

The effect of sewage irrigation on safety and hygiene of forage crops and silage

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Abstract

The aim was to evaluate the safety of summer forage crops in Israel irrigated with secondary-treated sewage water. Nitrates levels and the incidence of *Escherichia coli* and *Salmonella* in corn and sorghum intended for silage were determined, as well as the fates of *E. coli* and added nitrates, during ensiling.

E. coli and *Salmonella* were found in 9 and 1, respectively, out of 41 samples of forage crops that had been irrigated with sewage water. *E. coli* disappeared from the silage following the decrease in pH, but was found in decaying parts of commercial silages in which the pH increased.

The concentration of nitrates in summer forage crops was usually below the critical level, which is toxic to cattle. The lower parts of the plants contained more nitrates than the other parts. The highest levels of nitrates were found in plants, which were irrigated with captured flood water.

The conclusions of this study are that nitrates, *E. coli* and *Salmonella* from forage crops irrigated with sewage water are not likely to pose a health risk to cattle if the ensiling process is adequate.

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1. Introduction

Summers in Israel are dry and forage crops (corn and sorghum) must be irrigated. These crops are preserved by ensiling, which is a preservation technology for moist whole-plant

Abbreviations: DM, dry matter; CFU, colony forming units; LP, *Lactobacillus plantarum*

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forage crops that is based on lactic acid fermentation under anaerobic conditions. Many factors may affect the safety and quality of forage crops and silages; they include uncontrolled growth of microbial spoilers or pathogens. Good management practices and Hazard Analysis and Critical Control Points (HACCP) should be applied to forage crops and feedstuffs, as they are the first step in the human food production chain (Lindgren, 1999).

Among the factors considered hazardous in forage crops and silages are nitrates and pathogenic enterobacteria such as *Salmonella* and toxin-producing *Escherichia coli* (Wilkinson, 1999). A slow decline in silage pH favors the growth of enterobacteria in silage, whereas rapid ensiling hastens their elimination (Bach et al., 2002; Heron et al., 1993). Of special concern is pathogenic *E. coli* O157:H7; if conditions in the silage are favorable for its growth it may cause intestinal disorders and mastitis in animals that consume that silage (Lindgren, 1991). *Salmonella* may be fatal to young animals (Sheinbaum and Tromp, 1982). The presence of excess nitrates in crops may evolve from manure fertilization, and nitrate toxicity may cause reproduction problems or even be fatal to cattle (Hill, 1999), and gases derived from nitrates in silages may cause respiratory disorders in silage workers (“silo-fillers disease”). Spoelstra (1985 and 1987) showed that various strains of enterobacteria and lactobacilli can reduce nitrates, especially when the ensiling fermentation is slow.

Because of the acute water shortage in Israel, the use of recycled semi-purified (secondary treatment) sewage water in forage irrigation has increased dramatically: in fact, most of summer forage crops are irrigated with this water. Such water may contain factors, which are hazardous to both animal and humans. Sewage water might contain high levels of nitrates, which may be absorbed by plants and be toxic to animal and humans (e.g. Mondal et al., 1999; Wilson et al., 1999). Microbial populations in sewage water may vary, but the coliform level in treated sewage water can reach up to 10^5 CFU per 100 ml (Icekson-Tal et al., 2002; Israeli Ministry of Health, personal communication).

The purpose of the present study was to evaluate the effect of sewage irrigation on nitrates levels and on the incidence of *E. coli* and *Salmonella* in corn and sorghum that are intended for use as silage in Israel, and to follow their potential changes during ensiling.

2. Materials and methods

2.1. Sampling of summer forage crops

Forage corn and sorghum were sampled from 41 fields across Israel in summer 2003 (June 22 to September 14). The sampled fields were irrigated with secondary-treated sewage water, some of them with captured flood water; the plants received either drip or sprinkle irrigation. The plants were brought to the laboratory and the cobs and heads were removed.

For microbiological evaluation, analysis was applied to whole plants or to the lower third, according to whether they had received sprinkle or drip irrigation, respectively. The various plant parts were manually cut into 2–3-cm pieces with ethanol-sterilized clippers. Samples from two plants from each field were taken aseptically for *E. coli* and *Salmonella* determination. *Salmonella* was determined also in a few soil samples taken near the irrigation lines.

For nitrate determination two other plants from each field were taken. The plants were divided into four parts: lower, middle, upper and leaves, but very young short plants were analyzed whole, or divided into three parts: lower, upper and leaves. The various plant parts were chopped by hand and dried at 105 °C for 48 h. The dried material was ground through 1 mm sieve for nitrate determination.

