Sustainability of Land-Application of Class B Biosolids

Ian L. Pepper[a], Huruy Zerzghi[b], John P. Brooks[c], and Charles P. Gerba[b]

[a]The University of Arizona, Environmental Research Laboratory, 2601 E. Airport Drive, Tucson, AZ 85706;

[b]The University of Arizona, Department of Soil, Water and Environmental Science, 1177 E. Fourth Street, Shantz Building, Room 429, Tucson, AZ 85721;

[c]USDA ARS, Waste Management and Forage Research Unit, P.O. Box 5267, 810 Hwy 12 East, Mississippi State, MS, 39762.

[a]Corresponding author (ipepper@ag.arizona.edu).

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ABSTRACT

Land-application of Class B biosolids is routinely undertaken in the United States. However, due to public concern over potential hazards, the long term sustainability of land-application has been questioned. Thus, the objective of this review article was to evaluate the sustainability of land-application of Class B biosolids. To do this we evaluated: 1) the fate and transport of biological and chemical hazards within biosolids that could be harmful to human health; and 2) the influence of long term land-application on the microbial and chemical properties of the soil. Direct risks to human health posed by pathogens in biosolids have been shown to be low. Risks from indirect exposure such as aerosolized pathogens or microbially contaminated groundwater are also low. A long term land-application study showed enhanced microbial activity and no adverse toxicity effects on the soil microbial community. Long term land-application also increased soil macro-nutrients including C N and in particular P. In fact, care should be taken to avoid contamination of surface waters with high phosphate soils. Available soil metal concentrations remained low over the 20 year land-application period, due to the low metal content of the biosolids and a high soil pH. Soil salinity increases were not detected due to the low salt content of biosolids and irrigation rates in excess of consumptive use rates for cotton. Our conclusion based on these studies is that long term land application of Class B biosolids is sustainable.

INTRODUCTION

The world population of 6.8 billion people all produce sewage. In the developed world most of this is treated in wastewater treatment plants which results in large volumes of biosolids. In the United States, the term biosolids implies treatment to produce Class A or Class B biosolids that meet the land-application standards in the Part 503 Environmental Protection Agency regulations (EPA, 1994). Currently about 60% of all biosolids are land applied in the United States with most of the land receiving Class B biosolids (NRC, 2002). Class B biosolids are normally produced as a "cake" ($\simeq$ 20% solids) or as liquid biosolids ($\simeq$ 5-8% solids). In either case, Class B biosolids usually result from mesophilic anaerobic digestion, and by definition are likely to contain human pathogenic bacteria, viruses and protozoan parasites (Pepper et al., 2006).

Although the type of land and crops to which Class B biosolids are applied is controlled, public concern in some areas of the United States such as parts of California, has resulted in the banning of land-application of Class B biosolids. This has raised the issue of the sustainability of land-application in general, and in particular, application of Class B material. In some cases, public concern has been warranted due to the limited amount of data on the fate and transport of pathogens after land-application. Studies have been needed on indirect routes of exposure, such as contact with bioaerosols at some distance from land-application sites, or consumption of groundwater beneath sites where land application takes place. Because of this concern, over the past several years, the University of Arizona, National Science Foundation Water Quality Center has sponsored numerous studies on major biological issues related to land-application. In this review, we provide an evaluation of the relative risk of each hazard to human health based on a summary of the University of Arizona studies and other relevant studies.

Sustainability of land-application has also been questioned based on the long term effects of continuous biosolid applications on soil physical, microbial and chemical properties. To answer these issues we document a long term University of Arizona study on the effects of land-application on soil properties. Based on this study and a review of the literature, we provide an assessment of the sustainability of land-application of Class B biosolids.

POTENTIAL BIOLOGICAL HAZARDS ASSOCIATED WITH LAND APPLICATION OF CLASS B BIOSOLIDS

Occurrence of Known Human Pathogens in Class B Biosolids

The known principal pathogens of concern in Class B biosolids are shown in Table 1. Types of pathogens are categorized as: bacteria, viruses, protozoa, and helminth worms. Of these, *Salmonella*, enteric viruses and helminth ova concentrations are utilized to discriminate between Class A and Class B biosolids (EPA, 1994). Potentially, Class B biosolids may contain some or all of these known pathogens, and because of this, site restrictions are placed on land-application sites that are meant to preclude initial human contact with biosolids. Essentially site restrictions are implemented to allow for die off of human pathogens to non-detectable levels. Despite this, public concern has focused on indirect routes of exposure such as bioaerosols to off-site locations, or the leaching of pathogens into groundwater. In addition, from time to time, concerns have arisen about the presence of specific or emerging human pathogens that could be present in biosolids. An example of this is the *Staphylococcus aureus* controversy of 2002.

Incidence of *Staphylococcus aureus* in Sewage and Biosolids

S. aureus is the agent of a wide variety of skin and wound infections in humans and is commonly found in the nares and skin of warm blooded animals. Although there was no scientific documentation of *S. aureus* transmittal from wastewater or biosolids, claims were made that land-applied biosolids were the source of infections in residents living close to the application site (Lewis and Gattie, 2002). Staphylococci have been reported in aerosols from wastewater treatment plants using aeration treatments (Brandi et al., 2000). However, it was not confirmed that these were in fact *S. aureus*. However, raw sewage has been shown to be a definitive source of *S. aureus* in a more recent University of Arizona study, but it was not detected in Class A or B biosolids (Table 2), suggesting that the organism did not survive biosolid treatment (Rusin et al., 2003). In addition the organism was never detected in bioaerosols resulting from land-application sites. Based on these data, it was concluded that biosolids were not a significant source of *S.aureus* exposure (Rusin et al., 2003).

