PARASITOLOGICAL EXAMINATION OF COMPOST

U.S. ENVIRONMENTAL PROTECTION AGENCY

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Marketed and stockpiled compost and a mixture of compost and raw sewage sludge known to contain parasites were examined to compare the effectiveness of two concentration methods and one direct method for detecting helminthic forms and protozoan cysts. Nonviable helminthic ova and larvae were found in the stockpiled compost, and viable forms of helminthic parasites were found in the marketed compost. The formalin-ether centrifugal sedimentation method was usually more effective than the brine gravity flotation method for recovering both ova and larvae; the direct film method was the least efficient.

Municipal solid waste, with or without addition of sewage sludge, is being composted on a small scale in the United States today. Composting is merely a microbiological process wherein certain bacteria, fungi, and actinomycetes convert all biodegradable organic matter to a stable humus. Compost is being utilized mainly in agricultural and land reclamation projects.

The health hazards that may exist in composting and in the use of compost, especially when sewage sludge is added to municipal solid waste, have been the concern of many investigators.

Gotaas (10) and Golueke and Gotaas (9) have shown that a temperature of 140 F (60 C) for 1 hr should kill all nonspore-bearing pathogens. These reports indicated that *Ascaris lumbricoides* eggs were destroyed when the compost pile was subjected to temperatures of about 113 F (45 C) for 50 min, and *Endamoeba histolytica* and *Endamoeba coli* cysts were more easily destroyed than *Ascaris* eggs. Chang (3, 4) showed that 20 days at 45 C was required for unheated sewage sludge digestion to remove all nonhatching *Ascaris* ova, and that a contact time of 115 min at 45 C was needed to achieve a complete killing of *Endamoeba histolytica* cysts in water. Strauch (19), Hanks (11), and Fair and Geyer (6), however, have shown that municipal solid waste as well as sewage sludge can contain pathogenic organisms that endanger the health of man and animals. Krishnaswami and Post (15) stated that slaughterhouse waste waters often contain many helminthic parasites (ova, larvae, and adults) and these may enter receiving watercourses through disposal of waste.

Recently, Gaby (8) showed that *Endolimax nana, Endamoeba histolytica,* and *Necator americanus* ova inserted in composting refuse and sludge were killed within an 8-day exposure period at a minimum temperature of 120 F (49 C) at the 2- to 4-inch depth, and within 7 days at a minimum temperature of 131 F (55 C) at the middepth (2 to 3 ft); however, some morphologically intact parasitic ova such as those of *Ascaris, Trichuris, Necator, Ancylostoma,* and *Hymenolepis* persisted to the end of the 49th

day of the composting process. (Facilities were not available to determine the viability of these parasite forms.)

Our preliminary investigation was designed to find concentration methods, through comparative studies, that could effectively be used to recover helminthic parasites and their ova and larvae in composted waste.

The effectiveness of several methods presently used to recover animal parasites and ova from clinical specimens, sewage, vegetables, and compost samples were compared (1, 5, 7, 12, 16-18, 20, 21). Of these, two concentration methods (the brine gravity flotation method by Willis (21) and the formalin-ether centrifugal sedimentation method by Ritchie (17)) and also the direct film method by Harris and Coleman (12) were selected for comparison in experimental studies designed to recover and morphologically identify helminth ova and protozoan cysts in a compost--digested sewage sludge mixture. These methods were then applied in field studies to analyze stockpiled compost, with and without digested sewage sludge, and marketed compost.

It should be noted that the viability of ova was determined by embryo motility in direct observation with a light microscope. No testing was made to determine the hatchability of the ova recovered.

MATERIALS AND METHODS

Collection and Preparation of the Sample

Compost--digested sewage sludge mixture (experimental study). A 50-g sample of digested sewage sludge known to contain *Ascaris* and

Hymenolepis ova was mixed with 250 g of compost. The mixture was divided into three equal portions. After each of the three portions was thoroughly mixed, a final 10-g subsample was removed and placed in a 500-ml wide-mouthed flask containing 100 ml physiological salt solution and glass beads. The flask was placed on a rotary shaker at medium speed for 5 min to emulsify the material. The flask was then removed from the shaker and allowed to stand undisturbed for 45 min. Each of these portions was examined by three methods: brine gravity flotation, formalin-ether centrifugal sedimentation, and direct film.

Stockpiled compost (field study). Random 10- to 20-g samples from each of eight different piles of composted municipal solid waste, with and without the addition of digested sewage sludge, were collected with sterile tongs at a depth of 15 to 30 cm and placed in sterile, 32-oz, capped, glass bottles with wide mouth or in sterile, sealable, 18-oz polyethylene bags. The random samples from each of the eight compost piles were composited into eight 100- to 200-g samples. After thorough mixing, they were prepared as above.

