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Project Summary

Occurrence of Pathogens in Distribution and Marketing Municipal Sludges

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A study of the occurrence of microorganisms in distributed and marketed municipal sewage sludges was conducted in order to determine the levels of indicator and pathogenic organisms that might be present in these products. Samples were analyzed for a variety of bacteria, viruses, parasites and fungi in the indicator and pathogen categories. In the first part of this study, seven municipal sewage sludge compost products were sampled weekly for one year. Five of the sample products originated from one windrow composting facility and two products originated from one aerated static pile composting facility.

The products sampled at the windrow facility included the final compost produced at the municipal composting facility, and four commercially marketed, compost based soil amendment products. The two sampling points at the static pile facility were the final screened compost which was utilized in a number of bulk distribution programs and the "giveaway bin" which contained unscreened compost available to the public for home use.

The indicator microorganisms were frequently detected at high concentrations. Tremendous indicator variability was observed with some concentrations varying by as much as ten orders of magnitude. Data from the windrow site grouped into two strata. The first stratum included the final compost and the commercial product containing only screened final compost. Average concentrations of organisms were higher in the bagged compost than in the field compost samples, but the differences were not significant at the 95% confidence limits (C.L.). The second grouping of data consisted of bagged commercial products containing additional amendments; these products contained significantly higher concentrations of microorganisms than those in the first stratum.

Analysis of the indicator data from the static pile composting facility indicated that these two sampling points were also significantly different. The screened compost contained higher levels of bacteria than the material in the giveaway bin.

The only potential pathogens detected with regularity were bacterial. No protozoan cysts were found. Helminth ova were regularly detected but none could be shown to be viable. The most common ova observed were *Trichuris* and *Ascaris*. Many of the *Trichuris* were probably of non-human origin. Enteric viruses were confirmed in only two samples at very low levels.

The potential bacterial pathogens regularly detected were *Salmonella* and *Yersinia*. Salmonellae were detected at both facilities. *Yersinia* only occurred significantly at the static pile facility and were isolated in a pattern consistent with a seasonal occurrence. At the windrow facility, salmonellae were primarily isolated from amended compost products. Toxigenic *E. coli* were

randomly isolated. No *Campylobacter* were detected.

Total and fecal coliforms and fecal streptococci were shown to be good predictors of the presence of salmonellae.

In the second part of the study, 24 additional municipalities were sampled bimonthly for pathogen and indicator microorganism determinations. Final sludge products included composts, air-dried sludges and heat-treated sludges. Results from the 24 facilities also showed a great deal of variation in microbial densities. Air dried sludges often contained lower concentrations of enteric bacteria than composted sludges and as a group, were significantly lower than static pile composting sites. Salmonellae, *Yersinia* and toxigenic *E. coli* were randomly isolated, generally at low levels.

This study disclosed essentially no hazard associated with treated sludges from parasites or viruses. A potential health hazard associated with salmonellae was detected at both facilities sampled weekly. Results indicated that current composting practices may not insure complete elimination of pathogenic bacteria. The significance of *Yersinia* populations at the static pile facility was uncertain, but isolation patterns suggested a seasonal occurrence. Relatively few salmonellae were detected in final compost from the windrow facility, but significant increases in bacterial populations, including salmonellae, occurred during subsequent production of commercial soil amendment products. These increases were consistent with a regrowth phenomenon. Conventional indicator organisms appeared to be reasonable predictors for the presence of salmonellae. Final sludge products derived from the presence of salmonellae. Final sludge products derived from the various treatment processes often did not contain detectable levels of the tested enteric pathogens, but the data suggest monitoring may be necessary to ensure consistent quality of sludge-based products destined for home use.

This Project Summary was developed by EPA's Health Effects Research Laboratory, Research Triangle Park, NC, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

The Clean Water Act of 1972 (PL 92-500) was enacted to improve the quality of the nation's water supplies. As a result, a by-product of sewage treatment, known as sludge, has increased in quantity as wastewater treatment improved and expanded. An estimated 7 to 9 million dry tons of sludge are produced annually, and this amount is predicted to increase significantly in the future. Disposal of this sludge has become a major function of publicly owned treatment works (POTW); disposal options, however, are often limited. Ocean disposal of sludge has been greatly reduced. Cost and air pollution considerations have curtailed the use of incineration, and transportation costs and the lack of available sites have lessened the popularity of landfilling as sludge disposal options.

An attractive alternative, rapidly gaining in popularity, is the beneficial use of sludge as a soil amendment. Sludge has been shown to be an excellent organic amendment for soils; it is also a source of nutrients and minerals for plants. Land application of sludge represents a significant and rapidly increasing option for disposal of sludge produced in the U.S.