Offsite Exposure of Bioaerosols to Residents Close to Land-Application Sites

Closely related to the *S.aureus* issue was the question of bioaerosols. Specifically, concern centered on the potential for movement of aerosolized pathogens away from a land-application site to local residential communities. The risk of infection to residents living close to land-application sites was identified as an area where data were lacking in the National Research Council report on land-application (NRC, 2002). A limited number of studies have been conducted on the generation of bioaerosols from biosolids land application. Notably, Sorber et al. (1984) concluded that little or no risk was associated with the land application of liquid biosolids based on the lack of pathogenic viral presence in large volumes of sampled air. Other studies have focused on large piles of biosolids, unloaded by trucks on site, and subsequently loaded with front-end loaders into biosolids spreaders or hoppers (Pillai et al., 1996; Dowd et al., 2000). Loading events proved to be sources of increased concentration of faecal microbial indicators such as, H_2S producing bacteria, and *Clostridium* spp. No risk analyses were conducted in the former study although the investigators concluded that the microbial indicator concentrations were below levels that could be construed as a risk to public health. The latter study conducted microbial risk analyses based on the use of complex transport models first proposed for the transport of chemical aerosols (Pasquill, 1961). Through the use of these models, aerosol concentrations could effectively be predicted at downwind distances from both point (biosolids pile) and area sources (a biosolids applied field) (Dowd et al., 2000). Because of the lack of data on bioaerosols, the University of Arizona undertook a large national study to evaluate community risks from aerosols derived from land-application sites (Brooks et al., 2005a,b; Tanner et al., 2005). Risks of infection from *Salmonella* and Coxsackie virus A21 were determined at various distances from land-applied biosolids. An example of the data generated is illustrated in Table 3, which shows the annual community risk of infection from aerosolized *Salmonella* from land application at a distance of 30 m from the site. Risks were determined using the β-Poisson infectivity model for ingestion of nontyphoid *Salmonella* spp. (Brooks et al., 2005a). Risks of infection for Coxsackie virus A21 were also low. The most likely explanation for risks being so low is that for community risk, fate and transport of pathogens are important factors, allowing for dilution and natural attenuation of pathogens due to environmental factors such as dessication and ultraviolet light. In addition, it was postulated that organisms were bound to soil and or biosolid particles with limited subsequent transport. Occupational risks to biosolid workers were also evaluated and were found to be low, but higher than community risks. The higher risks are due to enhanced duration of exposure and proximity to the land application site.

In a more recent study, the diversity of aerosolized bacteria during land-application of Class B biosolids was determined (Brooks et al., 2007a). Specifically, sequence analyses of clones obtained from community DNA extractions were evaluated as to their source. Essentially the bacterial diversity of clone sequences from the following samples were obtained: 1) aerosols resulting from tractor operations on an agricultural field prior to land-application (aerosol-soil); 2) aerosols obtained during land-application (aerosol-biosolids); 3) Class B biosolids; and 4) control soil (no biosolids). The most likely predominant source of aerosols obtained during land-application of Class B biosolids can be determined from Figure 1. Interpretation of the Venn diagram shows that the majority of bacterial aerosols obtained during land-application of biosolids appear to be associated with the onsite soil, not the biosolids.

Recent work has concentrated on determining the source of bioaerosols from land application sites, utilizing methods developed for source tracking fecal contamination in watersheds (Paez-Rubio et al., 2006; Baertsch et al., 2007; Paez-Rubio et al., 2007). Microbial source tracking (MST) methods were utilized, in which the authors used bacterial DNA from the class *Chloroflexi* and the phylum *Euryarchaeota* , which are typically present in high concentrations in biosolids (Baertsch et al., 2007). These MST methods consisted of screening concentrated aerosol samples for the presence of these organisms using polymerase chain reaction (PCR). In addition, the investigators also used innovative approaches to indirectly report bioaerosol concentrations associated with Class B biosolids sites in the desert southwestern United States (Paez-Rubio et al., 2006; Paez-Rubio et al., 2007). These approaches involved the use of particulate matter (PM) measurements, and bulk biosolid chemical and biological measurements. The biological and chemical composition of downwind aerosols were estimated based on PM_{10} and bulk biosolid measurements. Through these three studies it was determined that biosolids/soil derived particulate matter do in fact aerosolize during disking operations, often to concentrations greater than 1.5 mg m^{-3}. The investigators estimate that biosolids derived particulate matter make up approximately less than 1/1000th of this concentration particularly during disking operations in which soil is the dominant particle. Overall this research confirmed that the majority of aerosols obtained during land application arise from soil sources rather than biosolids.