Marketed compost (field study). Random samples were collected from five different marketed compost materials in a manner similar to the stockpiled compost. After thorough mixing, they were prepared in a manner similar to that of the compost--digested sewage sludge mixture.

Examination of Sample

Direct film method (12). Approximately 0.05 ml of the sediment from the suspended material was added to a drop of physiological saline

solution on the center of a slide and stirred well. Coarse fibers, paper, sand, etc., in the test material were removed, and a cover glass was applied. The specimen was then examined under low power of the microscope. If protozoa, ova, or larvae were found, a drop of Lugol's iodine solution was added to the film, and the organisms were tentatively identified.

Brine gravity flotation method (21). The original suspension was well mixed and strained through an 18- by 16-mesh nylon sieve into a 250-ml beaker. The sieve was washed with approximately 10 ml of physiological salt solution and divided into two 50-ml centrifuge tubes. One tube was centrifuged at 2,000 rpm for 1 min; the other was saved for the formalin-ether centrifugal sedimentation. After centrifugation, the supernatant was decanted and salt brine was added to fill the tube. The contents of the tube were stirred to produce an even suspension and allowed to stand entirely undisturbed for 30 to 60 min. With the use of a freshly flamed wire loop (4-mm i.d.), the material in surface film from several locations was placed onto a clean slide, covered with cover glass, and then examined under low power of the microscope.

Formalin-ether centrifugal sedimentation method (17). The original suspension, saved from the previous test, was centrifuged at 2,000 rpm for 1 min. The supernatant was decanted, and the sediment resuspended in approximately 10 ml of physiological salt solution. The suspension was transferred to a 15-ml conical centrifuge tube and centrifuged for 1 min at about 2,000 rpm. After centrifugation, the supernatant fluid was discarded, and 10 ml of 10% formalin solution was added. It was

allowed to stand 10 min or longer for fixation. After this, 3 ml of ether was added to the tube; the tube was stoppered, shaken vigorously for 20 to 30 sec, and centrifuged for 2 min at about 1,500 rpm. After centrifugation, the supernatant was decanted, and 0.05 ml of sediment was transferred to a slide. The preparation was covered with a cover glass and examined under low power of the microscope.

RESULTS

Compost-sewage sludge mixture. In experimental studies of compost--digested sewage sludge mixture, *Ascaris* ova were found in each of the three portions tested by the brine gravity flotation and the formalin-ether centrifugal sedimentation methods (Table 1). The efficiency for the recovery of helminth ova from the compost--sewage sludge mixture was compared on the basis of the number of *Ascaris* ova. The results showed that formalin-ether centrifugal sedimentation was the best for the recovery of *Ascaris* ova from the first subsample and for *Hymenolepis* ova from the second subsample. The two concentration methods were found to be equally efficient for the third subsample. As for the protozoan *Endamoeba coli* cysts, the formalin-ether centrifugal sedimentation method also appeared to be the most efficient.

Stockpiled compost. The occurrence of helminth ova and larvae in an 8-sample series of stockpiled compost of various ages is summarized (Table 2). These samples were shipped to our laboratory from a composting plant designed to decompose municipal solid waste, with or without addition of digested sewage sludge, by a batch-type, high-rate mechanical

TABLE 1. Recovery of helminth ova and protozoan cysts from compost--digested sewage sludge mixture (per 0.05 ml)

Method	Subsample		
	1	2	3
		Ascaris	
Direct film	0	0	0
Brine gravity flotation	2	2	2
Formalin-ether centrifugal sedimentation	4	2	2
		Hymenolepis	
Direct film	0	0	0
Brine gravity flotation	0	0	2
Formalin-ether centrifugal sedimentation	0	2	2
		Endamoeba coli	
Direct film	0	1	0
Brine gravity flotation	0	0	0
Formalin-ether centrifugal sedimentation	0	3	0

TABLE 2. Helminth ova and larvae in various-aged, stockpiled compost

Sample no.	Sewage sludge added	Curing (months)	Compost temperature (C)	Organisms recovered/0.05 ml	
				Ova	Larvae
1	Yes	1	38	*Hymenolepis diminuta* (26)[a]	-[b]
2	Yes	1	76	-	-
3	Yes	2	64	-	*Strongyloides* (1)[c]
4	Yes	3	66	-	-
5	No	6	49	-	-
6	No	6	63	-	-
7	No	6	63	-	-
8	No	7	64	-	-

[a]Recovered by the formalin-ether centrifugal sedimentation method.

[b]None found.

