Landfill leachate composition and toxic potency in semi-arid areas: an integrated approach with the use of physicochemical and toxicological data.

V. Tsarpali¹ and S. Dailianis¹*
¹Section of Animal Biology, Department of Biology, School of Natural Sciences, University of Patras, 26500, Patra, Greece

*Corresponding author: E-mail: sdailianis@upatras.gr, Tel: +32610-969213, Fax: +32610-969213

Abstract
The investigation of landfill leachate composition, as well as its toxic potency throughout a whole year period, showed seasonal alterations of its physicochemical parameters and its toxic effects. In specific, seasonal alterations of leachate composition and toxic potency seemed to be related with the amount of rainfall. Certain rainfall-related leachate parameters, i.e. NO₃⁻, TN, NH₄-N, TDS and the BOD₅/NH₄-N ratio, could merely reflect the leachate strength and toxicity, as verified by the significant correlations occurred among each of them with the toxic values observed in all species tested. This fact indicates that the above parameters could be used independently, or in combination for investigating and predicting seasonal leachate toxic potential, at least in the case of semi-arid areas, such as the most of the Mediterranean countries.

Keywords: BOD₅/NH₄-N ratio, biotests, leachate, rainfall, toxicity.

1. INTRODUCTION

Landfills are among the most widely practiced methods for the disposal of municipal solid residues [1]. During this process, a complex mixture of liquid effluent, commonly called leachate, is generated by the precipitation and penetration of water into the mass of residues undergoing biodegradation [2]. Leachate is characterized by high concentrations of organic and inorganic compounds [3]. The contact of such a complex mixture with the surrounding water bodies, could lead to environmental alterations [4,5], thus it is considered as an environmental matter of concern.

Different biotests have shown significant toxic effects of leachate in various organisms [2,4-5]. One of the known parameters to affect leachate strength and toxic potency is the local rainfall regime, but little it is known about the way that happens, especially in semi-arid climatic conditions, such as the most of the Mediterranean countries [6]. Since the knowledge of leachate composition is necessary in order to manage the long-term impacts of a landfill [1], the aim of the present study was to monitor leachate composition and toxic potency alterations with time and to what extent the local rainfall regime could mediate leachate strength and toxicity.

2. MATERIALS AND METHODS

2.1 Landfill area characteristics and climatic data

The municipal landfill site of Aigeira (Peloponissos, Greece) has operated since 2006 and covers a total surface area of 48 ha. It is an active landfill, receiving urban wastes from three towns (total population ranged from 12003 to 55990 inhabitants in winter and summer months respectively). During the year 2011, the amount of waste disposed into the landfill ranged from 343 to a maximum of 1127 tonnes/month. Climatic data, in terms of rainfall and temperature, for the current
area were kindly provided by the Hellenic National Meteorological Service (meteorological station in Velo, Korinthia, Greece). The mean temperature was ranged from 10°C (on January) to 29°C (on July and August), while there was a great fluctuation of rainfall, with maximum values in February (135.5 mm/month) and minimum values (0 mm/month) in June, August, November and December. During this period, there was a gradient decrease of leachate flowrate, with minimum values in August, October and December.

2.2 Leachate collection and handling

Leachate samples were regularly collected every 2 months (6 sampling dates/periods, from February till December), from a single released sampling point of the landfill during the first five days of each month. Leachate’s flowrate was daily measured, before the onset and after the end of the sampling period (0 and 5 days respectively, which means 6 measurements in all cases). Samples (at least 10 L) were collected in polyethylene containers and/or glass bottles sterilized by autoclaving (121°C, 20 min) and maintained under conditions with minimized exposure to oxygen. After transportation to the laboratory, all leachates were divided into 6 parts, stored at 4°C in the dark and each part was prepared for further analysis within a period of 2 days [7]. Regarding toxicity tests, samples were filtered through sterilized filter membranes (diameter 0.2 μm) and stored at 4°C in the dark, no longer than 2 days, before been used.

2.3 Chemical analysis of leachate samples

Physicochemical parameters were systematically monitored according to Standard Methods for the Examination of Waters and Wastewaters [7]. In the case of the BOD5/COD and BOD5/ NH4-N ratio units, each unit was calculated from the respective value of each parameter observed from each different measurement (N=6 in each case). Concentrations of metals were determined with the use of a Perkin Elmer AAAnalyst 300 Atomic Absorption spectrometer (AAS), after digestion of the samples with HNO3. The metal analysis method was verified with known concentrations of each metal tested (Pure Atomic Spectroscopy standards, purchased by Perkin Elmer Life and Analytical Sciences, USA). Values of each parameter tested are mean ± SD from 6 different measurements in each case.