Based on all of these studies, we conclude that the community risk of human infection from pathogenic bioaerosols emanating from biosolids is low. However, another concern related to bioaerosols has been the issue of endotoxin, which can also be aerosolized. Endotoxin, lipopolysaccharide derived from the cell wall of Gram-negative bacteria, is a highly immunogenic class of molecules that when introduced into the bloodstream or airways has demonstrated the ability to cause a broad range of health effects, such as fever, asthma, and shock (hence the suffix “toxin”) (Bradley 1979; Olenchock 2001; Michel 2003). Lipopolysaccharide is present ubiquitously throughout the environment, as Gram-negative bacteria continuously release lipopolysaccharide during both cell decay and active cell growth. Most surfaces contain some traces of endotoxin due to dust-associated endotoxin, and therefore most human populations come into contact with some endotoxin (Gereda et al., 2001; Sharif et al. 2004). Although endotoxin is present in everyday environments, it is primarily of concern as an aerosol, since most human endotoxin ailments are pulmonary associated.

Exposures to aerosolized endotoxin have been specifically studied regarding occupational exposures from cotton dust, composting plants, and feed houses (Clark et al. 1983; Rylander et al., 1983; Castellan et al. 1987; Smid et al. 1992; Epstein 1994; Donham et al. 2000). Exposures to levels of endotoxin as low as 0.2 endotoxin unit (EU) m^{-3}, derived from poultry dust, have been found to cause acute pulmonary ailments, such as decreases in forced expiratory volume (Donham et al. 2000). Chronic conditions such as asthma and bronchitis have been found to be due to exposures of endotoxin from cotton dust as low as 10 EU m^{-3} on a daily basis (Olenchock 2001).

Past studies that have been conducted evaluating environmental exposures to endotoxin have used methods such as membrane trapping of aerosolized endotoxin. Recently, a study compared two methods of collection of aerosolized endotoxin: traditional membrane trappings and collection via impingement (Duchaine et al. 2001). Results suggest differences between the two methods, and that impingement may result in higher percent recoveries and greater precision. This same study focused on aerosolized endotoxin exposure in occupational settings, specifically swine barns and sawmills. It was shown that swine barns were found to contain mean concentrations of endotoxin 10 times greater than that of sawmills, 4,385 and 740 EU m^{-3}, respectively. Endotoxin concentration ranged from a minimum of 208 to 17,063 EU m^{-3} for sawmills and from 2,026 to 11,297 EU m^{-3} for swine barns, as collected by impingement sampling.

To determine the extent of endotoxin attributable to land-application, aerosol samples were recently collected during land-application of biosolids, during tractor operations on an agricultural field (no biosolids), and from an aeration basin located within an open-air wastewater treatment plant (Brooks et al., 2006). Data in Table 4 show that the greatest source of endotoxin was from a wastewater treatment plant aeration basin, although differences were not significant. In addition, an evaluation of the agricultural operations shows that on average, more endotoxin is aerosolized during tractor operations without biosolids, than with biosolids. Therefore, the majority of endotoxin may in fact be of soil origin, Work conducted by Paez-Rubio et al. (2006, 2007) corroborated these findings. These data also agree with the Brooks et al. (2007a) study which showed that soil was the major source of aerosolized bacteria.

Off-site Exposure to Pathogens via Groundwater Contamination

The transport and fate of viruses in porous media have received significant attention (Goyal and Gerba, 1979; Gerba et al., 1981; Bales et al., 1991; 1993; 1997; Jin et al., 1997; Dowd et al., 1998). Such studies have focused primarily on the adsorption, transport, and fate of viruses in aqueous (e.g., sewage effluent) systems. Conversely, minimal information with regard to the transport of indigenous coliphage associated with land application of biosolids is available.

The potential for groundwater contamination with pathogens due to land-application of biosolids is dependent on transport through soil and the vadose zone. Such transport is affected by a complex array of abiotic and biotic factors, including adhesion processes, filtration effects, physiological state of the cells, soil characteristics, water flow rates, predation and intrinsic mobility of the organism (Newby et al., 2000). Recent work at the University of Arizona showed that viruses are embedded and/or adsorbed to biosolids, which likely influences the potential for release and subsequent transport of the virus through soil under saturated conditions (Chetochine et al., 2006). Overall, less than 8% of indigenous coliphage initially present in Class B biosolids were leached out of the biosolids/soil matrix. Such binding reduces the mobility of viruses, and limits the potential for significant leaching. An additional study also showed that dissolved organic carbon had no influence on viral transport (Cheng et al., 2007). In agreement with this, Pancorbo et al. (1981) seeded anaerobically digested sewage sludge with poliovirus type 1 to a concentration of 10^6 PFU/ml. They recovered 60% by use of a serial extraction protocol suitable for poliovirus. Their findings indicated that the seeded polioviruses were adsorbed to the solid component of the sewage. Wellings et al. (1976) and Stagg et al. (1978) both reported that indigenous viruses were solid associated in wastewater-activated sludge. Stagg et al. demonstrated that 85% of the indigenous coliphage was adsorbed to solids present in sewage effluent. Bales et al. (1993) also reported that organic matter inhibited transport of phage and human viruses due to hydrophobic interactions. In contrast to these studies, other researchers have demonstrated enhanced transport of virus in the presence of organic matter (Pieper et al., 1997; Jin et al., 2000; Jin and Flury, 2002; Bradford et al., 2006a). This enhanced transport has been attributed to the blocking of favorable attachment sites by organic matter (Guber et al., 2005a). Dissolved organic matter from manure suspensions has also been shown to enhance transport of bacteria in soil (Guber et al., 2005b). Filling of straining sites has also been used to explain this enhanced transport (Bradford et al., 2006b). The different results obtained by different researchers are most likely due to the different types and concentrations of organic matter used in these studies. In the University of Arizona study (Chetochine et al., 2006), phage were shown to be tightly sorbed to biosolids such that 58 sequential extractions of the same biosolid sample were required to remove all phage from the biosolids. Thus, adsorbed and/or embedded viruses are less likely to be transported. Based on this, groundwater contamination from land-application of biosolids does not appear to be likely other than in areas where karst soils predominate with the potential for preferential flow.

