RESEARCH NOTE/NOTA INVESTIGATIVA

THE INFLUENCE OF SOIL AMENDMENTS (FLY ASH AND STABILIZED BIOSOLIDS) ON *MELOIDOGYNE HAPLA* IN MICROPLOTS PLANTED WITH TOMATO (*LYCOPERSICON ESCULENTUM*)

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ABSTRACT

Jaramillo-López, P. F., M. A. Powell, and D. B. Hayden. 2011. The influence of soil amendments (fly ash and stabilized biosolids) on *Meloidogyne hapla* in microplots planted with tomato (*Lycopersicon esculentum*). Nematropica 41:141-149.

The use of amendments composed of fly ash and stabilized biosolids were evaluated for managing *Meloidogyne hapla* on tomato (*Lycopersicon esculentum* P. Mill.). Amendment ratios of biosolids and fly ash were established based on international guidelines and previous studies for the use of biosolids and fly ash in soils. The field trial included amendments of 3%, 6%, 9%, 12%, and 15% (w/w) of biosolids and 3%, 6%, 9%, 12%, and 15% w/w) of a 50:50 mixture of fly ash and biosolids. Changes in parameters that might affect nematode viability were monitored including electrical conductivity, pH, biocontrol agents (bacterial and fungal colony forming units), and plant yield. Tomato plants grown with amendments showed improved yields relative to controls in the presence of nematode infestation. None of the amendments altered EC, pH or CFU enough to impact nematode populations.

Key words: biosolids, fly ash, *Meloidogyne hapla*, soil amendments, tomato.

RESUMEN

Jaramillo-López, P. F., M. A. Powell, and D. B. Hayden. 2011. La influencia de enmiendas de suelo (ceniza volante y biosólidos estabilizados) sobre *Meloidogyne hapla* en microparcelas plantadas con tomate (*Lycopersicon esculentum*). Nematropica 41:141-149.

El uso de enmiendas de suelo compuestas por ceniza volante de carbón mineral y biosólidos estabilizados fue evaluado para manejar *Meloidogyne hapla* en tomate (*Lycopersicon esculentum* P. Mill.). Las tasas de aplicación de biosólidos y ceniza volante fueron establecidas según normativas internacionales para el uso de ceniza y biosólidos en suelo. El experimento en campo incluyó enmiendas del 3%, 6%, 9%, 12%, y 15% en peso de biosólidos y 3%, 6%, 9%, 12%, y 15% en peso de una mezcla de 50:50 de ceniza y biosólidos. Los cambios en los parámetros que pueden afectar la viabilidad de los nematodos fue monitoreada incluyendo conductividad eléctrica, pH, agentes de biocontrol (unidades formadoras de colonia de hongos y bacterias), y rendimiento de las plantas. Las plantas de tomate que crecieron en las enmiendas mostraron un incremento en rendimiento inclusive con infestación de nematodos al compararlos con los controles. Ninguna de las enmiendas alteró la conductividad, pH, o las unidades formadoras de colonias lo suficiente como para causar un impacto sobre las poblaciones de nematodos.

Palabras clave: biosólidos, ceniza volante, enmiendas de suelo, *Meloidogyne hapla*, tomate.

The plant-parasitic northern root-knot nematode (*Meloidogyne hapla* Chitwood) 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). Nematodes from the genus *Meloidogyne* affect tomato plants by infecting their roots, resulting in poor nutrient absorption and reduced yields (Hussey, 1985). Due to nematode ubiquity and resilience, they are very difficult to manage. Traditionally, nematode problems have been dealt with by applying heavy doses of chemical pesticides. 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). 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 infestations (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).

Due to banning and environmental limitations, new approaches to manage nematodes need to be developed. 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). Additionally, commercially available mixtures of biosolids and fly ash applied to soils under laboratory conditions have been shown to have several detrimental effects on plant-parasitic nematodes, for example increased pH and the release of ammonia (Zasada and Tenuta 2004; Zasada, 2005). The objective of this study was to determine if mixtures of stabilized biosolids and coal combustion fly ash have the potential to reduce populations of *M. hapla* under field conditions. Specific objectives were to: (i) determine if the physical and chemical changes in the amended soils produce nematicidal effects, (ii) determine the effects of the amendments on fungal and bacterial activity, and (iii) determine if the amendments affect tomato yield.

