

## Decontamination of Enteric Pathogens in Sewage Irrigated Soil by Solarization and Desiccation

<sup>1</sup>Nawal A. Hassanain, <sup>1</sup>Mohey A. Hassanain, <sup>2</sup>Fatma H. Abd-El-Zaher, <sup>2</sup>Azza Sh. Turkey, <sup>2</sup>Esam A. Hobballa and <sup>2</sup>Mohamed Saber M. Saber

<sup>1</sup>Department of Zoonotic Diseases, National Research Center, Giza, Egypt

<sup>2</sup>Department of Agricultural Microbiology, National Research Center, Giza, Egypt

---

**Abstract:** A surface sample representing a high contaminated loamy sand soil irrigated with sewage effluent since 30 years and was cultivated with artichoke was collected from Abu-Rawash sewage farm at Giza (Egypt). The enteric pathogens were effectively decontaminated from this sewage soil after 45 days of solarization and desiccation unless no more enteric pathogens were accessed to the soil through irrigation with sewage effluent.

**Key words:** Solarization • Pathogens • Soil • Decontaminate • Desiccation

---

### INTRODUCTION

The likely transmit of enteric pathogens from sewage soils to humans is of indisputable worry under Egyptian conditions due to the incidence of an extensive array of enteric pathogens in sewage soils and the prevalent use of manual labor in sewage farms who work in close touch with the contaminated sewage soils and somewhat low standards of hygiene. In addition to that it is worthy to mention that huge volumes of sewage effluent is disposed raw in canals and drains all over Egypt and hence reaches soils and causes severe adverse consequences contamination [1]. No doubt, sewage effluent treatment does not remove all enteric pathogens and in many cases pathogen re-growth is significant [2].

Solarization and desiccation is a widespread, non-chemical agricultural practice for disinfecting soils. Due to the prevalence of high summer temperatures all through more than 300 sunny days per year in most Egyptian regions, soil solarization might be considered as a potent tool to control soil enteric pathogens that challenge sustainable sewage farming. Our main concern in this work was reducing the levels of enteric pathogens in sewage soils to numbers that would not give rise to risk of infection.

### MATERIALS AND METHODS

**Sampling:** A surface sample representing a high contaminated loamy sand soil irrigated with sewage effluent since 30 years and was cultivated with artichoke was collected from Abu-Rawash sewage farm.

**Experimental:** In a pot experiment, the high contaminated sewage soil was exposed to natural sun solarization for 45 days under three treatments in four replicates. In the first treatment the soil was exposed to natural sun solarization and desiccation, *i.e.*, kept dry without irrigation. In the second treatment the soil was exposed to natural sun solarization and was periodically irrigated with water. In the third treatment the soil was exposed to natural solarization and was eventually irrigated with treated sewage effluent. A composite soil sample was prepared from the four replicates for each treatment initially and finally and subjected to microbiological analyses for the determination of total and faecal coliforms and the existence of certain enteric pathogenic bacteria.

**Microbiological Methods:** Total and faecal coliforms were estimated according to APHA [2]. Zoonotic pathogens including (*Salmonella*, *Shigella* and *Campylobacter*) and

*Vibrio* species were respectively detected on SS agar and charcoal cefoperazone desoxycholate agar and TCBS agar according to the scheme described by Fox [3] for *Salmonella* and *Vibrio* and by Murray *et al.* [4] for *Campylobacter*.

## RESULTS AND DISCUSSION

The existence of faecal coliforms in thirty four sewage soil samples collected from the surface and sub-surface layers from both El-Gabal El-Asfer and Abu-Rawash sewage farms in Egypt representing soils under different landscapes irrigated with sewage effluent for extended periods ranging between 0 and 82 years was previously confirmed by Saber *et al.* [5]. The found intensities of faecal coliform ranged from 158 to  $15 \times 10^2$  viable cell/gram dry soil after 25 and 82 years of sewage farming respectively and was always linked with the date of last effluent irrigate.