Antibiotic-Resistant Bacteria

The antibiotic era began following Alexander Fleming's discovery of penicillin nearly 80 years ago. After the first introduction of antibiotics, overuse has been an issue, as over-prescription of first generation antibiotics has led to many resistant bacterial strains (Monroe and Polk 2000, Lieberman 2003). Human bacterial pathogens such as *Salmonella, Shigella*, and *Campylobacter* can all be potentially present in biosolids, and as such may present a cause for concern, due to the potential for gene transfer, which could include antibiotic resistance (Rensing et al. 2002, Dzidic and Bedekovic 2003). Of these, pathogenic antibiotic resistant bacteria (ARB) strains may be of most concern as these organisms are already resistant to many commonly prescribed antibiotics (Low 2001, Marshall et al 2004). Non-pathogenic ARB can potentially transfer their resistance genes horizontally to pathogenic strains through antibiotic resistant plasmids, either in the environment or human host (Rensing et al 2002, Dzidic and Bedekovic 2003, Salyers et al 2004). Therefore, potentially when soil, water, or food that has been in contact with biosolids is consumed, there exists the possibility of these ARB, and subsequent transfer of resistance genes occurring within the host. To date however, this has not yet been demonstrated.

The evaluation of the influence of land-application of biosolids on the incidence of ARB within soil was recently investigated (Brooks et al., 2007b). Table 5 illustrates the influence of land-application of Class B biosolids on the incidence of soil bacterial resistance to four antibiotics: ampicillin; cephalothin; ciprofloxacin; and tetracycline. Data from soil samples collected prior to land-application, and up to 450 days following land-application show that the influence of biosolids on the incidence of soil-borne antibiotic resistant bacteria was negligible.

Risk of Infection from *Salmonella* Regrowth in Biosolids

Regrowth of *Salmonella* in biosolids during storage after it has left the wastewater treatment plant is one of the more recent concerns with respect to land-application of biosolids. Many laboratory studies have been conducted in the past, focusing on survival and potential growth of inoculated organisms in sterile and nonsterile biosolids and compost. Regrowth of inoculated salmonellae in sterile biosolids is commonly documented, but few studies have documented survival and regrowth of indigenous pathogens in biosolids after levels have decreased below levels of detection. Due to limited available data regarding regrowth of salmonellae in biosolids used for land application, there are conflicting views on this topic. Some studies have shown that regrowth does occur, while other studies have shown that regrowth does not occur. These differences are most likely due to whether indigenous salmonellae are studied or whether biosolids are reseeded with salmonellae. Recently the regrowth potential of both Class A and Class B biosolids and biosolid amended soil was evaluated at the University of Arizona (Zaleski et al., 2005a; Castro del Campo et al., 2007).

Regrowth of *Salmonella* does not normally occur in Class B biosolids (Zaleski et al., 2005a). However during solar drying of Class B liquid biosolids in concrete lined drying beds, we did observe regrowth. Specifically during the solar drying, the initially Class B biosolids material (both aerobically and anaerobically) attained Class A status with respect to *Salmonella* levels (<3MPN per 4g). However, following rainfall events the biosolids became saturated and hence anaerobic, allowing regrowth of *Salmonella* to $\simeq 10^5$/g (Zaleski et al., 2005b). For Class A biosolids, regrowth also occurred when biosolids were maintained under saturated anaerobic conditions (Castro-Del Campo et al., 2007). This is consistent with other cited studies. For example Burge et al. (1987) observed that growth of *Salmonella* in Class B biosolids required moisture content greater than 20%. This also agrees with the earlier study of Thomason et al. (1975) who also detected *Salmonella* within wet environments. No regrowth occurred from Class A or B biosolids when the material was added to soil, regardless of whether samples were saturated or not (Zaleski et al., 2005b). The risks of infection from *Salmonella* from direct contact with, or aerosolization from, land applied Class A biosolids in which regrowth had occurred or land applied Class B biosolids, are shown in Table 6. Risks from land-applied Class B were low regardless of whether the route of exposure was from the ingestion of *Salmonella* following direct exposure, or from ingestion following inhalation of an aerosol. Note that *Salmonella* infections due to inhalation has not been documented at this time. In contrast, risks from contact with Class A biosolids following regrowth in the initial biosolids were significant. Therefore, care must be taken to prevent regrowth in Class A biosolids prior to land-application. Regrowth can be prevented by covering biosolids and precluding saturated anaerobic conditions (Gerba et al., 2007).