Field experiment: Forty-two microplots (12 l plastic pots) filled with different soil and amendment ratios were established in a random design at the Environmental Sciences Western (ESW) Field Station, Ilderton, Ontario, Canada. Pits for placement of the pots were dug using a tractor auger with a spacing of 0.75 m between pots and 1.5 m between rows. The soil (Bryanston Silt Loam) used for the experiment was altered by the addition of brick sand resulting in a texture of 46% sand, 46% silt, and 8% clay (sandy loam). 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 pots filled with treatments (Table 1). One tomato seedling was

Table 1. Treatment ratios used to produce growth media for field microplot experiment with *Meloidogyne hapla* (N) and tomato (*Lycopersicon esculentum* Mill). (Summer 2008).

Treatment	Description
CT-N	Control (soil only) without inoculum
CT+N	Control (soil only)[z]
CTF-N	Control with chemical fertilizer (F) without inoculum
CTF+N	Control with chemical fertilizer[z]
BS-3	3 % biosolids with 97 % soil[z]
BS-6	6 % biosolids with 94 % soil[z]
BS-9	9 % biosolids with 91 % soil[z]
BS-12	12 % biosolids with 88 % soil[z]
BS-15	15 % biosolids with 85 % soil[z]
A50-3	3 % of a 50% biosolids:50% fly ash mixture and 97 % soil[z]
A50-6	6 % of a 50% biosolids:50% fly ash mixture and 94 % soil[z]
A50-9	9 % of a 50% biosolids:50% fly ash mixture and 91 % soil[z]
A50-12	12 % of a 50% biosolids:50% fly ash mixture and 88 % soil[z]
A50-15	15 % of a 50% biosolids:50% fly ash mixture and 85 % soil[z]

[z]Treatments that received *M. hapla* inoculum

transplanted to each pot and the pots placed in the previously dug pits (microplots). Fertilized 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 microplot. The (CTF) microplots were subsequently fertilized with the same rate two additional times, one at the middle of the growing season and one after the plants had fruited as recommended by Keith McKell, Soil Smith, Ltd, London, Ontario, Canada.

Amendment components: Stabilized biosolids were obtained from the municipal sewage settling ponds from the town of Glencoe, Ontario, Canada. The biosolids were dewatered, transported to the ESW, placed on a concrete pad for drying and subsequently crushed with a roller attached to a tractor. The material was screened using a 0.635 cm screen and the <0.635 cm fraction was used to prepare the treatments.

The fly ash used came from the Lambton coal-fueled generating station (St. Clair River, St. Clair Township, Ontario, Canada) stockpiled at the ESW and used as received.

Treatment preparation: Treatment 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). Treatments were prepared from soil+fly ash+biosolids and mixed using an electric cement mixer according to the ratios in Table 1. The materials were added to the hopper of a rotating cement mixer in the order: soil-biosolids-fly ash in order to prevent clumping. The treatments were mixed approximately 5 minutes or until homogeneous. After treatments were prepared samples were taken for baseline analyses and pots were filled.

Meloidogyne hapla inoculum: Eggs of *M. hapla* were extracted from roots of infested tomato plants that had been kept in the greenhouse following the procedure by Hussey and Barker (1973) followed by centrifugal flotation. Transplanted tomato plants were allowed to grow in the microplots for 20 days before initial inoculation. All treatments were inoculated with the exception of the control (soil only, CT-N) and control with fertilizer (CTF-N). Each microplot received two, 5 ml aliquots of nematode (eggs and juveniles) suspension delivered with a 5 ml pipette in two holes 7 cm deep and 5 cm away from the main stem of the plant. Each microplot received approximately 11,320 eggs and 270 juveniles. Pots were reinoculated 51 days after initial inoculation using homogenized (separated, chopped and mixed) infested roots from the same tomato plants from which the initial inoculum was obtained. A 15 cm deep hole was made 5 cm away from the main tomato stem and 100 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 infested roots was processed using a 1% NaOCl solution. Each 100 ml of infested roots contained approximately 2.9 million *M. hapla* eggs.

