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RESEARCH SUMMARY

The Fate of Ivermectin in Manure Composting

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ABSTRACT

Ectoparasites and anthelmintics used to control external and internal parasites in livestock are largely excreted in manure in concentrations that are lethal or sub-lethal to beneficial organisms in the ecosystem. The objective of this study was to determine whether composting would reduce the concentration of ivermectin found in the manure of de-wormed horses. The effect of ivemectin on the composting process was also investigated. Manure and bedding from 60 horses at Oxley Equestrian Facility, Cornell University, Ithaca, NY de-wormed with 114 mg ivermectin paste each, was collected over a 3-day period in 2011 for composting. The presence of ivermectin did not appear to affect the composting process as shown by thermophilic temperatures and proper mass loss. There was a severe drop in ivermectin concentration in the manure/bedding mixture within the first few days of composting indicating exponential decay. This decay occurred at a rate of 1.8% per day with a half-life of 3.6 days. After 175 days of composting, ivermectin concentration decreased from an average of 1.59 mg/kg in the initial mixture to 0.6 mg/kg in the composted material and was not detected in the soil below the pile. However, potentially mineralizable nitrogen, a measure of microbial activity in soil, decreased in the soil on which the pile was built.

INTRODUCTION

Composting is a self-heating, aerobic process that accelerates the degradation of organic material by the successive action of a diverse group of microorganisms, including mesophiles, thermophiles, actinomycetes, and fungi. Because of the activity of microorganisms and the thermophilic temperatures reached, composting results in the destruction of pathogens in manure and carcasses (Bonhotal et al., 2006; Schwarz et al., 2010). However, pathogens are not the only concern in manure composting. Many pharmaceuticals used in livestock operations are not rapidly or completely degraded in the animal's body and can be present in the manure of the animal. Thus, their fate in composting can be a concern, depending on the ultimate use of the compost product. Destruction of antibiotics, steroids and other hormones in manure composting has been studied. Chlortetracycline and monensin (antibiotics found in livestock feed) were found to degrade rapidly in turkey manure composting (Dolliver et al., 2008), and Arikan et al. (2007) showed that composting rapidly reduces concentrations of extractable oxytetracycline in manure from therapeutically treated beef calves. Hakk et al. (2005) showed an 84 and 90% reduction of estradiol and testosterone, respectively, over 139 days of composting chicken manure, hay, straw and leaves. Little work, however, has been done on the fate of other drugs used in livestock operations.

Ectoparasites and anthelmintics are chemical formulations used to control external and internal parasites such as ticks, flies, lice and worms. The compound most commonly used is ivermectin (a macrocyclic lactone) which is highly lipophilic, and following administration is stored in animal fat tissue from where it is slowly released, metabolized and excreted, primarily in the feces (Khan et al., 2007). Because there was no apparent degradation of ivermectin over a 45 day period in fecal patties left in the environment, concern has been raised that the use of anti-parasitic agents in livestock may adversely affect harmless or beneficial organisms which breed in or feed on dung. In addition, ivermectins are known to kill adult insects and larval stages directly, as well as to exert sub-lethal effects on growth, molting, metamorphosis and reproduction (McKellar 1997). According to Lumaret & Errouissi (2002) the fecal concentration of ivermectin found in horse droppings was all above concentrations that are lethal or sub-lethal to many dung breeding invertebrates of benefit to the ecosystem. In horses, oral administration of ivermectin showed that it remained above the detectable level in manure for 40 days (Perez et al., 2001; Lumaret & Errouissi 2002). However, macrocyclic lactones are susceptible to aerobic biodegradation in soils under suitable conditions (Khan et al., 2007) thus would be expected to do the same in the composting process. The purpose of this study was to determine the fate of ivermectin concentration in composting manure.

MATERIALS AND METHODS

Treatment and Composting

Sixty horses at Oxley Equestrian Facility, Cornell University, Ithaca, NY were dewormed with Durvet Ivermectin Paste 1.87% on 12 September 2011. Each syringe contained sufficient paste to treat one 586 kg horse at the recommended dose rate of 200 µg ivermectin per kg body weight. Each of the 60 horses was dosed with a full syringe containing 114 mg ivermectin. Maximum concentration of ivermectin in horse manure after oral dosing at 0.2 mg/kg body weight is expected to be reached approximately 2.5 days post administration (Perez et al., 2001) reaching 90% of the total drug excreted in the feces at 4 days post-treatment (Lumaret and Errouissi 2002). Based on these figures, it was decided that manure and bedding would be collected to build the compost pile beginning 24 hours after deworming continuing through 96 hours post-deworming, building the pile with 3 full days of manure and bedding.