LONG TERM EFFECTS OF LAND-APPLICATION OF LIQUID CLASS B BIOSOLIDS ON SOIL PROPERTIES

Sustainability of any process implies that the process can be maintained indefinitely. Further, sustainability necessitates addressing the needs of the present without compromising the ability of future generations to meet their needs. Within this context, the sustainability of land application of Class B biosolids needs to be evaluated over long time periods, and the influence of repeated annual applications of biosolids on soil properties must be documented. Fundamentally, soil quality is maintained through the integration of soil physical, microbial, and chemical properties.

Influence on Soil Physical Properties

Soil physical properties are controlled by soil texture, which is intrinsic to any given soil, and soil structure, which is highly variable. Long term additions of biosolids have been shown to improve the physical properties of the amended soil in several studies. Aggelides and Londra (2000) documented improved physical properties including: saturated and unsaturated hydraulic conductivity; water retention capacity; soil resistance to penetration; bulk density; total porosity; pore size distribution; aggregation and aggregate instability. This study was conducted in a semi-arid climate and improvements were greater in a loamy soil than in a clay soil. Similarly, even three years of land application of biosolids improved the physical properties of a clay loam soil (Tsadilas et al., 2005). This field study was conducted in Mediterranean conditions in central Greece and documented enhanced water retention capacity, available water, and improved infiltration rates. The improvement of physical properties is most likely due to the increased soil organic matter content following land application. Organic matter content has been positively correlated with water retention and infiltration rate, and negatively correlated with bulk density and aggregate instability index (Tsadilas et al., 2005). Although the increased soil organic matter content that occurs following an application is always likely to improve soil physical properties, care must be taken to avoid compaction that can occur due to increased traffic operations. Stamatiadis et al. (1999) documented soil compaction and decreased water infiltration rates following land application of liquid municipal sewage sludge in eastern Nebraska. However, provided that land application is carefully managed i.e., avoidance of traffic over saturated soils, than land application is likely to be sustainable with respect to soil physical properties.

Influence on Soil Microbial Properties

The influence of long term land-application on the soil microbial community and soil chemical properties was evaluated in a University of Arizona 1986-2005 field study (Zerzghi, 2008). Essentially this 20 year study provides one basis for the evaluation of the sustainability of land-application since it is one of the longest studies of its kind. This replicated field plot study had 4 treatments: i) control (no amendment); ii) inorganic fertilizer control; iii) biosolids at an agronomic rate (1X) based on the nitrogen requirements for the growth of cotton (160 kg N/ha). The land applied biosolids contained 8% solids; and iv) biosolids at a 3X rate. Twenty annual land applications were applied from 1986-2005. All plots were utilized for the growth of cotton and furrow irrigated as necessary for optimum plant growth. Water supplied as irrigation was approximately 100 cm per growing season, since the consumptive water use requirements for cotton are 92 cm per season. This is approximately twice the evapotranspiration requirements for cotton allowing for leaching of soluble salts through the soil profile. The influence of land-application on the soil microbial community was evaluated in terms of microbial numbers, microbial activity and microbial diversity. The survival of indicator and pathogenic organisms was also determined. Finally note that some parameters were not only evaluated at the termination of the 20 year study, but also earlier in the study (Artiola and Pepper, 1992; Brendecke et al, 1993).

Land-application of Class B biosolids resulted in numbers of heterotrophic bacteria, fungi and actinomycetes that were similar to numbers found in control plots. This is not unexpected since the surface soil samples for microbial analyses were collected in December 2005, nine months after the last application of biosolids in March 2005. During this interval, a crop of cotton was grown and harvested, and all available microbial substrate would have been utilized. The lack of adverse affects of 20 years of land application of biosolids on soil microbial numbers agrees with earlier analyses within this study that were conducted in 1990 after 4 annual land applications (Brendecke et al., 1993). In these analyses, 4 years of land application had no significant effect on numbers of soil bacteria, fungi or actinomycetes. In addition, soil respiration rates (as measured by CO_2 evolution) were similar in land applied and control plots. In a recent British study, 3 years of land application at 3 different sites likewise resulted in no observed effect on soil respiration rates, or biomass carbon concentrations (Gibbs et al., 2006a). In addition, numbers of indigenous clover *Rhizobium* were also unaffected by biosolid additions. After 20 years of land application in the University of Arizona study, total coliform and fecal coliform counts did not exceed 3 MPN/g in plots that received biosolids. Control and fertilizer control plots did not contain detectable indicators, or coliphage, *Salmonella* and enteroviruses were not detected in any plots.

These data agree with other studies on the survival of indicators and pathogens introduced into soil via land application of biosolids. Specifically, limited survival of *E.coli* and other enteric organisms in biosolids-amended agricultural soil was documented in laboratory and field studies (Lang and Smith, 2007; Lang et al., 2007; Lang et al., 2003; Pepper et al., 1993). Lang and Smith (2007) concluded that the indigenous soil biota were involved in pathogen reduction processes in biosolid amended soil. Survival of enteroviruses in biosolid-amended soils was also evaluated earlier in the University of Arizona study. Specifically the duration of survival of poliovirus type 1 was less than 7 days in the summer months, and 7-14 days during winter months (Straub et al., 1993). Thus it is not surprising that viruses were not detected in the U of A study, 9 months after the last land application.