Sampling: Soil samples were taken at the beginning (baseline) and at the end of the experiment. All soil samples were processed and analyzed for each variable as described below.

Electrical conductivity (EC) and pH: A 20 g aliquot from each replicate for each treatment was transferred to a 100 ml beaker and 40 ml of deionized water was 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 transferred to a 50 ml graduated cylinder (to accommodate the probe) using a conductivity meter probe for the range 0-1999 µS (HI 8033 Handheld EC/TDS Meter, HANNA Instruments, India, Pvt. Ltd).

Bacterial and fungal colony forming units (CFU): 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 subsample 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 was transferred to a test tube containing 9.9 ml of a peptone blank and vortexed for 30 seconds. One hundred µl of final suspension was plated onto each medium. Triplicates of each media were plated for either bacteria or fungi. 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.

Tomato harvesting: 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 removed from the soil and placed in a shed. Above ground biomass was weighed before pots were taken to the laboratory for determining below ground biomass and processing for nematode egg extraction.

Extraction of M. hapla eggs: Roots of tomato plants were processed following the procedure by Hussey and Barker (1973). Resulting suspensions were standardized to 100 ml and a 15 ml aliquot of each was pipetted 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 the eggs from 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 number of eggs is expressed as eggs per gram of fresh root.

Statistical analysis: Data were subjected to (one-way) ANOVA followed by Tukey's range test (SPSS Statistics 17.0, Chicago, Illinois).

pH: For all the treatments, baseline and harvest pH ranged from 7.9-8.1 and 7.9-8.3, respectively, indicating that the amendments had little effect on pH (Table 2). This is consistent with the findings of Powell and Hart ("unpub. data") who showed that the pH of the fly ash was 8.3 (n=6) and the biosolids was 7.9 (n=20). The data are similar to the field soils with pH of 8.0.

Electrical conductivity: The EC was affected by the addition of biosolids or fly ash plus biosolids (Table 2). At baseline, average EC was 329 μS cm^{-1} in treatments receiving fertilizer and 231 μS cm^{-1} in those without fertilizer. With the addition of biosolids mixed with fly ash, average EC increased to 545 μS cm^{-1} and there was a marked increase with increasing amendment rate going from 298 (A50-3) to 849 (A50-15) μS cm^{-1}. The change in EC with the addition of biosolids was more pronounced, averaging 590 μS cm^{-1} and ranging from 361 (BS-3) to 938 (BS-15) μS cm^{-1} with increasing rates of amendment.

At harvest, the same trends were noticed for each group of treatments. For the controls, there was little change between fertilized and unfertilized treatments with EC of 114 and 107 μS cm^{-1}, respectively. Biosolids plus fly ash treatments had an average EC of 138 μS cm^{-1}, ranging from 119 (A50-3) to 153 (A50-15) μS cm^{-1}. The biosolids only treatments averaged 252 μS cm^{-1}, and ranged from 129 (BS-3) to 375 (BS-15) μS cm^{-1}.

The data indicate that the mixtures of biosolids plus fly ash had less impact on EC than those with only biosolids and that there was a decrease in EC in all groups going from baseline to harvest.

Bacterial and fungal CFU: There are no discernable trends in bacterial CFU with the addition of either biosolids or mixtures of biosolids plus fly ash at baseline (Table 2). Average CFU for controls with and without fertilizer were similar at 1.3^6 and 1.5^6 CFU g^{-1} of dry soil, respectively. Amendments of biosolids or biosolids plus fly ash averaged 2.1^6 and 2.0^6 CFU g^{-1} of dry soil, respectively. While most of the treatments in either group are similar, there are exceptions noted only for the highest amendment within each group; BS-15 with 3.4^6 CFU g^{-1} of dry soil and A50-15 with 2.4^6 CFU g^{-1} of dry soil.

