# INTESTINAL ENTEROCOCCI AS INDICATORS OF HUMAN INFLUENCE ON THE SOIL

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## Introduction

A group of intestinal enterococci (Ie) includes the Gram-positive facultatively anaerobic bacteria that belong to four species: *Enterococcus faecalis, E. faecium, E. durans* and *E. hirae*. These species are members of the normal intestinal microflora and are consequently present in the faeces of human and warm-blooded animals. Therefore, Ie are routinely used as bacterial indicators of faecal contamination in food, drinking and recreational waters [1]. There are few advantages to use Ie as indicators of faecal pollution as compared to well known intestinal bacterium *Escherichia coli*: Ie do not multiply in environments; Ie survive longer in environments; Ie are more resistant to adverse environmental factors (drying, chlorination, sodium chloride, alkaline pH). The Ie are generally not virulent. Nevertheless, multidrug-resistant *E. faecalis* and *E. faecium* have emerged in 21st century as leading causes of hospital-acquired infections [2].

The **aim** of this work was to examine the Ie as indicators of the influence of human solid waste on subjacent soil.

## Materials and Methods

In total 19 soils under the anthropogenic influence in Croatia were analysed: 15 influenced by illegal dumpsites of human solid waste and 4 agricultural soils fertilized with animal manure. Three samples were control soils without the visible anthropogenic influence (Fig. 1, Table 1).

Soil samples were aseptically taken in sterile plastic bottles and processed in the laboratory within 5 h after collection. The pH value was measured in soil suspension (1:2.5) in distilled water.

The fresh wet soils were analysed in technical triplicate after its suspension and dilution in sterile peptone water, following the filtration trough the 0.45µm sterile membrane filters. Membrane filters were incubated on Slanetz Bartley agar (Biolife) at 37°C/72 h and Ie were confirmed as brown colonies (Fig. 2) on bile esculin azide agar (Sigma-Aldrich) after incubation at 44°C/4 h [3].

## Results and Discussion

The pH values of soils varied from 4.9 to 9.7 (Table 1). The Ie were not detected (<1 CFU/g) in control soils saved from visible human activities.

The Ie also were not detected in agricultural soils fertilized with swine or poultry manure. The Ie are regularly present in animal manure [4]. However, the use of animal manure as a soil fertilizer in prescribed quantity has no appreciable influence on the occurrence of Ie in agricultural soil.

All soils influenced by illegal dumpsites contained the Ie at concentration from 1.3 to 6.2 log CFU per 1 g of wet soil (Table 1). The presence of Ie in soils influenced by illegal dumpsites is explained by the leaching of Ie from the human solid waste by storm waters and its consequent infiltration into subjacent soil.

Since Ie could survive in environment for prolonged periods [1,4], Ie in soils could be used as indicators of illegal dumps of human solid waste. The human solid waste resulted in the presence of Ie as bacterial indicators of faecal contamination, which represent the negative anthropogenic influence on the subjacent soil.

More stringent monitoring and prevention of illegal dumps is needed to avoid the negative human influence on the soil.

**Table 1.** Description of collected soil samples and corresponding numbers of intestinal enterococci (Ie).
0 - below detection limit (< 1 CFU/g); mean values of Ie are presented while standard deviations for all samples were in the range ± 0.1 to 0.3 log CFU/g.

| Description | Sample name | Depth (cm) | pH | Ie (log CFU/g) |
|---|---|---|---|---|
| Controls | Mljet-pasture | 0-20 | 7.3 | 0 |
| | Istra-organic vineyard | 0-20 | 5.4 | 0 |
| | Savudrija 1-pasture | 0-20 | 8.4 | 0 |
| Illegal dump sites | Susak 1 | 0-20 | 8.3 | 1.9 |
| | Susak 2 | 20-40 | 8.3 | 1.4 |
| | Samobor | 0-20 | 7.3 | 6.2 |
| | Bakar 1 | 0-10 | 8.5 | 2.8 |
| | Bakar 2 | 0-10 | 7.9 | 1.7 |
| | Sovjak 1 | 0-20 | 9.7 | 3.4 |
| | Sovjak 2 | 0-20 | 6.8 | 1.6 |
| | Plomin | 0-20 | 7.9 | 1.5 |
| | Sv. Lovrec 2 | 0-20 | 7.8 | 2.0 |
| | Sv. Lovrec 4 | 0-10 | 7.7 | 1.3 |
| | Loborika 1 | 0-10 | 7.2 | 1.8 |
| | Loborika 2 | 0-10 | 6.8 | 2.5 |
| | Morinje 1 | 0-10 | 7.2 | 2.4 |
| | Morinje 2 | 0-10 | 8.2 | 2.4 |
| | Zaton | 0-20 | 7.9 | 1.9 |
| Agricultural soil fertilized with animal manure | Medjimurje 1-swine manure | 0-30 | 6.1 | 0 |
| | Medjimurje 2-swine manure | 30-60 | 5.9 | 0 |
| | Medjimurje 3-poultry manure | 0-30 | 4.9 | 0 |
| | Medjimurje 4-poultry manure | 30-60 | 4.9 | 0 |

