd |
| As | 0.62 | 6.07 |
| B | 1.78 | 344 |
| Ca | 16170 | 12899 |
| Cd | 0.03 | 0.09 |
| Cr | 0.48 | 3.21 |
| Cu | 9.38 | 7.22 |
| Fe | 302 | 353 |
| K | 240 | 121 |
| Mg | 780 | 1329 |
| Mn | 98 | 11 |
| Ni | 1 | 2.52 |
| P | 171 | 588 |
| Pb | 6 | 2.01 |
| Zn | 22 | 7.82 |

*North pond, City of Glencoe, ON

** Lambton county power generating station, Lambton, ON