Journal of Environmental Management 119 (2013) 13–19

Contents lists available at SciVerse ScienceDirect

Journal of Environmental Management

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

Impact of calcium carbonate and temperature on survival of *Escherichia coli* in soil

M.B. Farhangi [a,*], A.A. Safari Sinegani [a], M.R. Mosaddeghi [b], A. Unc [c], G. Khodakaramian [d]

[a] Department of Soil Science, College of Agriculture, Bu-Ali Sina University, Hamadan 65174, Iran
[b] Department of Soil Science, College of Agriculture, Isfahan University of Technology, Isfahan 84156-83111, Iran
[c] Plant and Environmental Sciences, New Mexico State University, Box 30003 MSC 3Q, Las Cruces, NM 88003-8003, USA
[d] Department of Plant Protection, College of Agriculture, Bu-Ali Sina University, Hamadan 65174, Iran

ARTICLE INFO

Article history:
Received 19 August 2012
Received in revised form
3 January 2013
Accepted 27 January 2013
Available online 19 February 2013

Keywords:
$CaCO_3$
Temperature
Escherichia coli NAR
Survival
Decay rate

ABSTRACT

Spreading of waste organic matter on agricultural lands is considered to enhance soil microbial activities and physical properties and improves soil nutrient status. However, organic wastes have also been shown to be a source of microbial contaminants including pathogens. Related risks are governed by pathogens' survival and transport particularities. We evaluated the significance of high levels of $CaCO_3$, common in arid and semi-arid soils, on survival of *Escherichia coli* NAR at different temperatures. Amendments of 0, 5, 10, 15 or 25 g $CaCO_3$ were mixed into variable soil amounts to obtain 100 g soil–$CaCO_3$ mixtures. Both sterile and non-sterile soil mixtures were tested. Suspensions of a nalidixic acid-resistant *E. coli* strain (*E. coli* NAR) were added to the mixtures at a rate of 10^6 cell g^{-1} soil. Mixtures were incubated at 4, 15, or 37 °C at the soil's field capacity for water (i.e. 0.13 g g^{-1}). Each treatment was tested in triplicate. Persistence of culturable *E. coli* NAR was verified throughout the incubation period. The recovery rates of culturable *E. coli* NAR were significantly correlated to $CaCO_3$ concentrations ($P < 0.05$). Incubation temperature (T) was the most significant factor ($P < 0.01$). In non-sterile mixtures the largest decline in survival rates of *E. coli* NAR was measured for treatments with larger $CaCO_3$ content (i.e. 15 and 25%). Interaction of temperature and $CaCO_3$ was significant for *E. coli* NAR die-off. Sterilization of soil caused non-uniform fluctuations in the effect of treatments. The maximum calculated decay rate for *E. coli* NAR was 0.83 d^{-1} for the 15 g $CaCO_3$ non-sterile mixture incubated at 37 °C while the minimum was 0.09 d^{-1} for the control unamended sterile soil incubated at 15 °C. A combination of high temperature, large $CaCO_3$ concentrations and a non-sterile, biologically active soil created the least favorable conditions for *E. coli* survival.

1. Introduction

Spreading of sewage sludge on agricultural lands has been gaining attention in past decade as it improves soil nutrient status and enhances microbial activity (Johansson et al., 1999). Sewage sludge has been shown to improve soil physical properties including porosity, structure, bulk density, aggregation, and especially water holding capacity (Logan et al., 1996). On the other hand pathogens including bacteria, viruses, protozoa, annelid and nematode helminths and fungi have been found in sewage sludge (Epstein, 1998). Although different sludge stabilization processes provide reduction of pathogen population, complete elimination is rarely (if ever) achieved (Ngole et al., 2006). In many arid regions in developing countries the main source of drinking water is groundwater abstracted from dug or drilled wells (Foppen, 2002). Moreover, wastewater collection and treatment systems are uncommon in these countries and therefore there is a real risk for the abstracted water being contaminated with pathogens and pose a health risk through the food chain (Foppen, 2002).