EPA has promulgated regulations (40 CFR 257) specifying acceptable sludge treatments depending on the ultimate use of the sludge and the amount of public exposure. These treatments are termed Processes to Significantly Reduce Pathogens (PSRP) and Processes to Further Reduce Pathogens (PFRP). PSRP are generally standard treatment processes that incidentally reduce pathogens, such as anaerobic digestion. PFRP are processes specifically intended to reduce pathogens, as well as provide sludge stabilization, such as high temperature composting. Various restrictions are placed on the land disposal of sludges that have received only a PSRP treatment. Under current guidelines, a sludge subjected to PSRP followed by a PFRP may be used without restrictions.

Although the use of sludge as a soil amendment is attractive, it is not without potential health risks. Toxic chemicals, including heavy metals and industrial organics, may enter the food chain and present long-term health risks. Pathogenic microorganisms present in sewage and the resulting sludges increase the potential for disease transmission. These concerns must be mitigated if the full resource potential of sludge is to be realized.

In many areas, toxic chemicals are not present or their levels may be reduced to acceptable levels through source control programs. Pathogenic microorganisms, however, enter the wastewater from infected individuals. These organisms often concentrate in the resulting sludges due to their density or through adsorption to larger particles. The pathogens are thus a normal component of sludge and cannot be reduced by source control. The treatment processes employed at the POTW must effectively eliminate, or reduce to acceptable levels, the pathogenic microorganisms present in sludge before the material can be released for use.

The relative public health risk associated with the beneficial use of sludge is directly related to the extent of public exposure. Agricultural use for feed crops and silviculture in limited access areas present minimal risks. Risk increases if the sludge is used on food chain crops or public access areas.

Many programs are currently distributing and marketing (D & M) PFRP-treated sludges for home use on lawns and ornamental and vegetable gardens. Of the various reuse options, home use of treated sludge via some form of marketing or distribution program would appear to present the greatest potential for significant health effects due to increased exposure. The routes of exposure may take various forms, including handwork in gardens,and eating uncooked vegetables grown in sludge amended soils. Perhaps at the highest risk of ingesting pathogenic organisms are very young children playing in yards and gardens that have been treated with sludge products.

A number of review articles have discussed the occurrence and significance of pathogens in sewage and sludge and the effectiveness of various treatment options. Some pathogenic or potentially pathogenic microorganisms have been shown to regrow in treated sludges, but the significance of this phenomenon has not been determined. The pathogenic microorganisms of concern in sewage and sludge are members of four basic groups; these are the bacteria, fungi, parasites, and viruses.

Many different bacterial pathogens may be present in sewage and sludge. In the major concern category, *E. coli*

(pathogenic strains), *Salmonella* sp., *Campylobacter jejuni, Yersinia enterocolitica, Leptospira* spp., *Shigella* spp., and *Vibrio cholerae.*

The pathogenic strains of E. coli are often the cause of "travelers' diarrhea" and may cause serious gastroenteritis and diarrhea in children under five years of age. These strains have also been involved in outbreaks of gastroenteritis resulting from contaminated water supplies. Three types of pathogenic *E. coli* have been recognized; they are enterotoxigenic, enteropathogenic and enteroinvasive. It has been estimated that pathogenic *E. coli* represent less than 1% of the fecal coliform population. Little is known about the occurrence or fate of enteropathogenic *E. coli* in sludge and sludge treatment processes. The potential for pathogenic *E. coli* regrowth *in sludge products is unknown*, but certainly possible.

In contrast to the paucity of data concerning pathogenic *E. coli* in sludges, the salmonellae have been widely studied. The previously cited reviews summarize much of this work. It has been estimated that up to 2 million people per year acquire *Salmonella* infections, and the rate has been increasing in recent years. For this reason one of the prime concerns is the potential for salmonellae regrowth.

Studies have found that salmonellae would grow to high levels in sterilized sludge. Other experiments demonstrated regrowth of indigenous salmonellae within the naturally occurring mixed microbial population of compost. Although the salmonellae increased more than three orders of magnitude within five days, the effect was transient, and the salmonellae were returning to background levels after three weeks. Others have concluded that the active microflora of moist compost would eliminate contaminating salmonellae after six weeks, and that once a sludge product is applied to the soil, regrowth would not likely be a problem.

Nevertheless, the salmonellae remain an important concern with home use of sludge products. Most authorities indicate that a fairly high infective dose is required to initiate a *Salmonella* infection; however, there is evidence that this may be an overgeneralization. Salmonellosis outbreaks that may have been initiated by infective doses as low as 10-100 cells have been described in the literature. It would certainly appear imprudent to overlook moderate salmonellae populations because of "conventional wisdom" concerning infective doses.