2.2. Sampling from feeding centers

Silages, total mixed rations and broiler litter used for cattle feeding were sampled from nine farms in central Israel. Silage samples were taken from the top layer and from the “silage shoulders” (the contact zone between the top of the silage and the walls of a bunker silo), which are the parts most susceptible to air penetration and spoilage. Three samples were taken from each silage. Measurements included determination of DM, pH, and *E. coli* populations.

2.3. Ensiling experiments with nitrates

Two ensiling experiments were performed in order to monitor nitrate decomposition during ensiling. The corn was taken from fields irrigated with secondary-treated sewage water. The first experiment used young moist corn at 195 g/kg DM. The second experiment used corn at the one-third milk line stage (when two-third of the kernels are still watery) at 280 g/kg DM. The whole plants were chopped to 2–3 cm using a Wintersteiger® chopper (Austria). The chopped crop was ensiled in 1.5 l glass jars (Weck®, Germany) equipped with a lid that allows gas to escape but not to enter. The jars were stored at ambient temperature (25–28 °C). Three jars from each treatment were sampled three times during the first week of the experiment and the remaining three jars were sampled after about one month. The silages were analyzed for pH, DM and nitrate level, and the final silages were also analyzed for lactic acid and volatile fatty acids. Treatments included the following:

1. Control (no additives).
2. Addition of 27 g of KNO₃ (Carlo Erba, Italy) to 10 kg of freshly chopped corn, to obtain an estimated nitrates content of 5 g/kg in DM. The chemical powdered KNO₃ was added to the chopped crop, which was spread over a 1 m × 2 m area, and mixed thoroughly.
3. Addition of 54 g of KNO₃ to 10 kg of corn, as above to obtain a nitrates concentration of 10 g/kg nitrates in DM.
4. As for treatment 3, plus the addition of silage inoculant *Lactobacillus plantarum* MTD1 (Ecosyl, UK) added at 10⁶ CFU/g. The inoculant was suspended in 25 ml of distilled water and sprayed over the corn and mixed thoroughly.

2.4. The fate of *E. coli* during ensiling

Two ensiling experiments were performed to study the effect of ensiling on the elimination of *E. coli*. The first experiment used corn at the one-third milk line stage at 280 g/kg DM, and the second corn at three-quarter milk line stage (when most of the kernel contains solid starch) at 370 g/kg DM. In both experiments the corn was taken from fields irrigated with sewage water. The ensiling protocol was similar to that described above for the nitrates.

3. Analytical procedures

3.1. Chemical analyses

Dry matter (DM) was determined by oven drying. Fresh crops were dried at 105 °C and silages at 60 °C for 48 h. Lactic acid was determined spectrophotometrically, according to Barker and Summerson (1941). Volatile fermentation end-products were determined in aqueous extracts with a gas chromatograph using a semi-capillary FFAP column (Hewlett Packard, Germany) over a temperature range of 40–230 °C. Nitrates were determined by extracting 250 mg of dried ground material in 250 ml of distilled water for 1 h. The suspension was filtered through Whatman number 1 paper and the nitrates were determined with nitrate-test analytical strips (Reflectoquant[®] 1.16971, Merck) in a special reflectometer (Merck).

3.2. Microbiological analyses

Lactobacilli were determined in pour plate Rogosa agar (Oxoid) incubated for three days at 30 °C.

E. coli was determined in Chromocult TBX[®] agar (Merck, Germany) using the double layer technique. The plates were incubated for 24 h at 42 °C. Green colonies were counted as *E. coli*.

The presence of *Salmonella* was determined according to Israeli Official Standard Method No. 885. The determination involved enrichment steps in peptone water, Rappaport and tetra-cyanide broths, plating in brilliant-green agar from which pink colonies were selected and inoculated in triple sugar iron (TSI) and lysine-iron (LI), (Difco, USA) agar slants. The occurrence of typical changes in colors and the formation of a black precipitate were taken to confirm the presence of *Salmonella*.

3.3. Statistical analysis

Statistical analysis of the nitrate results in forage samples included a two-way analysis of variance. One source of variation was plant part and the second was the irrigation method. The analysis was performed with GLM procedure of statistical analysis system (SAS, 1982).