Percolation through the vadose zone has been found to be a significant mechanism for removing microbial contaminants (Gerba and Bitton, 1984). Several experiments have shown that microorganisms in septic tank effluent may be nearly completely removed after percolation through a relatively short distance in unsaturated porous media (Bouma et al., 1972; Ziebell et al., 1974). Jiang et al. (2005, 2006) noted that bacteria retention occurred mostly in the top layer of a sand column (top 0.1 m for their case study). The main retention mechanisms are physical straining or filtration within the pores of the solid matrix (Tan et al., 1992; Wan et al., 1994; Wan and Tokunaga, 1997; Schäfer et al., 1998; Mosaddeghi et al., 2009, 2010). Depending on its physiological state a bacterial cell may attach to surfaces where it may grow or can be

* Corresponding author. Tel.: +98 811 4227013; fax: +98 811 4227012.
E-mail address: M.farhangi@basu.ac.ir (M.B. Farhangi).

0301-4797/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jenvman.2013.01.022

dislodged into the aqueous solution and transported to new regions. Soils as filtration media for percolating wastes carrying bacterial contaminants may allow their survival and growth and thus serve as a temporary sink and a source for delayed leaching of bacteria into the subsurface (Ralfs, 2007).

Several factors have been reported to affect the survival of fecal coliforms (FC) in soils. According to Gerba and Smith (2005), animal and human waste-borne pathogenic bacteria may survive in soil from 2 months to 1 year at common or optimum conditions, respectively. Safari Sinegani and Maghsoudi (2011) reported survival of *E. coli* to range from 40 days to 3 month in soils treated with manures, depending on the source of manure and soils water content. The 40 days survival interval was noted for soils treated with poultry manure or sewage sludge while in saturated soils treated with cow manure *Escherichia coli* survival extended to over 90 days. Kirby et al. (2003) also reported that soil moisture, temperature, and texture affected the survival of FC both in laboratory and in field studies.

Survival of bacteria decreases with increasing temperature (e.g. Kristiansen, 1981; Stenstrøm and Hoffner, 1982). Unc and Goss (2006) found that survival of *E. coli* in soils amended with swine or cow manure decreased as temperature increased. van Donsel et al. (1967) reported 90% reduction of FC in the soil to occur within 13.4 days in winter but within only 3.3 days in summer. Foppen and Schijven (2006) reviewed extensively studies reporting on effects of temperature on *E. coli* die-off rates. Despite the heterogeneous conditions among the various experiments reviewed, the dependency of the die-off rate coefficients on temperature was similar; the increase in the die-off rate coefficient per degree Celsius rise was apparent and comparable in most experiments. Inactivation or die-off rate quantitatively relates microbial inactivation or dying-off in terms of $\log_{10}$ decline per day (John and Rose, 2005).

Much research focused on *E. coli* O157:H7, the most dangerous serovar of *E. coli* known in cattle manure (Jones, 1999). While varying among soil types, survival of this serovar in soil might be extensive (Guan and Holley, 2003). In laboratory it survived for at least 56 days at 25 °C (Mubiru et al., 2000), and under fluctuating environmental temperatures (−6.5 to 19.6 °C), its presence was detected for up to 99 days (Bolton et al., 1999).

The extended survival of fecal coliforms in organic soils compared with that in mineral soils has been proposed as possibly due to the higher water-holding capacity of organic soils (Gerba and Bitton, 1984). Semi-arid and arid region soils have low organic matter and are rich in carbonates with calcic or petrocalcic horizons commonly occurring. More than 50% of the latter can be $CaCO_3$ (Ruellan, 2002). Cuthbert et al. (1950) showed that *E. coli* survive several weeks in limestone (pH = 5.8–7.8) while dying in a few days in a peat soil (pH = 2.9–4.5), indicating the importance of pH as well as water content.