At harvest, bacterial CFU without fertilizer did not change appreciably relative to baseline and averaged 1.6^6 CFU g^{-1} of dry soil. However, bacterial CFU increased significantly in the fertilized treatments averaging 2.1^6 CFU g^{-1} of dry soil. Bacterial CFU at harvest increased over baseline for both the biosolids and biosolids plus fly ash mixtures. Biosolids only treatments averaged 2.6^6 and biosolids plus fly ash averaged 2.2^6 CFU g^{-1} of dry soil. As with the baseline samples, there were no trends noted within each treatment group, or with increasing rate of amendment.

Fungal CFU exhibited significant differences in the baseline samples in the order fertilized (5.1^4 CFU g^{-1} of dry soil) < unfertilized (5.7^4 CFU g^{-1} of dry soil) < biosolids plus fly ash (6.3^4 CFU g^{-1} of dry soil) < biosolids only (8.3^4 CFU g^{-1} of dry soil). Only the biosolids treatments showed a trend within the group, increasing from 4.8^4 to 12.4^4 CFU g^{-1} of dry soil as amendment rates increased. At harvest, unfertilized controls decreased slightly (4.9^4 CFU g^{-1} of dry soil) and biosolids remained approximately the same (8.4^4 CFU g^{-1} of dry soil) while the fertilized treatments (7.2^4 CFU g^{-1} of dry soil) and biosolids plus fly ash (9.5^4 CFU g^{-1} of dry soil) increased relative to the baseline. The trend of increased CFU with increased rate of application seen in the baseline biosolids plus fly ash treatments was not noted at harvest. However, the treatment with the highest application rate (A50-15) had appreciably higher CFU at 17.0^4 g^{-1} of dry soil and this one sample is responsible for the high average for the group.

Meloidogyne hapla eggs: Fertilized and unfertilized treatments which received inoculum contained 1408 and 920 eggs/g fresh root, respectively. The fertilized and unfertilized treatments without inoculum contained no *M. hapla* eggs.

Biosolids treatments averaged 1441 eggs/g fresh root and exhibit a trend, increasing with increasing rates of application, going from BS-3 (1089 eggs/g of fresh root) to BS-9 (1709 eggs/g of fresh root) but then decrease at the two highest application rates (BS-12 and BS-15).

Biosolids plus fly ash treatments (average 2166 eggs/g of fresh root) also showed a partial trend, increasing from 1142 to 4606 eggs/g of fresh root with increasing application rate from A50-3 to A50-12 and then decreasing at the highest application rate (A50-15 with 889 eggs/g of fresh root).

Tomato yield and biomass: Control treatments without fertilizer had lower average yields (1888 g/plant) than those with fertilizer (2420 g/plant). For those treatments receiving nematode inoculum the yield was similar with and without fertilizer (CT+N with 2075 g and CTF+N with 2182 g/plant). However, there was a large difference between the fertilized and unfertilized treatments that did not receive inoculum (CT-N with 1700 g and CTF-N with 2658 g/plant). Biosolids treatments gave the highest average yield (3180 g/plant) but no significant trend was noticed within this group even though the treatments with smaller application rates do show a slight increase in yield over the higher application rates. The opposite is true for the biosolids plus fly ash treatments, where there is a slight, but variable, trend towards higher yields with increasing application rates. This group had an average yield of 2969 g/plant.

As with yield, the above ground biomass decreased in both fertilized and unfertilized treatments that received inoculum; 274 g for fertilized treatments and 170 g for unfertilized with inoculum and 349 g and 216 g without inoculum. The highest average above ground

Table 2. Effect of treatments at baseline or harvest on *Meloidogyne hapla* (eggs/g of fresh root); bacterial and fungal colony forming (CFU g-1 of dry soil); pH and electrical conductivity (µS cm-1); tomato yield (g/plant); above and below ground biomass (g). (n=3 for all treatments, P values <0.05). Baseline (July 8/08) and Harvest (Oct. 14/08).