4. Results

4.1. The incidence of *E. coli* and *Salmonella* in forage crops and in silage and feedstuffs

E. coli was found in 9 out of the 41 sampled fields, but their populations in the crop were small: 15–4000 CFU/g crop. Both samples from each field did not always agree. *Salmonella* was detected in only one sample, which was the one that contained the largest *E. coli* population. *Salmonella* was found in a few soil samples that were taken near the irrigation lines.

In silage, *Salmonella* was found in only one out of five samples tested. This sample was from the same farm in which *Salmonella* was found in fresh corn plants; also *E. coli*

Table 1
The effect of irrigation method on nitrate levels in corn and sorghum

Crop	Irrigation method	Number of samples	Mean (g/kg DM)
Corn	Flood water	7	25
	Sprinkle	25	11
	Drip	55	7
Sorghum	Flood water	6	16
	Sprinkle	26	6
	Drip	49	3

was found in 10 out of 24 silage samples; the counts varied from 10 to 1.7×10^7 CFU/g. The pH of the samples with high *E. coli* counts was above 5.0, and these samples were considered spoiled. *E. coli* was found in both total mixed rations that were sampled (10^2 and 10^4 CFU/g), but not in either of the two samples of treated broiler litter.

4.2. Nitrate levels in forage crops

Nitrate levels in the forage crop plants were usually not higher than 20 g/kg DM (Table 1). The irrigation method and the plant part had significant effects on nitrate levels (for both $Pr < 0.0001$) and, in fact the highest levels were found in plants, which were irrigated with captured flood water. The lower parts of the plants contained higher nitrate concentrations than the middle, upper parts or leaves (Table 2). This result was expected for drip irrigation, since the lower parts of the plants are directly exposed to the water source. However, statistical analysis revealed that for corn the interaction between irrigation method and plant part was significant ($Pr < 0.01$): under sprinkle irrigation the higher parts of the plants also contained high levels of nitrates. The low parts of young plants tended to contain higher levels of nitrates (up to 50 g/kg DM), and in general, corn contained more nitrates than sorghum.

4.3. The effect of ensiling on nitrates levels

The pH of the corn silages decreased quickly and was below 4.0 within a few days of ensiling. The nitrate additions to the corn were intended to provide nitrate concentrations in

Table 2
Nitrate levels in different plant parts of corn and sorghum

Crop	Plant part	Number of samples	Mean (g/kg DM)
Corn	Lower	25	19.5
	Middle	15	8.8
	Upper	22	6.2
	Leaves	25	2.4
Sorghum	Lower	31	9.2
	Middle	10	4.0
	Upper	25	2.0
	Leaves	15	1.7

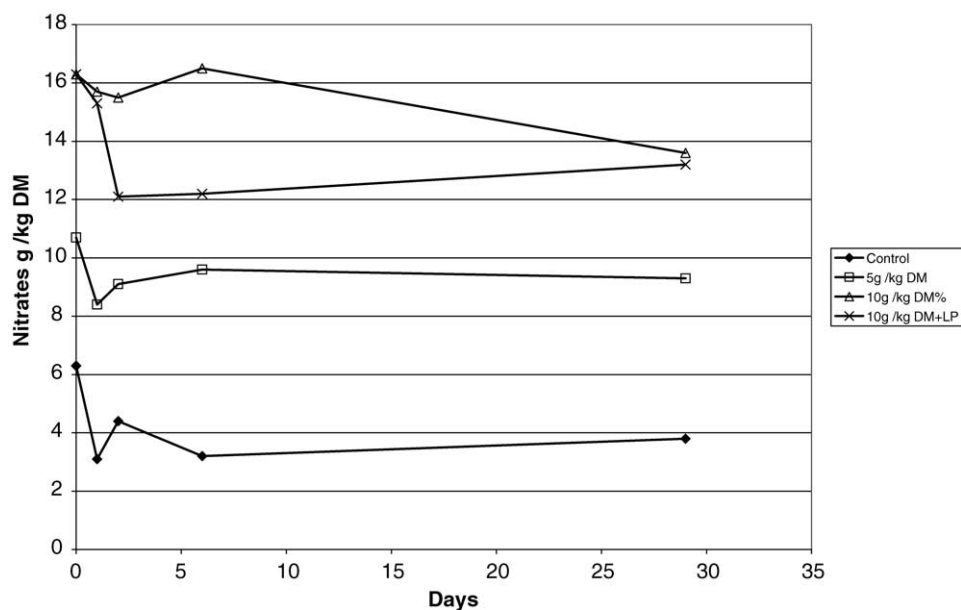


Fig. 1. Nitrates levels in corn silages at dry matter content of 195 g/kg. DM, dry matter; LP, *Lactobacillus plantarum*.