Santamaría and Toranzos [6] evidenced that the main enteric pathogens associated with sewage farming are *Salmonella* sp. (salmonellosis), *Vibrio cholerae* (cholera), *Shigella* sp. (dysentery), *Campylobacter jejuni* (campylobacteriosis) and *Escherichia coli*, particularly *E. coli* O157:H7 causing hemorrhagic colitis and hemolytic uremic syndrome, which proved to be able to survive in soil for several months causing persistent biological contamination. Abd-el-Naim and El-Houseini [1] found that 30% of 50 persons representing sewage workers, handlers and/or living close to Gabal-el-Asfer sewage farm suffered from typhoid and paratyphoid which were detected in 50% of their stools. The most influential factors controlling the survival of enteric pathogens in soils are colloids content, pH, cations, moisture, temperature, biological antagonism, bacteriophage, organic matter, solarization etc.

Fruits and vegetables frequently come in contact with soil post-harvest and thus might become contaminated with soil enteric pathogens present in sewage effluent. In a comprehensive survey of outbreaks with an identified food source, produce outbreaks accounted for 13% of outbreaks and 21% of associated illnesses from 1990 through 2005 [7]. In all produce outbreaks, *Salmonella* and *E. coli* were responsible for 18 and 8%, respectively. In vegetable outbreaks, *Salmonella* bacteria are the prime cause of 21% [8]. Unicomb *et al.* [9] determined the frequency of *Campylobacter* outbreaks in Australia that occurred between January 2001 and December 2006 and stated

that for 16 food borne outbreaks salad was associated with two. Shuval *et al.* [10] reported the existence of *V. cholerae* in the irrigated soils.

It is widely agreed upon that it is not realistic to verify the existence of all pathogenic organisms in sewage soils. For this reason, the indicator microorganism notion was reputable since some years ago. Regulatory agencies generally rely on tests for faecal coliform bacteria to indicate contamination. Although faecal coliforms themselves are not pathogenic, they indicate that pathogens could exist and possibly flourish. EPA recommended *E. coli* as a sensitive measure of faecal pollution. However, no single indicator microorganism could predict the presence of all enteric pathogens for all types of soils and different host-associated fecal contamination. If there are true correlations between indicator microorganisms and enteric pathogens, it is necessary to define to what extent and under which circumstances these microorganisms could be used as reliable indicators of fecal contamination [11, 12]. In general, soils that contain any coliform bacteria are reported as "total coliform positive." If the test results indicated a "total coliform positive", a faecal coliform test should be performed. Faecal coliform bacteria indicate contamination by human or animal waste. It is unacceptable for faecal coliform bacteria to be present in any concentration.

Data appeared in Table (1) and graphically illustrated in Fig. (1) show the effect of solarization and desiccation associated with ultra-violet exposure and high temperature on the survival of total and faecal coliforms in a soil sewage for 30 years at Abu-Rawash sewage farm. Multiple tube fermentation method [2] results showed that the initial total coliforms and faecal coliforms in the sewage soil were 988 and 606 CFU/gram respectively. The total coliforms counts enumerated after 45 days under the three treatments were decreased respectively to 0, 20 and 170 CFU/gram in soils exposed to solarization and desiccation, solarization and irrigation with water and solarization under irrigation with sewage effluent. The faecal coliform counts exhibited more or less the same trend as it was found to decrease to 0, 0 and 40 CFU/gram, respectively in soils exposed to solarization and desiccation, solarization and irrigation with water and solarization and irrigation with sewage effluent.

In harmony with our gained results, Stapleton and DeVay [13] found that solarization reduced the microbial population in soil by 58-87%. Hussein [14] also recorded a decrease in total microbial flora and fungi reached

Table 1: Effect of Solarization and Desiccation on the counts of total and faecal coliforms in a sewage soil (CFU/gm)

Treatments	Total coliforms		Faecal coliforms	
	Initial	Final	Initial	Final
Solarized and desiccated soil	988	0	606	0
Solarized soil irrigated with water	988	20	606	0
Solarized soil irrigated with sewage effluent	988	170	606	40

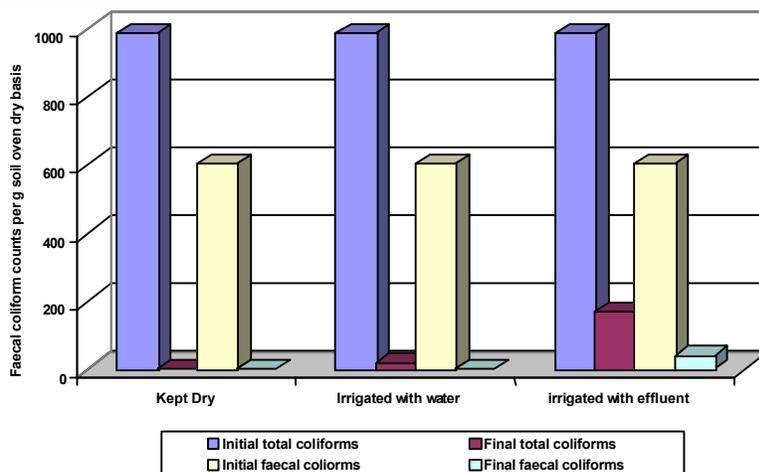


Fig. 1: Effect of solarization and desiccation on the counts of total and faecal coliforms in a sewage soil CFU/gm)