2.4 Toxicity tests with the use of microbiotests and the microalgal Dunaliella tertiolecta

Leachate toxicity test were performed with the use of the crustaceans Artemia franciscana and Thamnocephalus platyurus, as well as of the estuarine rotifer Brachionus plicatilis. All organisms were hatched from cysts derived from Screening Toxicity test supplied by MicroBio Tests Inc. (Artoxkit M™, Thamnotoxkit F™ and Rotoxkit M™ respectively). Toxicity tests were performed according to the Standard Operational Procedures, in terms constant levels of pH, temp, sal and DO and culturing media previously described. In order to verify the experimental procedures, reference tests were primarily performed with the toxicant K2Cr2O7 (stock solution of 100 mg/L diluted in distilled water). The results, expressed as 24h LC50 endpoints (the percentage of the leachate concentration that causes 50% of mortality in each species tested within a period time of 24h), are the mean ± SD from 6 replicates in each case. Moreover, the observed endpoints were transformed into toxic units (TU = 1/E(L)C50 x 100) and ranked into one of five classes according to Sprague and Ramsay [8].

Leachate inhibitory effects on the microalgal flagellate D. tertiolecta (strain CCAP19/6B) was investigated according to well-known protocols and guidelines [9]. In brief, stock cultures of algae (1x10⁴ cells/ml) were exposed to different concentrations of leachate (0, 6.25, 12.5, 25, 50, 70 and 100% v/v) for a period of 72 h, under constant conditions. pH values were maintained constant (pH
8.3± 0.3) throughout the experimental period, while salinity was maintained at 35‰ with the addition of proper amount of NaCl in any case. The experimental cultures were checked for cell number, growth rate (μ) and inhibition rate (% I) every 24 h. Each experimental procedure was carried out in duplicate and the results expressed as IC₅₀ (the percentage of the leachate concentration, % v/v, that causes 50% of growth inhibition per day [24, 48 and 72h IC₅₀]) are the mean ± SD from 6 replicates.

2.5 Statistical analysis

Data sets were checked for homogeneity of variance (Levene's test of equality of error variances) and the significant differences between parameters tested were assessed by ANOVA (Bonferonni test, p<0.05, IBM SPSS 19 Inc. software package). For the estimation of both LC₅₀ and IC₅₀ endpoints, log-transformed values were analyzed by Probit analysis (p<0.05). The sensitivity of species to leachate toxic potency was investigated non-parametrically with the use of Mann-Whitney u-test (p<0.05). Pearson rank correlation analysis (N=36, p<0.05) was performed, in order to investigate significant relationships among rainfall data with all the leachate physicochemical parameters tested. The obtained rainfall-related parameters were used in Principal Component Analysis (PCA), in order to evaluate potential differences among them. The Factor Scores of the first two components extracted were used for investigating possible seasonal differences among sampling dates/periods. Since PCA allows the reduction of the number of the parameters used for further correlations, the parameters with the larger weights (in terms of absolute values) for the PC1 were further used for investigating the relationships with the toxicity values obtained in all species tested (Pearson rank correlation analysis, p<0.05), (for further details see Pablos et al. [10]).

3. RESULTS AND DISCUSSION

3.1 Physicochemical parameters and metal content in leachate samples

pH and DO values measured in leachate samples showed slight variations throughout the year (p<0.05) with values ranged from 8.11 to 8.74 and 0.06-0.48 mg/L respectively. On the other hand, Sal, Cond, TDS, COD, NH₄-N, Cu, Cd, Cr, Pb and As showed seasonal alterations throughout the year, with highest values in samples from October and December (Table 1A,B). In addition, CT, NO₃-, TN, NH₄-N, PO₄⁻³-P, P₂O₅ and T-Ph levels measured in leachate samples showed a gradient increase throughout the year, with highest values in samples from October and December (Table 1A). On the other hand, SO₄²⁻ as well as TSS and VSS measured in samples from October and/or December showed low values, compared to the previous months/periods. Similarly, both BOD₅/ NH₄-N and BOD₅/COD ratios showed a gradient decrease throughout the year, with lowest values in samples from October and December (Table 1A). The levels of both Hg and Mn showed seasonal fluctuations, with low Hg levels in samples from February and high values in samples from December and August, while low levels of Mn were measured in leachate from February and April, compared with those occurred in samples from August and October (Table 1B).