On 12 September 2011 (day -4), stalls containing manure and sawdust bedding were cleaned out and piled on the manure pad. The pile was then sampled to obtain ivermectin concentration pre-worming (control) by taking grab samples from ten locations around the pile at varying depths to insure that the sample was representative of all 60 horses. Grab samples were thoroughly mixed in a bucket, sub-sampled and place on ice. Oxley Equestrian Facility staff dewormed all 60 horses as described previously. The following day stalls were cleaned and the manure pile was sampled (day -3). An additional grab sample of manure only was taken from the stall of one horse to quantify the concentration of ivermectin excreted day 1 post-deworming. The manure pad was cleaned off and all manure and bedding was removed for disposal. Over the next three mornings manure and bedding remained on the manure pad to accumulate stall cleanings. The pile was sampled as previously described on day -2 and day -1.

On 16 September 2011, 7500 kg of manure and bedding (days 2, 3 and 4 post-deworming) were transported to the compost site. Soil was sampled in triplicate where the pile would be built for potential mineralizable nitrogen and ivermectin concentration using the soil sampling procedure recommended by Cornell University's Soil Health Laboratory. The location for the windrow was on

grassy soil where composting had not occurred previously. Manure and bedding were unloaded from the truck to form a windrow in a north/south direction. Dimensions were approximately 3.7 m wide by 6 m long by 1.4 m high (31 m3). A submersible stainless steel micro data logger was set to record pile temperatures beginning at 11:30 am and reading every 4 hours was put into a hole dug in the top of the pile. Temperatures were taken at six spots around the bottom of the pile (on the East and West sides approximately 0.5 m off the ground by inserting a 1-meter compost thermometer into the pile parallel to the ground and at six spots around the top of the pile (approximately 0.3 m from the top of the pile) by inserting the probe into the pile at a 45 degree angle to the side of the pile (figure 1). Additional temperatures were taken via the compost thermometer on days 3, 4, 5, 6, 7, 24, 55, 84, 115, 145, 175, 181 and 182.

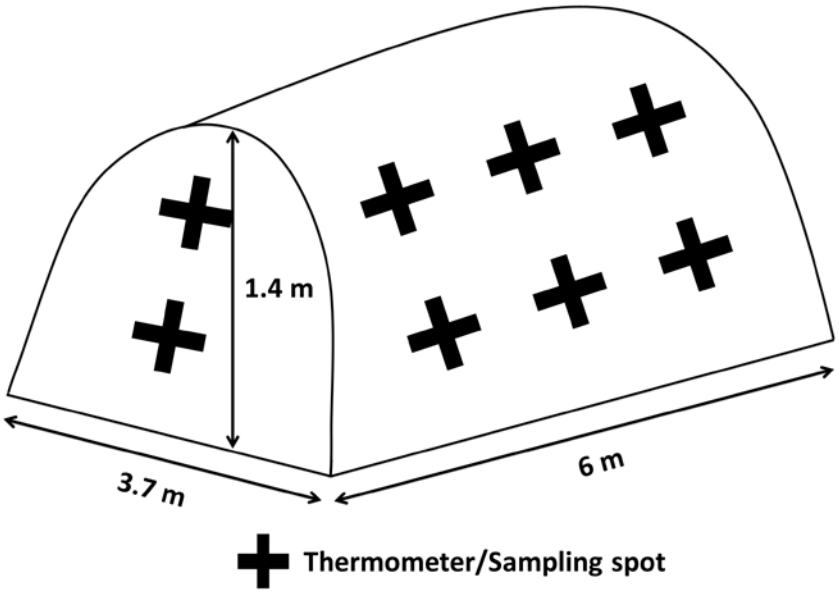

Figure 1. Diagram of where temperatures and samples were taken and dimensions of the compost pile

Triplicate samples of mixed compost occurred on days 0, 3, 4, 5, 6, 12, 24, 55, 115 and 175. Grab samples were taken from 8 spots in the compost pile at approximately the same points where temperatures were taken, alternating between the top and bottom of the windrow, mixed thoroughly in a bucket then split into three subsamples. The compost pile was turned on days 12, 40, 59, 80 and 98. On day 180, the pile was turned into a different spot and reformed. On day 182, triplicate soil samples were taken from where the pile had been built.