DM of 5 and 10 g/kg, on the assumption that the corn initially contained 330 g/kg in DM. However, the corn used in these experiments was much more moist (DM concentrations of 195 and 280 g/kg), and therefore the initial levels of nitrates were higher than anticipated, more so in the moister crop. The endogenous nitrate content of the plants (3–6 g/kg DM) also spiked the values somewhat. The sum of the added nitrate levels, calculated according to the real DM contents of the plants, plus the natural nitrate contents, were close to the analytical values, which were obtained.

Figs. 1 and 2 show the changes in nitrate content during the ensiling process. The results reveal that in the corn silages, ensiling did not reduce the nitrate levels and that the addition of *L. plantarum* did not enhance nitrate reduction. This result is in contrast to previous findings with moist grass silages, in which such cultures reduced the nitrate content (Spoelstra, 1985; Weissbach et al., 1993). Table 3 gives the fermentation products in the corn silages. The nitrate-treated silages contained less lactic acid and more ethanol than the others, as reported in the literature (Weissbach et al., 1993), but not more acetic acid.

Table 3

Fermentation products of corn silages with or without 10 g/kg nitrates in DM (g/kg DM \pm S.D.)

Dry matter (g/kg)	Treatment	Lactic acid	Acetic acid	Ethanol
195 \pm 4	Control	94 \pm 9	23 \pm 3	Not found
	Nitrates	77 \pm 7	23 \pm 6	3 \pm 1
280 \pm 4	Control	74 \pm 8	12 \pm 2	Not found
	Nitrates	66 \pm 3	13 \pm 4	2 \pm 0

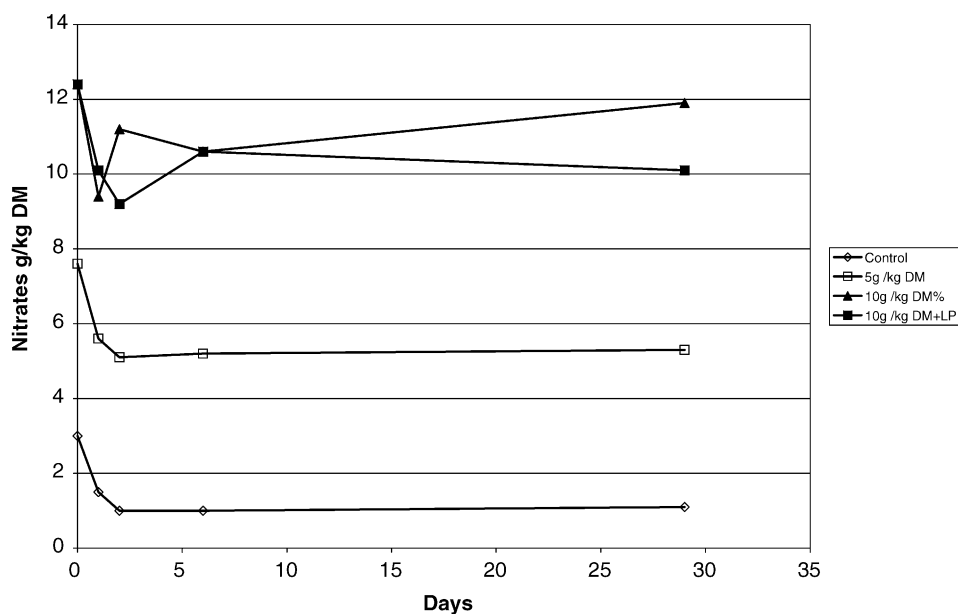


Fig. 2. Nitrates levels in corn silages at dry matter content of 280 g/kg. DM, dry matter; LP, *Lactobacillus plantarum*.

4.4. The effect of ensiling on elimination of *E. coli*

The pH of the corn silages decreased quickly and was below 4.0 as soon as 2–5 days after ensiling. In the first ensiling experiment the initial population of *E. coli* on the fresh corn (pH 5.8) was 9×10^3 CFU/g. By one and six days after ensiling, the pH decreased to 4.2 and 3.8, respectively. On day 6 and one month after ensiling, the *E. coli* populations were 8×10^3 CFU/g and below the detectable level of 10 CFU/g. In the second experiment the initial of *E. coli* population was 1.1×10^4 CFU/g. Five days after ensiling the pH decreased from 5.8 to 3.8, and the number of *E. coli* had fallen below the detectable level. One month later no *E. coli* were found.