66.7 and 89.0% respectively after six weeks of solarization. He concluded that the higher the reduction in microbial population the longest the period of solarization. Needless to say solarization and desiccation affect the whole microbial population in soil both indigenous and zymogenic. Gelsomino and Cacco [15] monitored compositional shifts in the genetic structure of indigenous soil bacterial communities represented by bacteria, actinomycetes, a- and b-Proteobacteria after 72-day plot-scale soil solarization. Beneath the polyethylene film the average soil temperature at 8-cm depth reached 55-85°C compared to 35-38°C in non-solarized soil. Eubacterial DGGE profiles revealed that soil solarization was the main factor inducing strong time-dependent population shifts in the community structure. They noticed similar behavior in DGGE patterns of b-proteobacterial and actinomycete populations and to a lesser extent in DGGE profiles of a-Proteobacteria. They evidenced an increased bacterial richness by DGGE fingerprints in 16- and 36-day samplings, followed by a decrease appearing in 72-day samplings. They ascribed their findings in addition to the direct thermal effect on soil microflora to solarization-induced changes in the physico-chemical properties of soil microbial habitats and the decreased competitiveness of dominating bacterial species. Scopa and Dumo [16] studied the effects of solarization on soil microbial

biomass, soil respiration and soil enzymatic activity and found that soil solarization appeared to be an effective practice able to control pathogens, even though it might cause serious stress on the soil microbial biomass.

The frequently registered increases in soil temperature following solarization seemed to be a major factor decontaminating enteric pathogens. In laboratory studies, as the temperature increased from 15 to 40°C, the inactivation rate of enteric pathogens increased significantly [17]. Also Pokharel [18] found that mild temperature increases during soil solarization were more selective towards thermophilic and thermotolerant (above 45°C) biota, including actinomycetes. These, however, might survive and even flourish under soil solarization, but poor soil competitors, such as many pathogens, are mostly killed by soil solarization. He added that solarization initially might reduce populations of certain beneficial soil microorganisms, but it was constantly noticed that their populations quickly decolonize the solarized soil.

It is worthy to stat that, in the present work, only the solarized sewage soil sample irrigated with sewage effluent contained *Salmonella*, *Vibrio* and/or *Campylobacter* species, both initially and after 45 days of treatment. These enteric pathogens were certainly originated from irrigation with sewage effluent. On the other hand, solarized sewage soils under either

desiccation or irrigation with water were found to be free from the three tested enteric pathogens after 45 days. Ögleni and Özdemir [19] examined the responses of *Salmonella* to heat drying, solar dehydration and found that their level fell below undetectable limits after 6 weeks.

It seems reasonable to conclude that successful bioremediation of enteric pathogens by solarization and desiccation depends generally on soil moisture, temperature and exposure time, which were always inversely related. Soil moisture favors cellular activities and growth of microorganisms, thereby making them more vulnerable to the lethal effects of high soil temperatures associated with soil solarization. Noteworthy, enteric pathogens always tend to naturally disappear from sewage soil with time as far as they were not enriched with additional sewage effluent irrigation [20, 21]. Such behavior is expected, as this group of microorganisms is naturally habituating the intestinal tract where an entirely different ecosystem subsists. However, this is not enough and bioremediation tools should be eventually practiced to ensure safe farming sustainability in sewage farms.

#### ACKNOWLEDGMENT

The authors would like to express their appreciations and gratitude to the authorities of Science and Technology Development Fund (STDF) for financing the present work through the project number 1425 contracted with the National Research Center on Bioremediation of Sewaged Soils.

