Microbial and chemical monitoring integrated approach applied to a potential landfill leachate contamination of groundwater

F. Piergiacomo[1], P.P. Limoni[2], L.E. Zuffianò[2], M.Polemio[2] L. Beneduce[1*]
[1]- Department of Agricultural, Food and Environmental sciences, University of Foggia, Foggia, Apulia, 71122, Italy
2- Research Institute for Hydrogeological Protection- IRPI-CNR, Bari, Apulia, 70126, Italy
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Presenting author email: luciano.beneduce@unifg.it

Municipalities landfill represents a viable and still the most commonly used method for solid waste disposal all over the world. The compaction of the waste layers and the biodegradation of organic matter in the management of solid wastes make the landfill a typical reducing-anaerobic environment (Kjeldsen *et al* 2002). The complex series of physical, chemical, and microbial transformations occurring in the decomposition of wastes, may also contribute to the transfer of pollutants to the percolating water (e.g., Christensen and Kjeldsen 1989).

In this context, severe impacts may arise from gas and leachate formation as groundwater pollution, air pollution and global warming emissions, along with unpleasant odors. The lack or insufficient leachate collection systems and engineered liners, made the risk of groundwater pollution as one the most severe environmental impact from landfills. Indeed, if not properly managed and collected as the more recent landfill directives provide for, landfill leachate could constitute one of the main ground and surface water pollutants, percolating through soil to water aquifers (El-Salam *et al* 2015).

Therefore, a comprehensive evaluation of the leachate impact on groundwater biological and chemical compositions, is fundamental to make accurate predictions on the impacts of landfills and on the perturbation sources of a polluted area (Kjeldsen *et al* (2002).

Molecular biology techniques are fast and reliable methods to assess microbial communities perturbation and presence of microbial indicator groups. Indeed, characterization of the patterns of species diversity is very important for understanding the underlying evolutionary and ecological processes shaping biodiversity across spatial and temporal scales in the environment (Levin, 1992). Leachate microbial communities are mostly represented by those of the anaerobic acid phase involved in the decomposition of cellulose and hemicellulose to methane and carbon dioxide in landfills under anaerobic conditions. Moreover, an excess in nitrogen compounds might bring to an unbalanced development of nitrogen cycling bacterial communities which metabolize them. Microbial groups related to faecal pollution of human or animal origin and other pathogens linked to civil and urban wastes could be also present.

The integration of biological indicators with the analytical determinations of chemicals is essential to support pollution sources hypothesis. Basing analyses on the most common type of landfill and wastes, which in general consists in a mixture of commercial, municipal, and mixed industrial waste, landfill leachate could be defined as a water-based solution involving Dissolved organic matter (DOM), volatile fatty acids, inorganic macro-components, heavy metals and xenobiotic organic compounds, with a variety of aromatic hydrocarbons, phenols, chlorinated aliphatic, pesticides, and plasticizers (Christensen *et al* 1994). Also, the concentration of Nitrogen (mostly in ammonia form) seems to constitute the major long-term pollutant in leachate (Kjeldsen *et al* 2002) and Ammonia-nitrogen and the alkalinity seem to be the primary cause of acute toxicity of municipal landfill leachate (Ernst *et al* 1994).

In this work, a potential infiltration of landfill leachate, deriving from a landfill site located in the province of Bari (southern Italy) , into groundwater was assessed through integrated use of biological and chemical methods. In particular, nitrogen and faecal microbial indicator were used as a proxy of potential pollution by leachate and biological determinants of aquifer contamination.

Materials and Methods

Landfill leachate was sampled directly from the collecting tank of the site, while groundwater samples were taken from 9 wells surrounding the landfill (5 in a radius of less than 50-500 m from the landfill and 4 in a wider range). Microbial count (total mesophilic at 30 °C and *E.coli*) were performed by MF method, while PCR was used to detect faecal indicators (*Bacteriodes* spp., *Bifidobacterium* spp., *Enterococcus* spp.) and N-cycle microbial groups (*Nitrobacter* spp., *Nitrospira* spp., AOB, AOA, denitrifiers, the latter by *NosZ* and *NirK* gene as targets). PCR-ARISA was performed to evaluate bacterial communities differences between groundwater samples and the leachate. The main hydro-chemical parameters (pH, EC, T, DO, Ca^{2+}, Mg^{2+} , Na^{+} , K^{+} , Cl^{-} , Fe^{+2}, Mn^{+2} SO_4^{2-} , NO_3^{-}, NO_2- and NH_4^{+}) were investigated too.

Results and discussion

In general, the microbial quality of the inspected wells was found to be high but differed in terms of total bacterial counts and faecal indicator levels (Tab.1).

Nitrogen cycle microbial group were found both in the leachate and in some of the inspected groundwater wells but in different taxa (AOB were present in the leachate while AOA were found in groundwater samples). ARISA analyses, stated that a significant difference of the bacterial communities between leachate and groundwater. Chemical parameters confirmed the absence of impacting contamination of the groundwater by landfill leachate. Also, ammonia and nitrate level of the sampled groundwater were found generally low and often below the limit of detection. The monitoring clearly pointed out that groundwater microbial and chemical quality was not influenced by landfill, nor potential human or animal faecal contamination, while soil nitrifying population support the hypothesis of impact of the intensive agricultural use of soil around the landfill area, contributing to groundwater increase of some parameters.

Conclusions

The development of effective management practices to preserve groundwater quality and evanctual remediation plans of polluted areas requires the identification of the sources and the understanding of the processes that influence pollution impact. Integrating chemical analyses with monitoring of microbial indicators by fast and sensitive molecular tools can contribute to an efficient environmental monitoring strategy and bring to a more secure management of landfill plants and related wastes.

Table 1. Summary of the analytical results obtained: ND = not determined; NI = not isolated in 100 mL;

Microbial Target	**Groundwater sample**									**Landfill leachate**
	Close (50-500 m radius)					Far (> 500 m radius)				
	11	12	13	14	15	2	5	3	8	
Microbial indicators of groundwater quality and potential fecal contamination:										
Mesophilic count (30° - UFC/100 mL)	300	100	3	500	3200	70	50	160	230	ND
E.coli (37°C–UFC/100mL)	ND	ND	ND	3	ND	ND	ND	ND	ND	ND
Indicators of potential fecal contamination (PCR):										
Bacteroides /Prevotella	-	+	-	+	+	-	-	+	-	-
Bifidobacterium spp.	-	-	-	-	-	-	-	-	-	-
Enterococcus spp.	-	-	-	-	-	-	-	-	-	-
Nitrogen cycle indicators associated with agricultural use of nitrogen fertilizers:										
Nitrobacter spp.	-	+	-	+	-/+	-	-	+	-	+
Nitrospira spp.	-	+	-	+	-	-	-	-	-	-
AOB	-	-	-	-	-	-	-	-	-	+
AOA	-	+	+	+	+	-	-	-	-	-
NosZ Denitro	-	+	-	+	-	-	-	-	-	+
NirK Denitro	-	+	-	-	-	-	-	-	-	+
More relevant physical and chemical main features of the groundwater										
EC (mS/cm)	873	953	1382	2813	1000	1195	845	766	762	12347
pH	7.5	7.3	7.5	7.5	7.3	7.8	7.7	7.5	7.4	8.2
NH4-N (mg/L)	<0.1	<0.1	<0.1	<0.1	0.3	<0.1	<0.1	<0.1	<0.1	101.9
NO3-N(mg/L)	40.9	33.7	32.9	17.2	38.8	36.8	56.0	33.7	20.9	27.5
Fe	<0.01	0.02	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.06	0.78

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