3.2 Toxicity of leachate samples collected throughout the year

In all cases, leachates appeared to have toxic effects on all species tested, showing type- and time-dependent alterations. Leachate samples from October and December showed the highest toxicity in all species tested (Table 2). Sensitivity of all species to leachate toxic effects, as obtained by the average LC₅₀ or IC₅₀ mean values from each species tested, was ranked as Thamnocephalus platyurus > Dunaliella tertiolecta > Brachionus plicatilis ≥ Artemia franciscana.
| Table 1. Physicochemical parameters (A) and metal concentrations (B) in leachate samples collected during sampling periods. |
|---|---|---|---|---|---|
| **A** | Feb | Apr | Jun | Aug | Oct |
| pH | 8.25±0.05 | 8.38±0.02 | 8.74±0.04 | 8.67±0.06 | 8.19±0.05 | 8.11±0.09 |
| Sal (‰) | 3.11±0.09 | 2.36±0.04 | 2.41±0.09 | 3.10±0.09 | 7.15±0.11 | 7.05±0.05 |
| Cond (μS/cm) | 3.75±0.05 | 3.95±0.04 | 4.55±0.01 | 5.91±0.07 | 12.49±0.03 | 8.65±0.04 |
| DO (ppm) | 0.23±0.02 | 0.18±0.02 | 0.28±0.02 | 0.18±0.02 | 0.06±0.04 | 0.48±0.13 |
| TDS (g/L) | 3.02±0.07 | 2.29±0.01 | 2.41±0.02 | 3.09±0.01 | 6.92±0.02 | 6.98±0.02 |
| TSS | 170±15.49 | 160±12.65 | 352.56±9.7 | 493.37±5.7 | 191.7±3.4 | 125.0±19.49 |
| VSS | 130±28.43 | 154.3±19.44 | 321±93.98 | 484±47.02 | 182.7±35.73 | 118.3±28.34 |
| COD (g/L) | 2.24±0.10 | 2.59±0.36 | 2.110±0.08 | 2.69±0.24 | 3.81±0.28 | 6.04±0.39 |
| BOD₅ (g/L) | 0.69±0.06 | 1.925±0.7 | 1.388±0.33 | 0.525±0.02 | 1.170±0.12 | 0.984±0.41 |
| Cl (g/L) | 0.708±0.07 | 1.378±0.49 | 2.026±0.10 | 2.020±0.22 | 2.868±0.31 | 3.495±0.32 |
| NO₃ | 10.7±0.2 | 7.1±0.1 | 9.6±0.4 | 11±0.2 | 25.8±1.3 | 23.7±6.9 |
| TN (g/L) | 0.337±0.02 | 0.328±0.04 | 0.322±0.05 | 0.296±0.04 | 1.207±0.07 | 1.107±0.02 |
| NH₄-N (g/L) | 0.171±0.03 | 0.163±0.06 | 0.144±0.03 | 0.082±0.08 | 1.308±0.01 | 1.744±0.14 |
| SO₄²⁻ | 232±85.30 | 231±77.90 | 308±18.0 | 330±1.30 | 195±4.00 | 293±3.10 |
| PO₄³⁻ | 2.9±0.3 | 9±1.6 | 7.7±2.0 | 8.5±0.4 | 15.6±0.6 | 12.6±0.3 |
| P₂O₅ | 6.2±0.0 | 20.7±3.7 | 12.2±3.9 | 19.6±0.9 | 35.7±1.4 | 28.7±0.8 |
| T-Ph | 7.1±1.2 | 7.0±0.2 | 15.1±0.5 | 15.7±1.4 | 21±0.8 | 21±1.4 |
| BOD₅/COD | 0.31±0.03 | 0.72±0.20 | 0.66±0.15 | 0.20±0.03 | 0.31±0.05 | 0.16±0.06 |
| BOD₅/NH₄-N | 4.05±0.36 | 11.93±5.22 | 9.59±2.23 | 6.46±0.77 | 0.89±0.08 | 0.58±0.29 |
| **B** | Cu | 0.68±0.14 | 0.13±0.02 | 0.15±0.03 | 0.36±0.05 | 0.41±0.11 | 0.39±0.07 |
| Cr | 0.91±0.28 | 0.48±0.04 | 1.58±0.10 | 2.28±0.67 | 2.87±0.66 | 2.32±0.69 |
| Cd | 0.03±0.01 | 0.04±0.01 | 0.14±0.04 | 0.13±0.04 | 0.17±0.06 | 0.10±0.08 |
| Hg | 0.001±0.00 | 0.003±0.00 | 0.006±0.00 | 0.009±0.00 | 0.008±0.00 | 0.007±0.00 |
| Zn | 0.27±0.10 | 0.82±0.20 | 1.39±0.37 | 0.81±0.36 | 0.16±0.03 | 0.64±0.11 |
| Pb | 0.88±0.50 | 0.75±0.22 | 2.04±0.31 | 1.88±0.62 | 2.91±0.27 | 1.52±0.23 |
| Mn | 1.56±0.1 | 1.02±0.14 | 0.81±0.05 | 0.41±0.03 | 0.46±0.09 | 0.78±0.06 |
| As | 0.002±0.00 | 0.02±0.01 | 0.01±0.03 | 0.04±0.02 | 0.05±0.03 | 0.11±0.05 |