**Fig. 1.** Example of control soil without the visible anthropogenic influence (sample Mljet, left) and soil influenced by illegal dumpsite of human solid waste (sample Susak1, right).

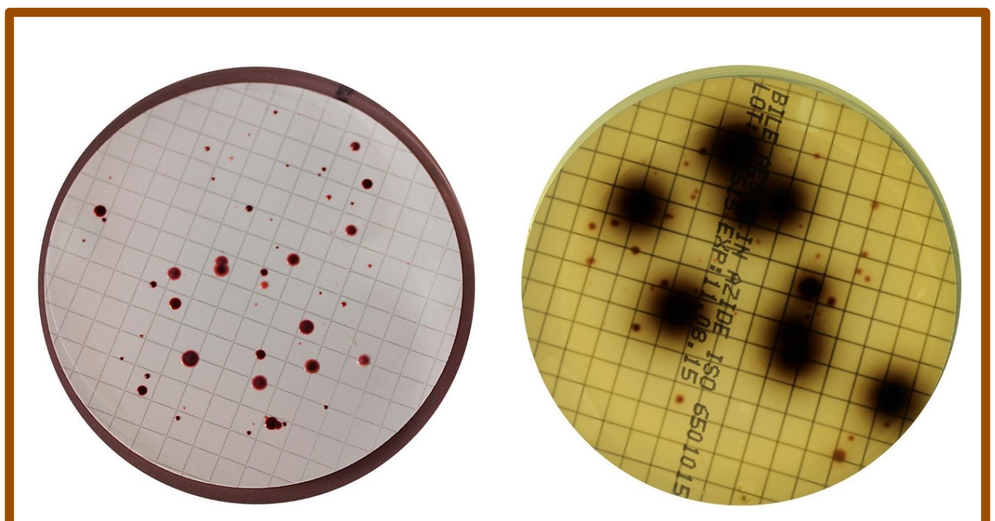

**Fig. 2.** Colonies of presumptive intestinal enterococci on Slanetz Bartley agar (left) and confirmed colonies of intestinal enterococci on bile esculin azide agar (right).

## Conclusions

- Ie were not detected (<1 CFU/g) in soils saved from visible human activities.
- Use of animal manure as a soil fertilizer had no appreciable influence on the occurrence of Ie in soils.
- Ie were present (> 1 CFU/g) in soils influenced by illegal dumpsites of human solid waste.
- Concentration of Ie above 1 CFU per 1 g of wet soil could be used as indicator of illegal dumps.
- More stringent monitoring and prevention of illegal dumps is needed to avoid the negative human influence on the soil.

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## References

1. Gilmore MS, Clewell DB, Ike Y, Shankar N. 2014. Enterococci: from commensals to leading causes of drug resistant infection. Massachusetts Eye and Ear Infirmary, Boston.
2. Raza T, Ullah SR, Mehmood K, Andleeb S. 2018. Vancomycin resistant Enterococci: A brief review. J Pak Med Assoc. 68:768-772.
3. HRN ISO 7899-2. 2000. Water quality - Detection and enumeration of intestinal enterococci, Part 2: membrane filtration method. Geneva: Organization for Standardization.
4. Solecki O, Jeanneau L, Jarde E, Gourmelon M, Marin C, Pourcher AM. 2011. Persistence of microbial and chemical pig manure markers as compared to faecal indicator bacteria survival in freshwater and seawater microcosms. Water Res. 45:4623-4633.