#### REFERENCES

1. Abd-el-Naim, M. and M. El-Houseini, 2002. Some environmental aspects of sewage sludge application in Egypt. In the Proceedings of 17<sup>th</sup> WCSS symposium, Thailand.
2. APHA (American Public Health Association), 1995. Standard Methods for the Examination of Water and Wastewater. 19<sup>th</sup> ed., APHA, Washington.
3. Fox, A., 2011. Bacteriology-Chapter 11, Enterobacteriaceae, Vibrio, Campylobacter and Helicobacter. In Microbiology and Immunology on-line. University of South Carolina School of Medicine.
4. Murray, P., E. Baron, M. Pfaller, J. Jorgensen, R. Tenover and I. Tenover, 2003. Campylobacter and Arcobacter. In: Manual of clinical microbiology. Washington, D.C: American Society for Microbiology Press, 5: 902-914.
5. Saber, M., A. Turkey, F. Abd-El-Zaher and D. Abd-El-Mowla, 2012. Biological characterization of sandy soils irrigated with sewage effluent for extended periods. International Journal of Basic and Applied Sciences, 1: 68-76.
6. Santamaria, J. and G. Toranzos, 2003. Enteric pathogens and soil: a short review. Int. Microbiol., 6: 5-9.
7. DeWaal, C. and F. Bhuiya, 2007. Outbreaks by the number: Fruits and vegetable 1990-2005. International Association for Food Protection, Orlando, FL. Poster Presentation P3-03, July, pp: 8-11.
8. U.S. Food and Drug Administration, 2005. Safe handling of raw produce and fresh-squeezed fruit and vegetable juices. <http://WWW.cfsan.fda.gov/~dms/prodsafe.html>.
9. Unicomb, L., K. Fullerton, M. Kirk and R. Stafford, 2009. Outbreaks of campylobacteriosis in Australia, 2001 to 2006. Foodborne Pathog. Dis., 6: 1241-1250.
10. Shuval, H., A. Adin, B. Fattal, E. Rawitz and P. Yekutieli, 1986. Wastewater irrigation in developing countries: health effects and technical solutions (World Bank Technical Paper) (51). In: UNDP (ed) UNDP project management report 6. (Integrated resource recovery series CLO/80/004) World Bank, Washington, D.C., pp: 27-57.
11. Bynum, V.P., 2001. Coliforms are non-pathogenic indicators of sewage faecal pollution in food, water and sludge. A short history lesson. Disease causing organisms in your food and water. Courtesy of EPA, FDA and USDA, 6/25/2011
12. Tyagi, V., A. Chopra, A. Kazmi and A. Kumar, 2006. Alternative microbial indicators of faecal pollution: current perspective. Iranian Journal of Environmental Health, Science and Engineering, 3: 205-216.
13. Stapleton, J. and J. DeVay, 1986. Soil solarization: a non-chemical approach for management of plant pathogens and pests. Crop Protection, 5: 190-198.
14. Hussein, H., 1991. Solarization, mechanical and chemical weed control in peanut (*Arachis hypogaea* L), Ph.D. Thesis, Faculty of Agriculture, Ain Shams University.
15. Gelsomino, A. and G. Cacco, 2006. Compositional shifts of bacterial groups in a solarized and amended soil as determined by denaturing gradient gel electrophoresis. Soil Biology and Biochemistry, 38: 91-102.
16. Scopa, A. and S. Dumontet, 2007. Soil solarization: Effects on soil microbiological parameters. Journal of Plant Nutrition, 30: 537-547.

17. Straub, T., I. Pepper and C. Gerba, 1992. Persistence of viruses in desert soils amended with anaerobically digested sewage sludge. *Appl. Environ. Microbiol.*, 58: 636-641.
18. Pokharel, R., 2011. Soil Solarization, an alternative to soil fumigants. Fact Sheet No. 0.505 Crop Series Soils. Colorado State University, Western Colorado Research Center.
19. Ögleni, N. and S. Özdemir, 2010. Pathogen reduction effects of solar drying and soil application in sewage sludge. *Turk. J. Agric. For.*, 34: 509-515.
20. Housing and Building National research Center, 2004. The Egyptian Manual as Guidelines for Treated Waste Water Reuse in Agriculture. Ministry of Housing and Utilities and New Communities, Cairo.
21. Saber, M., 2007. Strategic prospective for reuse of sewage effluents in agriculture. In the Proceedings of the Fourth ERD6 Conference, Faculty of Engineering, Shebin El-Kom, Center of Rural Development.