Microbial activity in the University of Arizona 20 year land application study was assessed by studies on the common microbial transformations: nitrification and sulfur oxidation, where rates of both microbial transformations increased in soils from the biosolid amended plots, and increased with increased rate of biosolids application. Dehydrogenase activity also increased with increased biosolid amendment. This is in contrast to trends earlier in this study, when after 4 years of land application, dehydrogenase activity was unaffected by biosolid additions (Brendecke et al., 1993). After 20 years of land application, bacterial diversity in all plots was evaluated through cloning and sequence analysis of bacterial 16S rRNA (Zerzghi, 2008). Here, data showed that the known number of identifiable species increased in the high rate biosolid plots, when compared to control (no biosolid) plots.

In summary, the soil microbial community did not appear to be adversely affected by 20 years of land-application. In fact, land-application appeared to have been beneficial as evidenced by increased microbial diversity, and enhanced microbial activity. In addition, no known pathogens were detected in soils sampled 9 months after the last biosolid application.

Influence on Soil Chemical Properties

At the termination of the 20 year land application study, soil chemical properties were also evaluated in all plots. Specifically, soil core samples were collected from 0-150 cm depths in 30 cm intervals. As in the case for surface soil samples collected for microbial analyses, samples were taken in December 2005, nine months after the 20th land application in March 2005. These soil core subsurface samples allowed for an evaluation not only of the influence of long term land-application of biosolids on soil chemical properties, but also the influence of land application on various parameters as a function of soil depth. Analysed soil chemicals clustered into three main groups: soil macro elements (N,P,C); heavy metals, and endocrine disruptors.

Soil Macro Elements

Soil nitrate concentrations in both biosolid amended plots and plots that received inorganic fertilizers for 20 years were significantly higher than control (no amendment) plots when averaged over all soil depths (0-150 cm). Total nitrogen increased in biosolid-amended soil (Table 7). Nitrate values in both biosolid and fertilizer treated plots exceeded 10 ppm NO_3-N at most soil depths down to 150 cm. These data indicate the potential for nitrate pollution of groundwater regardless of whether biosolids or inorganic fertilizer are applied to the soil. Land application of biosolids also significantly increased total and available soil phosphate concentrations, particularly in the surface (0-30 cm) soil. These data are not unexpected since several other studies have documented phosphate increases following land application (Mantovi et al., 2005). Increases in soil phosphate concentrations of biosolid amended soil were already evident in soil samples collected from the same study after 4 years of land application (Brendecke et al., 1993). Elliott and O'Connor (2007) recently stated that "phosphorus is at the forefront of biosolids-related issues that may adversely affect the long term sustainability of land-based recycling programs in the U.S." Such concern is based on the potential for water quality deterioration that can occur in surface waters due to eutrophication following phosphate accumulations in surface water runoff. However, these issues are more important in the eastern USA, since in the arid southwest, surface waters are rare. That notwithstanding, phosphorus management will continue to be important for sustainable biosolids recycling in the United States.

Total organic carbon significantly increased in biosolid amended soil after 20 years of land application (U of A study). This is in contrast to analyses of the same plots following 5 land applications. Data from samples collected in 1990 showed no differences in the soil total organic carbon in control versus biosolid amended soils (Artiola and Pepper, 1992). However dissolved organic carbon increases were detected in the 1990 biosolid-amended soil samples. These data illustrate how difficult it is to increase soil organic matter in soils of the arid southwest USA, due to high mineralization rates (Artiola and Pepper, 1992). But it is important to note that even modest increases in total organic carbon are important to soil fertility in soils which are traditionally low in soil organic matter (0.5-1%) (Fuller, 1991). Land application of biosolids has also been shown to increase soil organic carbon in other studies (Gibbs et al., 2006b; Mantovi et al., 2005).

No increases in soil salinity were observed following the 20 years of land application , no doubt in part because irrigation rates were in excess of consumptive water use rates for cotton, resulting in the leaching of salts through the soil profile.

Soil Heavy Metals

The biosolids applied in the University of Arizona 20 year land-application study contained relatively low levels of heavy metals (Table 8). After 20 annual land applications, significant but modest increases in available concentrations of some metals were detected including Cu, Cd, Zn, and Ni (Table 7). However, biosolid-amended soil concentrations of available metals were low and not hazardous. In addition, the metal concentrations found within biosolids have decreased over the past 20 years due to improved point-source controls. Finally, metal concentrations attenuated rapidly with increasing soil depth, and were generally similar to values found in control soils at a depth of 150 feet. Overall, the potential for metal contamination of soil or surface waters from land application of biosolids has decreased. Recently, Tian et al. (2006) concluded that application of biosolids for land reclamation at high loading rates from 1972-2002 only impacted surface water quality prior to the promulgation of the 40 CFR Part 503 regulations. After the promulgation, metal impacts on surface water quality were minimal. In addition to reduced concentrations of metals in biosolids, new understanding of trace element chemistry in biosolid-amended soil has shown that following termination of land application, available metal concentrations essentially remain constant provided the soil pH remains constant, or even decreases (Basta et al., 2005). Such decreases are thought to be due to sorption to inorganic oxide surfaces or very recalcitrant organics present in soil of non-biosolid origin (Basta et al., 2005).