[c]Recovered by the brine gravity flotation method.

aerobic bacterial digestion system that employed digestor tanks. The waste decomposed to compost in 6 to 10 days at temperatures of 66 C and above in the winter and at 71 C in the summer. Four to five days were required before temperatures reached their maximum values at all depths within the composting mass. The samples were collected from stockpiled composted material that had completed the digested cycle, had undergone a final grind, and had aged for various periods of time. Ova of *Hymenolepis diminuta* (26 per 0.05 ml) were found in a sample that had been cured for 1 month; rhabditiform larva of *Strongyloides* (1 per 0.05 ml) appeared in a sample collected from a pile that had been cured for 2 months. These developmental forms appeared to be microscopically nonviable. Note that temperatures of 38 and 64 C were recorded in the stockpiled compost at the respective time of sampling.

Marketed compost. The incidence of helminth ova and larvae in marketed compost materials is shown in Table 3. Of the five samples analyzed, one showed the ovum of *Trichuris trichuria* (1 per 0.05 ml), *Taenia* sp. (1 per 0.05 ml), *Hymenolepis diminuta* (2 per 0.05 ml), and rhabditiform larvae of *Strongyloides* (1 per 0.05 ml); another showed the larvae of hookworm (2 per 0.05 ml). The ovum of *T. trichuria* and the larvae of *Strongyloides* and hookworm were found to be viable. Compost (H) was derived from waste to which activated sewage sludge had been added before composting. Although the exact constituents of Compost (L) were not learned, the bulk of this compost was known to be composted animal excreta and peat moss.

TABLE 3. Recovery of helminth ova and larvae in marketed compost materials

Type of sample (source)	Organisms recovered/0.05 ml	
	Ova	Larvae
Compost (H)	-[a]	Hookworm, viable (2)[b]
Compost (H)	-	-
Compost (L)	*Trichuris trichuria*, viable (1)[b] *Taenia* (1)[b] *Hymenolepis diminuta* (2)[b]	*Strongyloides*, viable (1)[c]
Compost (F)	-	-
Milorganite	-	-

[a]None found.

[b]Recovered by the formalin-ether centrifugal sedimentation method.

[c]Recovered by the brine gravity flotation method.

DISCUSSION

Compost derived from municipal solid waste, with or without sewage sludge, contains a great variety of materials, most of which are either lighter or denser or smaller or larger than cysts, ova, and larvae of parasites. In using a concentration method, it was of importance to separate, as completely as possible, the cysts, ova, and larvae of parasites from all other elements of compost. This was accomplished by screening, by sedimentation, and by flotation. The two concentration methods, brine gravity flotation and formalin-ether centrifugal sedimentation, incorporated all three of these principles. The screening was achieved by filtering the sample through an 18- by 16-mesh nylon sieve; sedimentation, by treatment with 10% formalin and ether, followed by centrifugation; and flotation, by application of a saturated aqueous solution of sodium chloride. Our experimental studies showed that the formalin-ether centrifugal sedimentation method was usually more effective than the brine gravity flotation method for recovering both ova and larvae; the direct film method was least efficient. The viability of ova was based on direct observations of *in vivo* embryo motility with a light microscope.

To compare the parasite recovery ability of the two concentration procedures, a series of samples were examined: marketed and stockpiled compost, and a mixture of compost and raw sewage sludge known to contain parasites.

Nonviable helminth ova and larvae were demonstrated in various ages of stockpiled compost. One sample contained ova of *Hymenolepis diminuta*

and other larva of *Strongyloides*. Since no hatchability tests were made during this preliminary study, the significance of these parasite forms cannot be ascertained.

The incidence of helminth ova and larvae in marketed compost was significant. Two out of five samples were positive. One sample contained viable ova of *Trichuris trichuria* and viable *Strongyloides* larvae; viable hookworm larvae were found in another sample. As Chang (2) pointed out, if hookworm or *Strongyloides* larvae are present in the marketed compost, applying the product where humans walk barefooted or where the larvae may be carried by food or water can spread the infection. Gotaas and others (9, 10, 14), however, found that compost derived from municipal solid waste without sewage sludge is generally accepted as being innocuous from a health standpoint; but, the compost from solid waste and sewage sludge or from animal excreta has the potential hazard of a residual pathogen content.

Kabler (13) stated that the sludges derived from digestion processes are apt to contain large numbers of parasites. To render this material innocuous, it must be heat treated at 57.2 C for 1 hr or exposed to extended composting or drying. Drying for 12 to 15 months is sufficient to render *Mycobacterium tuberculosis* nonviable; however, the moisture content in sludge must be at a very low level to destroy completely the viability of the helminthic ova.

These preliminary findings indicate the need of further studies to determine the potential public health hazard of parasites in improperly disposed of solid waste and in compost derived from municipal solid waste

and sewage sludge. This is especially true in relation to monitoring all solid waste components for disease potential. Such studies would include: a standardized methodology for parasitological examination of waste materials; studies on survivability and transmission of parasites through disposed, untreated waste; studies on migration of parasites through soil from leached solid wastes; and, finally, studies of treatment methods and environmental factors affecting parasite destruction in solid waste.

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