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Conference Abstract

Validation of bacterial markers to discriminate against the source of nitrate contamination: a promising application within the EU Nitrates Directive

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Abstract

The European Community (EC) Nitrates Directive (ND) (Council Directive 1991/676/EEC) aims to avoid water pollution by nitrates from poor farming practices. Under the ND, Nitrate Vulnerable Zones (NVZs) have been defined as areas where the concentration of agricultural nitrates in surface or groundwater exceeds 50 mg/L. Groundwater is one of the main sources of drinking water in Europe, so ensuring its quality is of significant importance. Monitoring of water parameters, the identification of pollution, the development of good agricultural practice codes is included in ND as measures and action programs. ND provides for the attribution of areas affected by nitrate pollution to the NVZ in which farmers must comply with measures necessary to reduce nitrogen in excess and protect the quality of environmental matrices.

However, in some cases the quality of water bodies remains poor, suggesting that some action measures are ineffective or need improvements. Nevertheless, nitrates in excess in

groundwater might be related to industrial inputs, untreated waste discharge, and sewage spilling. Due to this, identifying non-agricultural contributions to nitrate groundwater pollution can be of great interest. With this aim, the employment of biomarkers such as microbes associated with the intestinal tract of a specific host is under study for identifying fecal pollution.

This strategy is known, in scientific literature, as Microbial Source Tracking (MST)(Furtula et al. 2011)The amplification of 16S rRNA genes of host-specific Bacteroidales allows discriminating against human and livestock fecal sources in samples from nitrate polluted environment.

The aim of this work is to evaluate the sensitivity and specificity of these intestinal biomarkers in groundwater samples collected along with the Apulian Region (Southern Italy) in order to assess the applicability of the MST in the employ of ND. A preliminary study was carried out to validate the performance of seven markers for MST using two different approaches: Polimerase Chain Reaction (PCR) and quantitative Real-time PCR (qPCR) assays. For both PCR and qPCR experiments selected primer sets were checked using fecal samples of known origin as positive controls. PCR assay was used for human (HF74 and *Enterococcus faecalis*) (Bernhard and Field 2000, Jackson et al. 2004), cattle (CF123) (Bernhard and Field 2000), equine (HoF597) (Dick et al. 2005), and pig (Pig163) (Dick et al. 2005) markers screening. Two additional markers were tested through Real-time PCR: human (Human- Bacteroides) (Seurinck et al. 2005) and zootechnical (BacPre I) (Kobayashi et al. 2012).

The results indicate that biomarkers can be considered reliable in distinguishing human from animal pollution. Nevertheless, our studies show that the tests conducted with the human *Enterococcus faecalis* biomarker do not discriminate the zootechnical source from the human one. In some cases, PCR-tested biomarkers cannot determine the source of contamination in environmental matrices due to the detection limit. Animal and human fecal markers were widely detected on eleven groundwater samples through Real Time PCR, highlighting the prevalent source of contamination in the environmental matrix.

This research provides evidence that MST technology is a valid tool for local authorities to identify the source of nitrate contamination and review uncertainties during the NVZs definition and the action program development required by the European Nitrate Directive.

Keywords

Microbial Source Tracking, water quality, biomarker, groundwater, Nitrates Directive, Real-time PCR, Bacteroidales, fecal pollution.

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