	Baseline				Harvest							
Treatment[w]	pH[x]	EC[y]	Bacterial CFU $(10^6)^z$	Fungal CFU $(10^4)^z$	pH[x]	EC[y]	Bacterial CFU $(10^6)^z$	Fungal CFU $(10^4)^z$	*M. hapla* eggs	Tomato yield	Above ground mass	Below ground mass
CT-N	8.05bc	228a	1.4a	5.3ab	8.32cd	107a	1.6	5.7	0a	1700a	216	23a
CT+N	8.09c	234a	1.5a	6.0ab	8.34d	107a	1.6	4.0	920a	2075ab	170	27ab
CTF-N	8.00abc	329abc	1.6a	5.6ab	8.23cd	114a	1.9	5.8	0a	2658ab	349	33abc
CTF+N	8.02abc	328abc	1.0a	4.5a	8.30cd	113a	2.2	8.6	1408a	2182ab	274	32abc
BS-3	7.99abc	361abc	1.9ab	4.8ab	8.08abcd	129a	2.6	7.2	1089a	3400b	378	33abc
BS-6	7.95abc	409bcd	2.1ab	6.5ab	8.10bcd	180ab	1.8	8.4	1189a	3548b	307	44abc
BS-9	7.88ab	539de	1.8ab	8.9bc	8.06abc	235b	2.5	7.4	1709ab	2911ab	355	42abc
BS-12	7.91abc	703fg	1.5a	8.9bc	7.93ab	341c	3.2	8.8	1617ab	2982ab	282	45bc
BS-15	7.85a	918h	3.4b	12.4c	7.82a	375c	2.7	10.3	1599ab	3061ab	402	51c
A50-3	8.07c	298ab	1.9ab	6.6ab	8.33cd	119a	2.1	8.8	1142a	2732ab	268	34abc
A50-6	8.03abc	408bcd	2.0ab	6.8ab	8.26cd	129a	2.3	7.2	1348a	2668ab	290	41abc
A50-9	7.92abc	486cd	1.7ab	5.0ab	8.26cd	136a	2.0	9.5	2844ab	3040ab	346	39abc
A50-12	7.87ab	683ef	1.9ab	6.2ab	8.24cd	151ab	1.9	4.8	4606b	3397b	308	48bc
A50-15	7.91abc	849gh	2.4ab	6.8ab	8.28cd	153ab	2.6	17.0	889a	3008ab	285	38abc
P-values	<0.001	<0.001	0.014	<0.001	<0.001	<0.001	>0.05	>0.05	0.003	0.015	>0.05	0.003

[w] Description of each treatment is given in Table 1.
[x] Soil suspension pH determined from a 1:2 soil:water (w/v) suspension.
[y] Soil suspension EC determined from a 1:2 soil:water (w/v) suspension.
[z] Number of CFU is the average count of 9 plates for each treatment.

biomass was seen in the biosolids treatments, averaging 345 g and while there was no appreciable trend with increasing rates of amendment, BS-15 was greatest with 402 g. For the biosolids plus fly ash treatments, the highest values were seen in the middle of the group (A50-9, 346 g, A50-12, 308 g) with a group average of 299 g.

Below ground biomass for controls with and without fertilizer and inoculum did not exhibit the same trends as the above ground biomass. Below ground biomass for the fertilized control treatments averaged 33 g and was similar with or without inoculum. The average for the unfertilized treatments was 25 g and, as with the fertilized treatments, there was little difference between those with and without inoculum. Average below ground biomass was slightly greater (43 g) for the biosolids treatments relative to the biosolids plus fly ash treatments (40 g). A slight trend was noticed in the biosolids treatments with increasing application rates going from 33 g for BS-3 to 51 g for BS-15. No trend was noticed in the biosolids plus fly ash treatments but there was a slight increase towards the middle of the group.