5. Discussion

This study was initiated in order to assess the safety of summer forage crops in Israel where they are irrigated with secondary-treated sewage water. Raw sewage water, as well as secondary-treated effluent may be a source of various pathogenic microorganisms and toxic chemicals. The cows in Israel are high-yielding dairy cows, which are intensively managed, and, therefore, are sensitive to any change in feed quality or hygiene. In order to evaluate the hygienic and safety status of forage crops, which are irrigated with secondary-treated sewage water, the populations of two microorganisms (*E. coli* and

Salmonella), and nitrates levels were chosen for examination. *E. coli* was chosen as a hygiene indicator for fecal contamination, and *Salmonella* and nitrates were chosen because of the potential health hazard posed to humans and cattle by this microorganism and chemical.

The results reveal that the sewage-irrigated forage crops in Israel contain *E. coli* and *Salmonella*, usually in small numbers. Furthermore, such microorganisms usually do not survive at a pH lower than 4.1 (Frazier and Westhoff, 1978), such as characterizes well-prepared and managed corn and sorghum silages. However, it was reported that *E. coli* O157:H7 might develop acid resistance following induction of an acid-tolerance response and, consequently, would be able to survive at a pH as low as 3.4 (Brudzinski and Harrison, 1998). In our experiments, *E. coli* disappeared during ensiling following the decrease in pH. However, we have found *E. coli* in samples taken from commercial silages in which the pH was between 6 and 7, and these silages were considered spoiled. Such samples were taken from the top layer or from the shoulders of the face; these are the parts of silages that are most susceptible parts to air penetration, which is followed by an increase in pH, and spoilage. Pathogenic enterobacteria in silage and in various feedstuffs may originate in the excreta from pigeons and other birds that live around dairy farms in Israel, or they may develop from endogenous populations, following an increase in pH. The effect of these microbial populations on animal health is not known, but such microorganisms can cause illness in cattle (Sheinbaum and Tromp, 1982) or even be transmitted to man (Russell et al., 2000).

Different plants can accumulate different levels of nitrates. Nitrate content in grass increases following N fertilization. The nitrate concentrations in grass and corn are normally 1–8 and 1–4 g/kg DM, respectively, but concentrations of 30 and 10 g/kg, respectively, have also been detected (Spoelstra, 1985). In temperate, rainy climates, where forage crops are very moist (DM below 200 g/kg) there may be an undesirable clostridial fermentation, which low nitrate levels (up to 3 g/kg in DM) help to prevent (Kaiser et al., 2002). However, in the subtropical Israeli climate, forage crops for silage are usually much drier and clostridia need cause no concern. High levels of nitrates may be toxic to cattle and their gaseous degradation products may be hazardous to silage workers; the critical nitrate concentration in the rations that is toxic to cattle is around 5 g/kg in DM (Page et al., 1990). Our present results showed that sewage irrigation does not necessarily enhance the nitrate levels in plants; the lower parts of the plants accumulated the highest nitrate concentrations; younger plants tended to exhibit higher nitrate concentrations than older ones; and corn contained more nitrates than sorghum. However, silage is usually made from mature plants harvested at the milk or dough maturation stage; therefore, even if their lower parts contain high levels of nitrates, the nitrates are diluted when the whole plants are chopped and ensiled. If animals graze on the stubble of such crops, the nitrates may present a problem and our results showed that if very high concentrations of nitrates accumulate in the plants the ensiling process is not likely to reduce them.

Some strains of *Enterobacteria* and *Lactobacilli* are able to reduce nitrates during ensiling (McDonald et al., 1991, Spoelstra, 1987). We have tried one strain of *L. plantarum* in the corn silages, but it did not result in any decrease in nitrate concentration in the corn silages; therefore, more strains should be tested for their capability to metabolize nitrates in silages.

6. Conclusions

The incidence of *E. coli* in forage crops irrigated with partially treated sewage water is around 25%. *E. coli* in silage disappears following the decrease in pH, but pathogens may be found in decaying parts of improperly fermented or stored silages into which air penetrated and caused the pH to increase, or in feedstuffs with pH values close to neutral.

The concentration of nitrates in summer forage crops in Israel is usually below the critical level that is toxic to cattle. We found that the lower parts of the plants contained more nitrates than the other parts, but their source was not necessarily the sewage water.

Overall, *E. coli*, *Salmonella* and nitrates from sewage irrigated forage crops do not seem to pose a health risk to cattle, but adequate management is required in order to ensure that silages are free from pathogenic enterobacteria.

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