Laboratory Analysis - Ivermectin

All samples (manure, manure/bedding, compost and soil) were collected in plastic bags, sealed and frozen until shipped to the laboratory. Analysis of ivermectin in compost was performed at the University of Pennsylvania's School of Veterinary Medicine's PADLS New Bolton Center Toxicology Laboratory. The analysis was performed on an API 4000 LC/MS/MS (AB Sciex, Foster City, CA) equipped with Prominence LC (Shimadzu). Compost used as a blank control was obtained from a local farm in Kennett Square, PA. All the reagents used were of analytical grade and water for sample preparation and mobile phase was ≥ 18 mohms (EMD Millipore, Billerica, MA).

A 5 g portion of compost or soil sample was vortexed for 10 seconds with 0.2 g of sodium chloride and 20 ml methanol. The mixture was sonicated for 20 minutes, shaken for 1 hour on a platform shaker and centrifuged for 8 minutes at 1500 rpm. The supernatant was transferred to a clean tube and the residue was re-extracted with an additional 20 ml methanol by vortexing for 15 seconds. The extracts were combined and a 1 ml portion was transferred to a tube containing 0.05 g PSA (primary, secondary amine bonded silica) sorbent. The tube was vortexed for 15 seconds, kept for 10 minutes followed by centrifugation at 13,000 rpm for 8 minutes. The supernatant was transferred to an autosampler vial for analysis. Any samples with concentrations higher than the spiked range were diluted with 50/50 acetonitrile/water and reanalyzed.

The samples were analyzed for ivermectin using API 4000 LC/MS/MS equipped with Allure C18 column, 5 µm, 50 mm x 4.6 mm (Restek, Bellefonte, PA). Mobile phase consisted of 5 mM ammonium acetate with 0.1% formic acid and acetonitrile in a gradient run at a flow rate of 0.4 ml/minute with initial conditions of 70% acetonitrile for the first 4 minutes, followed by an increase to 98% acetonitrile in the next 6 minutes, then returned to 70% acetonitrile to equilibrate for the next 5 minutes. The mass transition of 892.7/569.3 using collision energy of 22 V was monitored. The following ion source parameters were used: Temperature 450oC, Curtain Gas 10 psi, Gas1 30 psi, Gas2, 30 psi, CAD Gas 7 (positive), and IS voltage 5500 V. Ivermectin eluted at 9.8 minutes. Blank compost was spiked at 0, 0.1, 0.5, 1, 2.5 and 5 mg/kg to generate a quantitation curve (r=0.9995). The method detection limit of ivermectin was established at 0.1 mg/kg.

Laboratory Analysis – Potentially Mineralizable Nitrogen

Soil samples were sent to the Cornell Soil Health lab at Cornell University, Ithaca, NY for analysis of potentially mineralizable nitrogen (PMN). PMN is an indicator of the capacity of the soil microbial community to convert (mineralize) nitrogen tied up in complex organic residues into the plant available form of ammonium. Soil samples are incubated for 7 days and the amount of ammonium produced in that period reflects the capacity for nitrogen mineralization.

The mixed composite bulk soil sample was sieved and two 9 g soil samples were removed and placed into 50 ml centrifuge tubes. Forty ml of 2.0 M KCl was added to one of the tubes, shaken on a mechanical shaker for 1 hour, centrifuged for 10 minutes, and then 20 ml of the supernatant was collected and analyzed for ammonium concentration (“time 0” measurement). Ten ml of distilled water was added to the second tube, hand shaken and incubated for 7 days at 30oC. After the 7-day anaerobic incubation, 30 ml of 2.67 M KCl was added to the second tube, shaken on a mechanical shaker for 1 hour, centrifuged for 10 minutes and 20 ml supernatant collected and analyzed for ammonium concentration (“time 7 days” measurement). The difference between the “time 0” and “time 7 days” is the rate at which the soil microbes are able to mineralize organic nitrogen in the soil sample. Results are reported in units of micrograms nitrogen mineralized per gram dry weight of soil per week.

Statistical Analysis

Exponential decay of the concentration of ivermectin in the compost pile was conducted using nonlinear regression with α = 0.05 (JMP Pro 10.0.0 software, SAS Institute Inc., Cary, NC, 2012).