Endocrine Disruptors

Endocrine disrupting compounds (EDCs) are chemicals that interfere with endocrine glands or their hormones. Polybrominated diphenyl ethers (PBDEs) are compounds utilized as flame retardants for everyday household items including carpets and cushions. PBDEs are a known class of EDCs that are typically present at ppm levels in municipal biosolids produced in the USA. Data on the fate of PBDEs following land application are limited, but was recently evaluated at the University of Arizona. Following 20 years of land application, 50–70% of the (estimated) applied PBDE mass was accounted for in the upper two feet of receiving soil, suggesting that PBDEs are conserved in soil over periods of decades or longer, most likely due to the hydrophobic nature of PBDEs (Quanrud et al., 2007). Due to sorption, biodegradation of the PBDEs would be unlikely. However, although the consequences of PBDE accumulation in soil are unknown, it is important to note that PBDE concentrations in household dust are similar to concentrations found in biosolids (Quanrud et al., 2007).

Biosolids are also known to contain other endocrine disrupting compounds including the estradiol and estrone (Drewes and Shore, 2001) and can contain other synthetic endocrine disruptors present in pharmaceuticals and personal care products (Daughton and Ternes, 1999). However to date, little is known of the fate and transport of these compounds following land application. Lorenzen et al. (2006) showed that a wide range of endocrine disruptors including 4-nonylphenol, ethynylestradiol, estradiol and estrone are rapidly degraded from biosolids or animal wastes following land application. Roberts et al. (2006) also showed that 4-nonylphenol was rapidly mineralized in soil. In addition, they found that plant uptake of nonylphenol was minimal. Finally, they concluded that “the spreading of nonylphenol contaminated waste to soil probably poses a very low environmental risk to freshwater ecosystems and human health.” Based on this land application of biosolids is likely to be sustainable with respect to endocrine disruptors.

SUMMARY: SUSTAINABILITY OF LAND-APPLICATION OF CLASS B LIQUID BIOSOLIDS

The overall conclusion we have reached based on all of our land-application studies over the past two decades and an in depth review of other relevant land application studies is that land-application of Class B biosolids is sustainable. Specifically, the risks to human health posed by many microbiological entities within biosolids have been shown to be low if current EPA regulatory guidelines are followed. In addition, risks from indirect exposures such as aerosolized pathogens or contaminated groundwaters appear to be particularly low. This is not to say that the risks are zero, but that the risks or concerns can be managed to safeguard human health and provide a sustainable environment. Of course, vigilance is always necessary as new contaminants continue to emerge, and their presence and fate in biosolids need to be assessed to ensure current practices and guidelines are protective. Long term land-application in the University of Arizona study showed enhanced microbial activity and diversity. Overall these are positive beneficial effects, and there was no evidence of adverse toxicity effects on the soil microbial community with respect to numbers, activity or diversity. Long term land-application also increased soil macro-nutrients including C, N, and P. In addition, increases in available metal concentrations were modest, and no increase in soil salinity was observed. This lack of increased salinity was likely due to leaching of salts out of the root zone, following row irrigations throughout each growing season. Since irrigation rates were in excess of consumptive water use rates for cotton, such leaching may ultimately impact groundwater quality. To date there does not appear to be any chemical entity likely to limit land-application other than phosphate loadings in areas sensitive to surface water contamination.

Also in the University of Arizona study, there was no evidence of long term persistence of enteric pathogens in the soil, nor migration of pathogens to groundwater. In addition it is well documented that endocrine disruptors such as 4-nonylphenol and estrone are rapidly degraded in soils. Other hydrophobic endocrines such as PBDEs are strongly sorbed to soil colloids and are relatively immobile in soil. Finally it should be noted that the University of Arizona study was conducted in the arid southwest and as such results are site specific. Application of these findings to other locations needs to be conducted carefully.

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FIGURE CAPTION

Figure 1. (A) Number of shared orders associated with unique aerosolized clone isolates detected from downwind of aerosolized soil without biosolids, from downwind of biosolids land-application operations when compared to onsite soil; (B) when compared to Class B biosolids; (C) Number of shared orders associated with unique clone isolates detected from onsite soil, Class B biosolids when compared to aerosols collected during biosolids land-application operations; (D) when compared to aerosolized soil without biosolids. (Source: Brooks et al., 2007a)

Table 1. Known principal pathogens of concern in Class B biosolids in the U.S.

BACTERIA	PROTOZOA
Salmonella sp.	*Cryptosporidium*
Shigella sp.	
Yersinia	*Giardia lamblia*
Vibrio cholerae	
Campylobacter jejuni	*Toxoplasma gondii*
Escherichia coli	
ENTERIC VIRUSES	HELMINTH WORMS
Hepatitis A virus	*Ascaris lumbricoides*
Adenovirus	*Ascaris suum*
Norovirus	*Trichuris trichiura*
Sapporovirus	*Toxocara canis*
Rotavirus	*Taenia saginata*
Enterovirus	*Taenia solium*
- Poliovirus	*Necator americanus*
- Coxsackievirus	*Hymenolepisnana*
- Echovirus	
- Enterovirus 68-91	
Reovirus	
Astrovirus	
Hepatitis E virus	
Picobirnavirus	

Table 2. Incidence of *Staphylococcus aureus* in sewage, biosolids, and bioaerosols.