While there is no information on the survival of fecal microorganisms in $CaCO_3$ rich soils we could find the work of Bashan et al. (1995) who studied the survival of *Azospirillum brasilense* in 23 types of plant-free sterilized soils obtained from a wide range of environments (containing 0.5–48.4% $CaCO_3$) in Israel and Mexico. Counts of *A. brasilense* declined rapidly in 35 days after inoculation in Israeli soils (arid, semi-arid, or mountain regions), but remained stable or even increased during the 45-day incubation in the arid soils from Mexico. Bashan et al. (1995) therefore reported $CaCO_3$ to be among the abiotic parameters that controlled survival of *A. brasilense* and that only at its largest concentrations $CaCO_3$ had a highly negative effect on viability of *A. brasiliense* in soil.

Although interactions of carbonates and bacteria have been studied for microbial-induced carbonate precipitation processes, in an engineering context, the role of this mineral on survival of FC has not been specifically evaluated. To the best of authors' knowledge, there is no published information on the combined effect of carbonate and temperature on survival of *E. coli*. The objective of our study was to elucidate the significance of calcium carbonate on survival of *E. coli*, a well-known indicator of FC, at different temperatures. Proper understanding the factors governing environmental survival of microbes and sludge-borne pathogens could offer an objective basis for defining appropriate management options.

2. Materials and methods

2.1. Soil collection and analysis

In October 2010 soil was collected from the surface layer (0–15 cm) of an agricultural site in Hamadan city, northwestern Iran. The collection area is in a semi-arid climate with annual precipitation of 328 mm, and annual average temperature of 13 °C (maxima of 35.5 °C recorded in August and a minima of −10.3 °C recorded in January). The collected soil was air-dried and passed through a 2-mm sieve before further analyses. Soil particle-size was analyzed by the hydrometer method (Gee and Bauder, 1986). Equivalent calcium carbonate (ECC) was measured by the back-titration procedure (Loeppert and Suarez, 1996). Soil acidity (pH) and electrical conductivity (EC) were determined in a 1:2 soil:water extract (Hesse, 1971). Organic carbon content (OC) was determined by the wet-oxidation method (Walkley and Black, 1934).

Heterotrophic bacterial and fungal populations in the soil were estimated by the plate count method. Soil samples were diluted in sterile 0.2% $Na_4P_2O_7$ solution. Nutrient agar (NA), Rose Bengal starch casein nitrate agar (RBSCNA), and modified potato dextrose agar (MPDA) were used for determination of total soil bacteria, *Actinomycetes* or fungi. Counts of microorganisms were expressed as $\log_{10}$ colony forming units (CFU) per gram dry mass of soil sample ($\log_{10}$ CFU g^{-1}). Basal respiration (BR) was measured as CO_2 rate evolved over 5 days per gram of soil dry mass (Alef and Nannipieri, 1995).

2.2. Soil–$CaCO_3$ mixtures preparation

The sampled soil was not calcareous with considerably low ECC (0.20 g 100 g^{-1}). The soil–$CaCO_3$ mixtures were prepared by mixing 5, 10, 15 or 25 g analytical-grade $CaCO_3$ (Merck, Darmstadt) into 95, 90, 85 or 75 g soil (dry mass basis) and poured into 150-mL polypropylene incubation vials. These $CaCO_3$ concentrations are not common for lime amending scenarios, where usually 1.35–9.78 Mg lime is added to a hectare (varying by soil type) for a unit change in the soil pH (CPHA, 2002), but occur in subsurface calcic or petrocalcic horizons in arid land soils. Thus our experimental setup considers such extreme conditions as a means to establish potential mechanisms/effects relevant for waste infiltration into such soils. Unamended soil samples were used as control. Half of each mixture was sterilized over 5 d through a 2 h daily autoclaving at 121 °C as per Unc and Goss (2006). Between each autoclaving, the mixtures were kept under aseptic conditions at room temperature to facilitate germination of spores. To compensate for any gravimetrically detected evaporative loss during sterilization processes sterile deionized water (less than 50 mL per 100 g mixture) was added to the mixtures. Heat sterilization allowed assessing the absolute impact of abiotic soil properties on *E. coli* NAR survival. The effectiveness of the autoclaving was evaluated by plating dilutions of autoclaved mixtures on Nutrient agar (NA). No growth was detected after 48 h for any mixture type.