Science of the Total Environment 565 (2016) 200–210

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Fate of classical faecal bacterial markers and ampicillin-resistant bacteria in agricultural soils under Mediterranean climate after urban sludge amendment

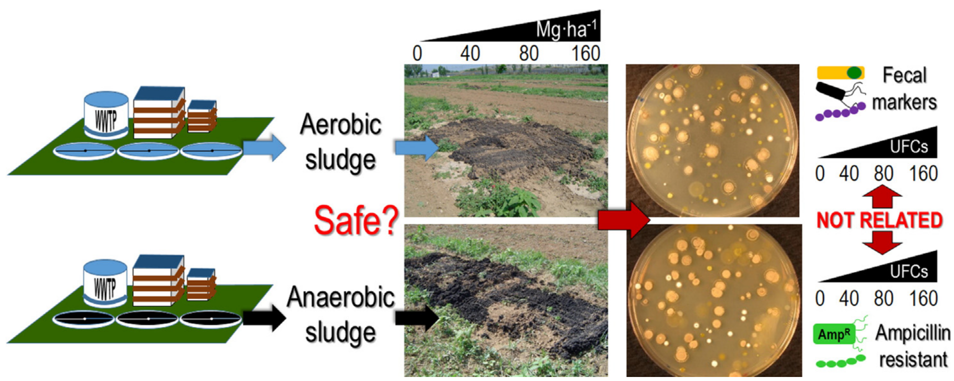
Clarissa Gondim-Porto, Leticia Platero, Ignacio Nadal, Federico Navarro-García *

Dpt. Microbiología II, Facultad de Farmacia, Univ. Complutense de Madrid, Madrid, Spain

HIGHLIGHTS

- Effect of biosolids on fecal and ampicillin-resistant bacteria (AmpR) in crop soil
- Bacteria in soils were analyzed every four months using culturing methods.
- Faecal and AmpR bacteria increased and persisted in amended soil for two years.
- The proportion of AmpR bacteria in amended soils differed with different biosolids.
- No correlation between classical faecal markers and AmpR bacteria was found.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 3 March 2016
Received in revised form 22 April 2016
Accepted 22 April 2016
Available online xxxx

Editor: D. Barcelo

Keywords:
Soil microbiota
Fecal bacteria
Ampicillin resistant bacteria
Urban sludge
Mediterranean climate

ABSTRACT

The use of sewage sludge or biosolids as agricultural amendments may pose environmental and human health risks related to pathogen or antibiotic-resistant microorganism transmission from soils to vegetables or to water through runoff. Since the survival of those microorganisms in amended soils has been poorly studied under Mediterranean climatic conditions, we followed the variation of soil fecal bacterial markers and ampicillin-resistant bacteria for two years with samplings every four months in a split block design with three replica in a crop soil where two different types of biosolids (aerobically or anaerobically digested) at three doses (low, 40; intermediate, 80; and high, 160 Mg·ha^{-1}) were applied. Low amounts of biosolids produced similar decay rates of coliform populations than in control soil (-0.19 and -0.27 log$_{10}$CFUs·g^{-1} dry soil month^{-1} versus -0.22) while in the case of intermediate and high doses were close to zero and their populations remained 24 months later in the range of 4–5 log$_{10}$CFUs·g^{-1} ds. Enterococci populations decayed at different rates when using aerobic than anaerobic biosolids although high doses had higher rates than control (-0.09 and -0.13 log$_{10}$CFUs·g^{-1} ds month^{-1} for aerobic and anaerobic, respectively, vs -0.07). At the end of the experiment, counts in high aerobic and low and intermediate anaerobic plots were 1 log$_{10}$ higher than in control (4.21, 4.03, 4.2 and 3.11 log$_{10}$CFUs·g^{-1} ds, respectively). Biosolid application increased the number of *Clostridium* spores in all plots at least 1 log$_{10}$ with respect to control with a different dynamic of decay for low and intermediate doses of aerobic and anaerobic sludge. Ampicillin-resistant bacteria increased in amended soils 4 months after amendment and remained at least 1 log$_{10}$ higher 24 months later, especially in aerobic and low and intermediate anaerobic plots due to small rates of decay (in the range of -0.001 to -0.008 log$_{10}$CFUs·g^{-1} ds month^{-1} vs -0.016 for control). Aerobic plots had relative populations of

* Corresponding author at: Dpt. Microbiología II, Facultad de Farmacia, Universidad Complutense de Madrid, Plaza de Ramón y Cajal s/n, E-28040 Madrid, Spain.
E-mail address: fnavarro@farm.ucm.es (F. Navarro-García).

http://dx.doi.org/10.1016/j.scitotenv.2016.04.160

ampicillin-resistant bacteria higher than anaerobic plots with different positive trends. Dose (22%) and time (13%) explained most of the variation of the bacterial populations. Dynamics of fecal markers did not correlate with ampicillin-resistant bacteria thus making necessary to evaluate specifically this trait to avoid possible risks for human and environmental health.

1. Introduction

The use of human and animal residues as organic amendments for agricultural fertilization is one of the most common practices in crop management. These residues introduce different amounts of essential elements such as carbon, nitrogen, sulfur, potassium and phosphorus, increase water retention and stabilize the soil structure. These aspects are especially important for soils in the Mediterranean region which are more prone to degradation through erosion due to its characteristic climatic variations like seasonally prolonged drought and extreme rainfall events (González-Hidalgo et al., 2007). Different types of organic amendments are available in the market but nowadays a suitable option is sewage sludge or biosolids from wastewater treatment plants. In fact, the use of these products in agriculture helps to solve a major problem in developed countries: the increasing generation of urban residues (Wang et al., 2008). Overall Europe, the annual production of sewage sludge is about 12 million tons (dry matter) being 42% of them used as soil fertilizer, while USA produced 6.2 million tons per year (Navia and Mittelbach, 2012). As a specific example, in 2009 the Spanish production of sewage sludge from wastewater treatment plants was 1.2 million tons, being used as agricultural amendment ~83% of the total (0.99 tons) (Secretaría General Técnica, 2010).