Campylobacter and *Yersinia* have been referred to as "pathogens of emerging significance." Although much work has focused on *Yersinia*, there is very little information concerning these organisms in sludges. One study reported 2 X 10^5 *Yersinia enterocolitica* per gram total suspended solids in one digested sample. A study in Seattle reported *Yersinia enterocolitica* levels of 10^7 to 10^9 per gram in various sludge samples. The significance of these numbers is unknown. Many *Y. enterocolitica*-like organisms, unusual *Y. enterocolitica* or atypical *Y. enterocolitica* have been reported. The pathogenicity of individual strains is unknown, and the role of these organisms in human disease has not been completely discerned. Yersiniosis occurs only sporadically in the United States.

Gastroenteritis caused by *Campylobacter jejuni* is much more common than previously thought and may approach the incidence of salmonellosis. *C. jejuni* is commonly found in the intestines of many animals, but the fate of these organisms in the environment is generally unknown. Waterborne campylobacteriosis has been documented, and the survival of the organisms in water has been studied to a limited extent. Limited experiments on the survival of *Campylobacter* in sludges from four wastewater treatment plants in Ohio found that seeded *Campylobacter* survived for seven days in sludge stored at 4°C, however, no indigenous *Campylobacter* were isolated from any sludge samples

Although the other bacterial pathogens listed as significant are responsible for a substantial amount of disease, they either have not been demonstrated in sludges, or sludge applied to land is not considered an agent of transmission.

A number of pathogenic or allergic fungi can be isolated from sludge. These include yeasts, such as certain specious of *Candida*, *Cryptocuccus* and Trichosporon, and pathogenic members of some filamentous genera, such as *Aspergillus, Phialophora, Geotrichum, Trichophyton* and *Epidermophyton. Aspergillus fumigatus*, an opportunistic pathogen to individuals with pulmonary problems and a strong allergen to many, may proliferate in some composting systems. This may be a consideration when selecting prospective composting sites. The general consensus, however, is that fungi in treated sludges present a minimal hazard. With the exception of the aspergilli, little work has been done to define the relationships of fungi in polluted environments or sludges. The significance, if any, of fungal types and diversity in compost is unknown. A highly diversified mycobiota probably indicates a stabilized environment. A lack of fungal diversity in treated sludge would not present a particular problem unless the fungus was potentially harmful. Dermatophytic fungi, for example, may be present in sludge at detectable levels. It is not known if the common dermatophytes can survive or proliferate in sludges. Conventional thought considers the dermatophytes to be parasitic, although there is evidence that some dermatophytes live a saprophytic existence. Adding large numbers of these organisms to home soils would be undesirable.

Parasitic infections present a potential health risk associated with home use of sludge due to the existence of highly resistant stages of the organisms and low infective doses. Two groups of parasites are of concern, the protozoa and the helminths. The common protozoan parasites include organisms such as *Entamoeba histolytica* and *Giardia lamblia. Giardia* infection has become endemic in some areas of the country and numerous outbreaks of giardiasis have occurred. *Ascaris* ova are the most commonly isolated nematode ova in sludge. Others include *Trichuris, Toxocara, Hymenolepis* and *Taenia,* to mention a few. In 1973, ascariasis was estimated to affect four million people in the United States.

Sludge digestion destroys protozoan cysts but not metazoan eggs. This observation was confirmed by recent studies in Seattle where *Giardia* was isolated from raw and waste activated sludges but not from digested or digested dewatered sludges. The Seattle study concluded that *Giardia* pose a negligible health risk from land application of digested sludges. In general, available evidence indicates that helminth ova are more resistant to environmental stresses than are protozoan cysts. The ova of *Ascaris* sp. are possibly the most resistant of the eggs or cysts commonly found in sewage. It has been proposed that *Ascaris* ova be used as an indicator of other parasites, however, the assessment of viability is important because intact non-viable ova may be detected. The inactivation rates of ova from three species of roundworms and a tapeworm

when stored in sludges have been studied. Both viability and infectivity of the ova were evaluated, and the conclusion was that long-term storage may be an effective method of eliminating parasite eggs. A number of studies have shown that composting is an effective method of eliminating parasite ova. In this context, EPA permits unrestricted use of sludges subjected to PFRP treatment such as composting. The World Health Organization has concluded that the risk of infection to man from parasites associated with the use of (untreated) sludge needs evaluation.