The addition of soil amendments, aimed to manage nematodes might also favor their viability by providing adequate soil conditions for their development. It is possible to increase target crop yields with the addition of soil amendments but that gain might be negated if the amendments also promote increased nematode numbers. This problem is complicated by the fact that the amendments can greatly influence indirect promotion by decreasing biocontrol organisms or increasing metals, or other chemical and physical parameters of the soil that either increase or decrease nematode numbers. An optimal amendment should be designed in such a way that its addition to the soil promotes yield and suppresses nematode populations while taking into consideration the parameters of the soil being used.

The soils for this study are classified as a heavy silt loam, so for the purpose of this study they were modified to a sandy loam by the addition of sand, which nematodes prefer over a heavier soil, Ensuring that textural influence was minimized. The in situ soils typically range in pH from 8.3-8.5. However, the measured pH of the growth media resulting after the addition of sand was fairly constant at 8.0.

The influence of pH on nematodes has been well studied. Practically, plant-parasitic nematode eggs hatch within the range of pH 4-8, greatly decreasing at lower or higher levels (Guerena, 2006). Nematodes thrive at pH 4-6 (Burns, 1970; Korthals *et al.*, 1996a, 1996b; Korthals *et al*., 1998; Bardgett *et al*., 1994, Bouwman *et al*., 2005), depending on the species. Non plant-parasitic nematodes favor pH >6 but plant-parasitic nematodes are greatly reduced at higher pH. Zasada *et al*. (2008) found that the addition of biosolids alone caused a decrease in nematode numbers as the pH reached 8.5. In lab experiments (Zasada and Tenuta, 2004) addition of a commercially available soil amendment decreased plant-parasitic nematodes due to pH increases and the concomitant formation of ammonia. Siddiqui (2005) reported that nematodes were suppressed at pH <5.8. The acidy of the fly ash and biosolids used to make the amendments in this study were 8.3 and 7.9, respectively, and the soil had a pH of 8.3-8.4. Therefore the amendments did not lower the pH of the resulting growth media to a point that would favor either nematode egg hatching or juvenile activity. This may partly explain why even though the microplots were inoculated with over 2.9 million eggs and 270 J2, the number of eggs/g of root recovered at harvest was in the range of 900-4500, well below the level that causes tomato plants to show nematode damage.

Electrical conductivity (EC) is also known to affect nematode survival. Vellidis *et al.* (2006) found that nematode numbers decreased in the range between 500 to 4 nematodes going from EC 5-100 $\mu S\ cm^{-1}$. Another study at higher EC values showed that nematodes were little affected at EC 1945 $\mu S\ cm^{-1}$ but that no nematodes survived above EC 4100 $\mu S\ cm^{-1}$ (Nkem *et al*., 2005). These and numerous other studies have shown that increasing EC results in lower nematode presence. In this study, the addition of biosolids alone or with fly ash increased the EC with increasing application rates at time of planting; unamended media had EC in the range 200-300 $\mu S\ cm^{-1}$ while amended media ranged from 500-1000 $\mu S\ cm^{-1}$. This increase would have affected the numbers of juveniles that survived. However, none of the amendments raised the EC over 1000 $\mu S\ cm^{-1}$, which is within the range of nematode activity. Further, at the time of harvest the EC values of the growth media had dropped to under 400 $\mu S\ cm^{-1}$ which is well below threshold values for nematode survival. Therefore, the addition of low EC biosolids and fly ash cannot be expected to act as a nematode inhibitor even though the amendments probably helped control nematodes at the time of planting.

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. Levels of 50-100% 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 factor that had the most significant effect in reducing nematode populations was the use of a resistant soybean cultivar but, at higher rates of application, increased pH and the production of ammonia also reduced nematode abundance. The present study used realistic application rates of 3-15% (wt.), and even though the numbers of *M. hapla* eggs inoculated were much higher, the tomato plants did not show visible signs of nematode infestation which might include stunting, wilting or chlorotic appearance. Nematode threshold levels in the present study 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% (w/w) and as high as 25% (w/w) have been shown to decrease microbial respiration and other microbiological activity (Wong and Lai, 1996). The maximum level of fly ash used in this study was 7.5% (w/w) being lower than those reported by Wong and Lai (1996) and therefore should not be expected to have influenced microbial activity (Schutter and Fuhrmann, 2001).