RESULTS AND DISCUSSION

Thermophilic temperatures (>40^{o}C) were reached in the compost pile at the first temperature recording (figure 2). They remained in the thermophilic range for 118 days before declining to mesophilic temperatures and remaining there through day 182 when the soil samples were taken. The pile had been turned into a new spot on day 180 and temperatures rose to thermophilic 15 days later for about 10 days. As the temperature did not rise again, the hot phase of composting was deemed to be finished. On day 180, when the pile was re-formed into a new spot, the new pile measured 2.4 m wide by 2.4 m long by 1.2 m high. Based on the dimensions of the pile when built there was a volume loss of approximately 64% between day 0 and day 180. Temperature and mass loss are parameters that indicate the process of composting is working properly. A compost pile that is working properly will heat up in a matter of days and hold that heat for several weeks to months. As microbes in the pile digest the feedstock, the mass of the pile will decrease. Based on temperatures reached and loss of volume, it was concluded that the presence of ivermectin in the manure had no detrimental effect on the composting process.

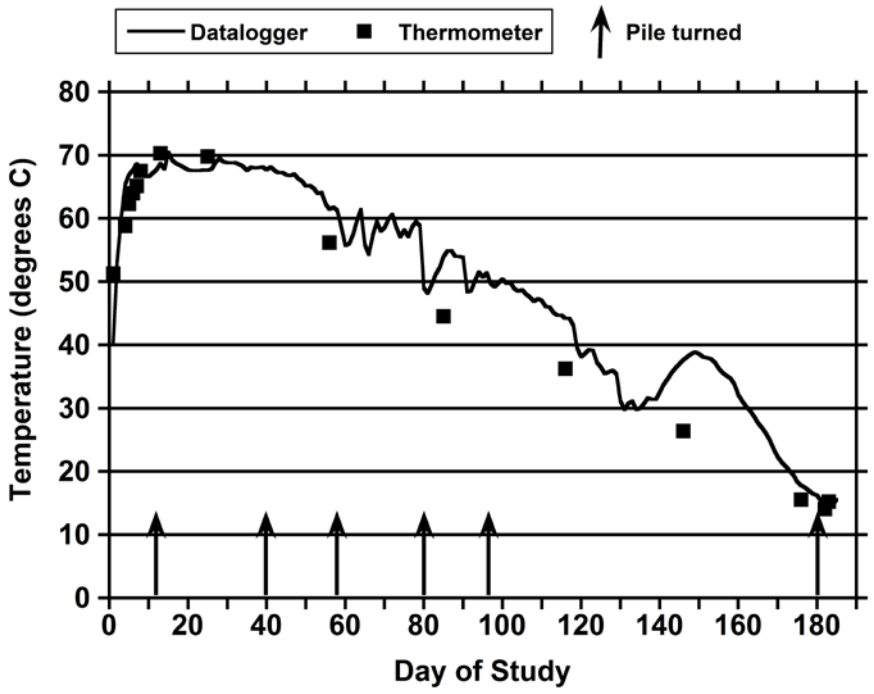

Figure 2. Average compost pile temperatures over time.

Table 1 shows concentration of ivermectin in manure mixed with bedding, compost and soil over the study period. The value for "day" is relative to pile building, making day -4 the day that the horses were dewormed. Ivermectin concentration in manure and in manure and bedding prior to deworming was non-detectable. Ivermectin concentration in the manure and bedding appeared to peak at day -2 (2 days post deworming) as expected.

Table 1: Mean ivermectin concentration from triplicate samples of manure plus bedding and compost (mg/kg)

Date	Day of study (relative to pile building)	Mean Ivermectin concentration (mg/kg)	Standard deviations
12 Sep 2011	-4	non-detect	
13 Sep 2011	-3	1.85	0.252
14 Sep 2011	-2	2.35	0.852
15 Sep 2011	-1	0.56	0.079
16 Sep 2011	0	0.38	0.144
19 Sep 2011	3	0.76	0.068
20 Sep 2011	4	0.55	0.273
21 Sep 2011	5	0.56	0.276
22 Sep 2011	6	0.38	0.089
28 Sep 2011	12	0.70	0.273
10 Oct 2011	24	0.26	0.061
10 Nov 2011	55	0.19	0.100
9 Jan 2012	115	0.10	0.031
9 Mar 2012	175	0.06	0.008