More than 110 enteric viruses are recognized and may be present in sewage. The list of enteric viruses is increasing and now includes rotaviruses and the Norwalk viral agent. Most viruses probably adsorb to the solids in sewage although the adsorption process has been shown to be type- and strain-dependent. The potential for transmission of viral diseases through sludge reuse programs does exist. Virus data collected during a compost-monitoring program indicated that composting was an effective means of reducing viral levels. The results also suggested that the composting procedures must be well-defined, with monitoring or process assurances that all of the composting mass is exposed to adequate temperature. If not properly controlled, virus survival could occur. Infective doses for the viruses are thought to be low. Therefore, reasonably thorough virus inactivation is necessary for home use of sludge products. Once destroyed, enteric viruses cannot re-establish themselves in sludge; regrowth is not a concern.

The World Health Organization and others have addressed the question of risk to health from use of sludge on land. Appropriate measures are available to manage the risk. A group of scientists meeting in Denver in 1983 pointed out that there appear to be no published records of adverse health effects on man associated with the land application of sludge.

The lack of information on health problems associated with D & M sludges may reflect the absence of a problem, the lack of intensive surveillance, or the inability to detect recurrent small-scale incidents of disease. In general, the levels of enteric disease in the U.S. are low because of good sanitation, personal hygiene, and extensive public treatment works. In recent years, however, waterborne outbreaks of disease and the rates of certain enteric diseases, such as salmonellosis, have been increasing.

Conclusions

All of the sewage sludge products examined were found to contain variable densities of indicator microorganisms. Some products contained bacterial pathogens at high frequencies and levels. Variability of microorganism concentrations was often great between different facilities and between different samples from the same facility. Many of the observed trends would not have been detected without a large number of samples collected over a long period of time.

Overall, the highest concentrations of microorganisms occurred in samples from static pile composting systems; the lowest concentrations were found in pelletized sludge from a heat drying process. Microorganism densities in aged anaerobically digested, air-dried sludges were as low as, or lower, than most of the composted sludges.

Composts modified with various materials to produce commercial soil amendments contained significantly higher concentrations of bacteria and fungi than the base compost material. The data suggested a nutrient-related regrowth phenomenon.

Potentially pathogenic bacteria, including *Salmonella* sp., *Yersinia enterocolitica* and toxigenic *E. coli* were detected. *Salmonella* sp. were the most frequent pathogen detected. The quantitative test for toxigenic *E. coli* indicated that these strains, when present, occurred at very low levels. However, the percentage of colonies that were toxigenic strongly suggested that the concentration of toxigenic strains was much higher than indicated. *Yersinia enterocolitica* occurred at very high densities in some samples. The isolation of *Yersinia* was consistent with a seasonal occurrence. The prevalence and density was higher in colder months. Based on a small number of tests, the *Yersinia* appeared to be avirulent (not causing disease).

No significant health hazard was associated with respect to *Campylobacter,* parasitic helminth ova or enteric viruses. The test for campylobacters in compost was relatively ineffective, but other available data suggest these bacteria would not survive composting or air drying. Helminth ova were detected regularly, but no indications of viability were observed. No protozoan cysts were found.

The fungus *Aspergillus fumigatus* was detected in products from most sample sites but usually at low densities. The highest concentrations of *A. fumigatus* occurred in composts from static pile composting facilities.

Given the considerable variation observed in microbial densities and the reasonably frequent isolation of salmonellae, bacterial monitoring to assure product quality may be of value for the home use of sludge and compost soil amendments. Regression analysis suggested that total or fecal coliforms or fecal streptococci may be suitable indicators for monitoring.

The occurrence of pathogenic bacteria in distributed and marketed municipal sewage sludge products represents a potential health hazard. However, the extent of risk associated with use of such products remains to be determined.

Recommendations

Factors associated with the extensive variability observed in the microbial populations need to be better delineated in order to institute appropriate control measures.

Significance of the relatively high microbial concentrations in static pile compost products should be determined. The influence of recycling wood chips should be further evaluated.

Additional studies on *Salmonella* regrowth are recommended. The effects of substrate additions should be evaluated. Laboratory regrowth experiments to date may not have adequately simulated field conditions.

Consideration should be given to establishing criteria and conducting research necessary for qualifying digested, air dried sludges as equivalent to PFRP treated sludges.

Further studies are recommended to quantitate toxigenic *E. coli* populations. Gene probe techniques may be applicable to this task.

The potential for sludge and compost to serve as a reservoir of pathogenic *Yersinia* in certain locations needs additional evaluation.

Bacterial limits may need to be established for the uncontrolled home use of sludge and compost products or appropriate educational material should be supplied to users of the products.

Studies should be conducted to determine the extent of risk, if any, of bacterial infections from the use of distributed and marketed municipal sewage sludge products.

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The complete report, entitled "Occurrence of Pathogens in Distribution and Marketing Municipal Sludges," (Order No. PB88-154 273/AS; Cost: $25.95, subject to change) will be available only from:
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