In this study at baseline, both bacterial and fungal CFU increased over controls due to the addition of the amendments. The largest increase at time of plantation occurred due to the addition of biosolids only followed by biosolids plus fly ash. The largest change in both bacterial and fungal CFU occurred in the highest treatment rates of biosolids or biosolids plus fly ash (15% w/w), which would indicate that the biosolids, rather than the fly ash, contributed more to microbial activity. This can be explained by the fact that biosolids are rich in microbes while fly ash is not. It should be noted that even though biosolids are the origin of the microbes, it is the addition of the fly ash with its fertilizing effect that aids in their survival (Schutter and Fuhrmann, 2001). In the case of bacterial CFU there is no correlation between *M. hapla* eggs and treatments. However, for the fungal CFU there was a significant reduction in *M. hapla* eggs at the highest application rate, indicating that the fungal microbes might be more important in controlling nematodes than the bacteria. These findings are consistent with other studies (De Leij *et al*., 1993; Townshend *et al.*, 1989).

Tomato yield was affected differently in the case of biosolids only relative to biosolids plus fly ash. While *M. hapla* eggs remained nearly the same in the biosolids treatments relative to the control with nematodes (>2%) there was a marked increase in yield of 45%. In the case of the biosolids plus fly ash, *M. hapla* numbers increased 54% over controls and there was a 36% increase in yield. These data suggest that treatments of biosolids alone do not appreciably increase nematode activity relative to controls that have received nematode inoculum at the levels of this study but that they do improve yield. Effectively, both the fertilizer added to the controls and the biosolids provided similar environments for nematode growth. In the case of mixtures of biosolids and fly ash, nematode numbers increased enough that yield was affected relative to the biosolids alone. When treatment data are compared, it appears that a 12% (w/w) application of a 50:50 mixture of biosolids plus fly ash is enough to increase yields in the presence of manageable numbers of nematodes. Once the application rate reaches 15% (w/w), nematode numbers drop significantly (from 4606 to 889) and yield is only slightly affected suggesting that a point of equilibrium was reached between application rate, nematode populations and tomato yield. This should be further investigated to determine if a higher application rate would decrease nematode numbers even more while increasing tomato yield. In the case of biosolids alone, an application rate of near 6% is sufficient for maximum yield. It should be noted that these results are specific to the soils and types of biosolids and fly ash used in this study.

Addition of organic amendments with narrow C/N ratios has been shown to enhance the activity of nematode biocontrol agents in soils (Rodriguez-Kabana *et al.*, 1987; Stirling *et al.*, 2003; Lazarovits, 2001). Organic amendments can be very diverse and so are the mechanisms by which these enhance biological control of phytoparasitic nematodes (Akhtar and Malik, 2000). The materials used in this study were analyzed for their elemental composition (Table 3) and applied to soils considering application rates that do not introduce high concentrations of heavy metals. The C/N ratios of the mixtures applied in this study were not considered as guidelines for their application. This is because the aim of this study was to apply realistic amounts of amendments that do not breach international guidelines on the use of amendments made from anthropogenically-generated byproducts. Such rates are also realistic when considering the economical feasibility of amendment application to soils.

The type and application rates of fly 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 fly 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.

Any future work on the impact of fly 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. Additionally, potential biocontrol agents that affect nematode populations and that flourish in the amendments should be identified.

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Table 3. 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[z] (n=20)	Fly ash[y] (n=6)
pH	7.87	8.32
% OM	9.5	Nd
NH4	114	Nd
NO3	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

[z]North pond, City of Glencoe, ON
[y]Lambton county power generating station, Lambton, ON

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