Nevertheless, the environmental implications of the use of sewage sludge as a crop soil amendment are still unclear, and its impact in global change, groundwater pollution by reactive nitrogen (Galloway et al., 2004; Tarrasón et al., 2008) or the appearance of emergent pollutants is being reevaluated (Eriksson et al., 2008; McClellan and Halden, 2010; Postigo et al., 2010). Among those aspects, microbial biosecurity is coming back into focus due to two important issues: the transfer and persistence of surrogates of pathogens or *Escherichia coli* O157:H7 to vegetables grown in soil treated with contaminated compost and irrigation water (Girardin et al., 2005; Oliveira et al., 2011; Oliveira et al., 2012) and the transfer and persistence of antimicrobial resistance or the evolution of antibiotic resistance traits in soil (Kümmerer, 2004) that could end in human beings.

The processes of sewage sludge production reduce the presence of pathogenic bacteria (*Salmonella*, *Shigella* and *Campylobacter*), parasites, protozoa and virus, but the intrinsic thermal resistance of some bacteria (coliforms, enterococci) (Bonjoch and Blanch, 2009), or the production of structures of resistance like spores, pose the question of their transmissibility and viability in soils (United States Environmental Protection Agency, 1995). The common strategy to evaluate the presence or persistence of pathogenic bacteria in biosolids has been the determination of microbial indicators that are present in human excreta like fecal or thermotolerant coliforms and fecal enterococci (Pepper et al., 2010). In amended soils, as in sewage sludge, fecal coliforms, *E. coli* or enterococci are used as microbial indicators. The main reasons are that coliforms are present in large numbers in human faeces and that enterococci survive longer outside the intestine, being, thus, easy markers to find and track (Sidhu and Toze, 2009). Although continuous research to find other suitable markers related to different human pathogens, like enteric viruses, protozoan parasites or helminthes, has been developed with variable results, research is still necessary (Sidhu and Toze, 2009; Environmental Protection Agency, 2012).

In the case of antibiotic resistances, the transfer to soil bacteria seems to be well established when manure from antibiotic-treated farm animals is used (Heuer et al., 2011). Nevertheless, the effect of urban sewage sludge is not clear yet: only a few studies indicate that antibiotic resistance markers or resistant bacteria remain temporarily in soils after amendment (Brooks et al., 2007; Munir and Xagoraraki, 2011; Rahube et al., 2014; Ross and Topp, 2015) and that can even be acquired by animals (Blanco et al., 2009). And, more importantly, none of them have been developed under the conditions of Mediterranean climate. For that reason, we decided to undertake a long-term study in Spain of the variation of microbial populations in crop soil amended with different amounts of urban sludge mimicking agricultural uses with particular attention to bacteria resistant to ampicillin, the most used antibiotic in human therapeutics in Spain (Campos et al., 2007). Our hypotheses are that 1) amendments would increase the presence and residence time of microbial markers, 2) ampicillin-resistant bacteria would increase in soil after biosolid amendment and 3) increases of ampicillin-resistant bacteria in soil could be tracked with classical bacterial markers, since this trait is not currently tested in sewage sludge nor in amended soils and neither standardized protocols nor recommendations exist for this type of analysis.

2. Materials and methods

2.1. Study site

Experimental plots were set up in an experimental farm ("La Isla") from the Institute for Crop and Food Research of Madrid (Instituto Madrileño de Investigación y Desarrollo Rural y Agrario, IMIDRA, Comunidad Autónoma de Madrid, Spain). This research station is located in Arganda del Rey, 20 km E from Madrid, Spain (40°18′52.9992″ N, −3° 29′ 51.2694″ W (40.314722,−3.497575)). During the period of study, the mean daily temperature was 15.5 °C with a mean annual rainfall of 388.4 mm, preferentially accumulated in spring and fall. These values were very similar to the records in the research station spanning the last 30 years (14.7 °C and 385 mm, respectively). As a typical Mediterranean climate, winter is cold and dry and summer registered the highest temperatures combined with dryness. Temperature and rainfall data are shown in Supplemental Fig. 1.

2.2. Organic amendments

Two types of sewage sludge were used, which were generated in two wastewater plants from Canal de Isabel II. Aerobic digested sludge (AE) was produced and air-dried in Campo Real treatment plant (Campo Real, Madrid, Spain), while anaerobic digested sludge (ANAE) was obtained after digestion without the addition of oxygen in the Guadarrama medio treatment plant (Brunete, Madrid, Spain). The chemical composition of sewage sludges is shown in Table 1. Levels of metals were below the average values in Spain (Ortega et al., 2009) and fulfilled national and European legislation for agricultural use (Council of the European Union, 1986; Real Decreto 1310-1990, 1990).

2.3. Experimental plots and sampling

The land on which the plots are located consists of Quaternary sediments from the Jarama River, basically sands and silts. These sediments are of alluvial origin, giving rise to a Calcaric Fluvisol, which today has the characteristics typical of Antrosol (IUSS Working Group WRB, 2006). The particle size distribution is characterized as Coarse Clay (7.78% fine sand, 41.28% silt, 23.61% sand and 27.34% clay) (Gonzalez-Ubierna et al., 2013). Soil composition was identical in all plots at the