Rate of decay of ivermectin during composting was determined using the average concentration (1.59 mg/kg) in manure and bedding prior to composting (days -3 to -1) and the concentration of ivermectin in compost (days 0 through 175) with JMP's nonlinear fit model (figure 3). Exponential decay of ivermectin during composting occurred at a rate of 1.8% per day with a half-life of 3.6 days ($p<0.0001$, $r2=0.42$). Halley, et al. (1989) studied ivermectin degradation rates in feces/soil mixtures and found that when stored indoors at room temperature, ivermectin degraded slowly with half-lives between 93 and 240 days. It was also slow when exposed to outdoor, winter weather. In contrast, when exposed to the summer weather, ivermectin in soil and feces/soil mixtures degraded rapidly, with half lives of 1 to 2 weeks.

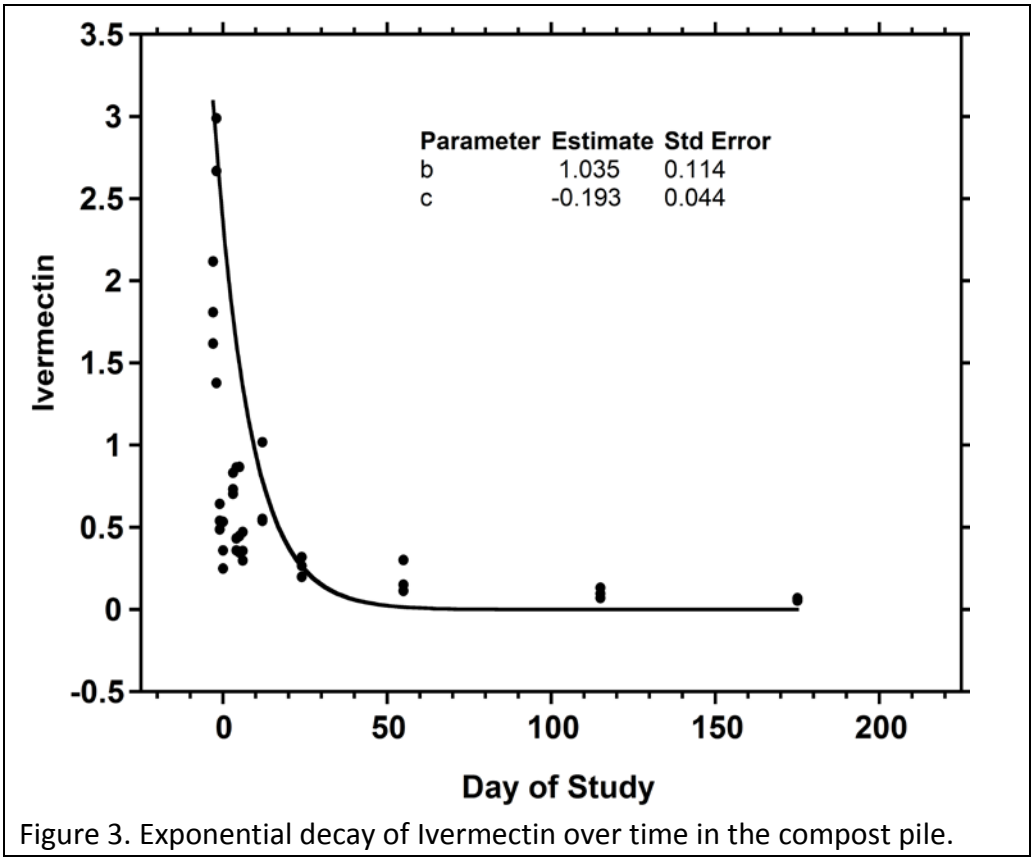

Figure 3. Exponential decay of Ivermectin over time in the compost pile.