Sample	**Number of Samples**	**Incidence of *S.aureus***
Raw sewage	3	1/3 positive
Undigested primary sewage	2	2/2 positive
Class B biosolids—anaerobic mesophilic	6	All negative
Class B biosolids—aerobic mesophilic	1	Negative
Class B biosolids—aerobic mesophilic, lime	9	All negative
Class A biosolids—aerobic thermophilic	1	Negative
Class A biosolids—anaerobic thermophilic	1	Negative
Class A biosolids—heat dried pellets	4	All negative
Class A composted biosolids	1	Negative
Bioaerosols from land-application sites	27	All negative

Source: Adapted from Rusin et al. (2003).

Table 3. Annual community risk of infection from aerosolized *Salmonella* during land-application of Class B biosolids[1] (Modified from Brooks et al., 2005b).

Annual Risk					
Number of *Salmonella*					
500 *Salmonella* g^{-1} Biosolids		**50 *Salmonella* g^{-1} Biosolids**		**5 *Salmonella* g^{-1} Biosolids**	
Exposure Time					
1h	***8h***	***1h***	***8h***	***1h***	***8h***
4.5×10^{-8}	3.6×10^{-7}	4.5×10^{-9}	3.6×10^{-8}	4.5×10^{-10}	5.6×10^{-9}

[1] Risk of infection based on 6 days per year of land-application at a distance of 30.5 m from the application site during loading conditions.

Table 4. Aerosolized endotoxin concentrations detected downwind of various operations (Modified from Brooks et al., 2006).

Source of Sample	**Number of Samples Collected**	**Endotoxin Concentration ($EU\ m^{-3}$)**		
		Range	**Average**	**Median**
Ambient background	12	2-4	2.6	2.5
Biosolids - loading	39	6-1808	344	92
Biosolids - land-application	24	5-143	34	6
Wastewater treatment plant - aeration basin	6	294-891	627	639
Tractor on field - no biosolids	5	284-659	470	491

EU = endotoxin units

Table 5. Percentage of heterotrophic plate count (HPC) bacteria exhibiting antibiotic resistance from soil collected at a land-application site (Modified from Brooks et al., 2007b).

	% Resistant^1			
Time (days)	**Ampicillin**	**Cephalothin**	**Ciprofloxacin**	**Tetracycline**
One day prior to land application	12.1	12.4	1.6	1.1
0	5.3	7.9	0.9	1.4
7	11.5	11.5	2.8	2.0
14	5.4	6.1	1.5	0.6
30	14.6	13.6	4.8	3.1
60	8.7	10.7	1.9	1.6
90	8.2	8.4	2.1	1.8
120	8.4	11.0	2.2	2.0
150	9.1	11.1	2.0	1.8
180	5.3	6.6	2.7	1.3
450	6.7	10.5	2.3	1.9

Mean Value Post Biosolid Land-application (7-450 days)	8.7	9.9	2.3	1.9

[1] Antibiotic concentrations of cultured plates: ampicillin = 32 µg ml^{-1}; cephalothin = 32 µg ml^{-1}; ciprofloxacin = 4 µg ml^{-1}; and tetracycline = 16 µg ml^{-1}.

Table 6. Risk of infection from *Salmonella* from direct contact with, or aerosolization from, land applied Class A (following regrowth) or Class B land applied biosolids (Modified from Gerba et al., 2007).

Class of Biosolids Land-Applied	**Number of *Salmonella*/gm**	**Route of Exposure**	**Risk of Infection**
B	105	Ingestion of 50 mg via direct contact	6×10^{-6}
B	105	Ingestion of 480 mg via direct contact	6×10^{-5}
B	500	Ingestion of 50% of aerosol[1] inhaled	1×10^{-4}
A	10^{6}	Ingestion of 50 mg via direct contact	5×10^{-3}
A	10^{6}	Ingestion of 480 mg via direct contact	3×10^{-1}
A	10^{6}	Ingestion of 50%[1] of aerosol inhaled	1×10^{-2}

[1] 8 hr exposure per day for 6 days annually at 30m distance from the site.

Table 7. Influence of 20 years of land-application of liquid Class B biosolids on soil chemical properties. Adapted from Zerzghi, 2008.

Soil Chemical Parameter	**Influence of Land-Application**
pH	None
Electrical Conductivity (EC)	None
% $CaCO_3$	Values increased with rate of biosolid addition and soil depth
Total N	Higher in biosolid plots
Total Organic Carbon	Higher in biosolid plots
Total P	Higher in biosolid plots
Available P	Higher in biosolid plots
Total Metals: Cu; Cd; Zn	Higher in biosolid plots
Total Metals: Pb; Ni	No discernable effect
DTPA Metals: Cu; Zn; Pb; Ni	Higher in biosolid plots
DTPA Cd	No discernable effect
Total Metals: As; Hg; Mo; Se; Cr and B	No discernable effect
Polybrominated diphenyl ethers (PBDEs)	Higher in biosolid plots

[1] DTPA = diethylene triamine penta acetic acid

Table 8. Representative heavy metals in land-applied biosolids in the University of Arizona study.

Metal	**Range (mg kg^{-1} dry biosolids)**
Zn	800-1590
Cu	568- 957
Pb	89-221
Ni	26-51
Cr	32-95
Cd	7-15
Ag	3-60