Soil samples were taken prior to building the compost pile to determine PMN, a measure of soil biological activity that indicates the extent of microbial populations, and then again on day 182 in order to see if the soil microbes were affected by composting the manure and bedding. The Cornell Soil Health Lab uses scoring function to aid in interpreting soil health. A scoring function is a curve that assigns specific scores between 0 and 100 to the values measured. A score of 100 is the best (highest) while a score of 0 is the worst (poorest). Figure 4 shows the scoring function for PMN. Zero to 30 corresponds to deficiency of an indicator, implying that it will constrain soil use, greater than 30 to less than 70 corresponds to the intermediate region of the indicator, and 70 to 100 denotes that the indicator value is at an optimal level. Prior to building the pile, average PMN in the soil was 32.7 µg nitrogen/g soil (dry weight)/week (scoring function of 100), indicating that there was high microbial activity in the soil prior to building the compost pile. At day 182, after the pile was moved, average PMN in the soil decreased to 7.8, indicating very low microbial activity (scoring function of between 10 and 20 depending on the type of soil). There could be several reasons for this. Lack of oxygen and/or soil compaction from the pile above or organic acids leaching from the pile above could have reached toxic levels. The heat of composting could have killed off the micro-organisms that thrive at cooler temperatures in the soil, the soil micro-organisms directly under the compost pile could have moved into the pile, or ivermectin from the pile could have leached into the soil and affected microbial activity. When any composting pile is removed from an area of natural ground, that plot of soil typically remains barren for weeks. A compost pile temporarily reduces the biological productivity of the soil beneath it. In addition, as no ivermectin was detected in the soil under the compost pile after the pile was moved, ivermectin was most likely not the reason for decreased PMN.

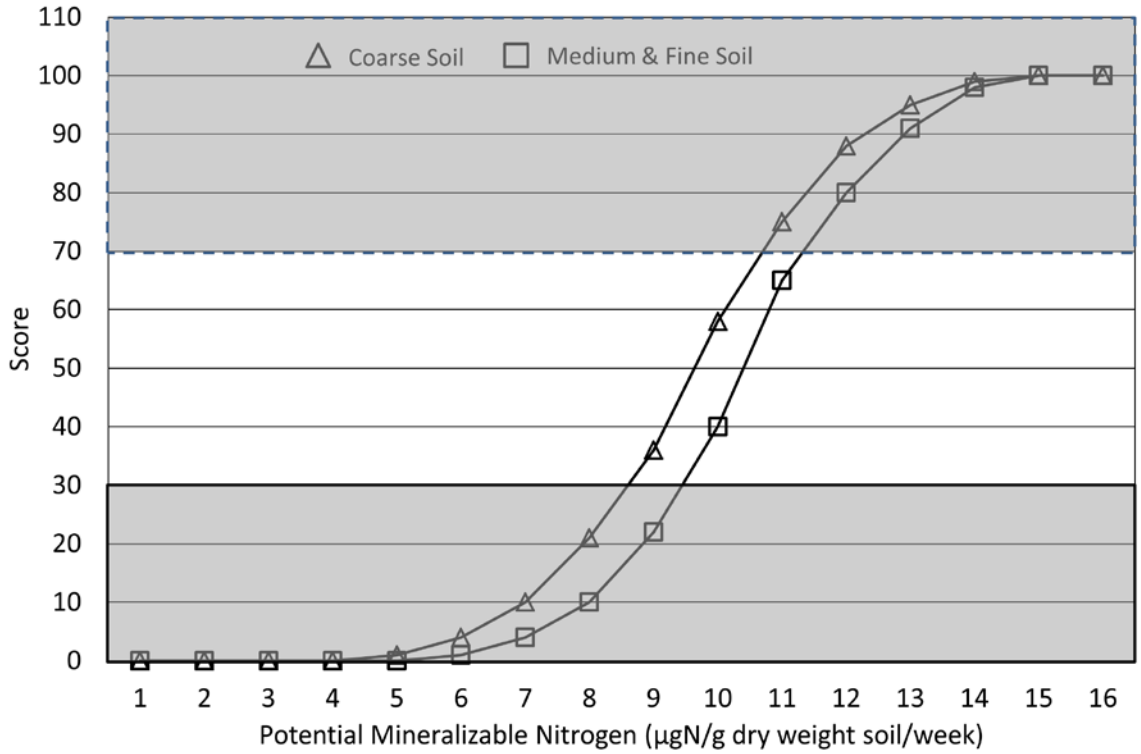

Figure 4. Scoring function for potentially mineralizable nitrogen.

Composting manure and bedding from animals that have been de-wormed with ivermectin decreases the concentration of ivermectin such that the resulting compost product can be used without harm or negative effect to beneficial insects in the environment. As ivermectin has been found to persist in manure for 45 days or more, composting is a good management technique to use for manure after de-worming, or if animals are continually de-wormed. In addition, as the ivermectin concentration decreased almost entirely in the first 24 days of composting, subjecting manure and bedding from de-wormed animals to a thermophilic phase through piling would allow horse owners to field-spread the manure prior to finishing composting.

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