All concentrations are given in mg/L. Values are mean ± SD from 6 independent measurements in each case. Values with the same letter indicate statistically significant difference from each other (Bonferroni test, p<0.05) in any case.

| Table 2. Leachate toxicity on *T. platyurus*, *A. franciscana*, *B. plicatilis* and *D. tertiolecta*. |
|---|---|---|---|---|
| **T. platyurus** | **A. franciscana** | **B. plicatilis** | **D. tertiolecta** |
| 24h LC₅₀ | 72h LC₅₀ | 24h LC₅₀ | 72h LC₅₀ | 24h LC₅₀ | 72h LC₅₀ | 24h LC₅₀ | 72h LC₅₀ |
| Feb | 8.67 | 62.13 | 75.11 | 70.98-79.20 | 1.3 | 32.09 | 21.9-54.2 |
| (6.61-12.03) | (39.61-113.74) | (41.89-192.75) | (83.31-90.43) | (86.85-105.82) | | (7.2-11.6) |
| Apr | 9.06 | 72.31 | 72.09 | 65.86-77.92 | 1.4 | 42.5 | 33.3-56.7 |
| (7.49-11.23) | (41.89-192.75) | (83.31-90.43) | (86.85-105.82) | (86.85-105.82) | | (27.3-65.5) |
| Jun | 4.63 | 86.79 | 92.20 | 87.53-101.08 | 1.0 | 40.2 | 13.1-83.0 |
| (2.71-5.63) | (83.31-90.43) | (86.85-105.82) | (86.85-105.82) | (86.85-105.82) | | (9.3) |
| Aug | 8.36 | 95.24 | 92.95 | 89.15-100.54 | 1.0 | 43.3 | (27.4-33.67) |
| (7.74-8.97) | (86.85-105.82) | (86.85-105.82) | (86.85-105.82) | (86.85-105.82) | | (7.2-11.6) |
| Oct | 2.56 | 54.64 | 30.98 | 27.42-33.67 | 1.8 | 9.3 | | |
| (1.99-2.87) | (50.52-59.25) | (50.52-59.25) | (50.52-59.25) | (50.52-59.25) | | (50.52-59.25) |
3.3 Significant relationship among leachate physicochemical and ecotoxicological parameters

Pearson rank correlation analysis (N=36, p<0.05) showed that there was a strong negative correlation among the amount of rainfall obtained throughout the year with the majority of leachate parameters measured in samples from each sampling date/period. Moreover, rainfall seemed to be positively correlated with leachate flowrate, BOD$_5$, Mn as well as with the BOD$_5$/COD and the BOD$_5$/NH$_4$-N ratios in each case (Table 3). PCA analysis resulted in a partial grouping of leachate parameters tested. Regarding seasonal differences among sampling dates/periods, the scatter plot graph conducted with the Factor Scores of the first two components extracted, showed a clear grouping among sampling dates/periods (Fig. 1). Parameters with the larger PC1 weights were Sal, Cond, TDS, COD, BOD$_5$/NH$_4$-N ratio, phenols, chlorides, N-derived parameters, in terms of NO$_3$-, TN and NH$_4$-N, PO$_4^{3-}$, P$_2$O$_5$ and As. Pearson rank correlation analysis, performed with those parameters, showed significant relationships among each of them with the toxicity values obtained in all species tested. Leachate salinity was routinely normalized before executing toxicity tests and excluded for the aforementioned analysis. In specific, Cond, TDS, NO$_3$-, TN and NH$_4$-N values showed a significant negative correlation with 24h LC$_{50}$ and/or 72h IC$_{50}$ values observed in all species tested. Similarly, the BOD$_5$/NH$_4$-N ratio showed a significant positive correlation with 24h LC$_{50}$ and/or 72h IC$_{50}$ values observed in all species tested. COD, T-Ph, Cl, PO$_4^{3-}$-P and P$_2$O$_5$ showed a significantly negative correlation with LC$_{50}$ values obtained in *T. platyurus* and *B. plicatilis*, as well as with 24h IC$_{50}$ values obtained in *D. tertiolecta*, while As showed a negative correlation with *B. plicatilis* and *D. tertiolecta* (Table 4).

![Figure 1](image-url)  
**Figure 1.** Scatter plot of the first two PC extracted factor scores for the rainfall-related leachate physicochemical parameters. Symbols with different colors indicate grouped-variables for each sampling date/period.

**Table 3.** Correlation coefficient (Pearson t-test, p<0.05) among rainfall (RF) data with each of the leachate parameters obtained during sampling dates/periods (N=36).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>RF</th>
<th>Sal</th>
<th>Cond</th>
<th>TDS</th>
<th>TSS</th>
<th>VSS</th>
<th>COD</th>
<th>BOD$_5$</th>
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<tbody>
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<td>FR</td>
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<td>-0.625**</td>
<td>-0.633**</td>
<td>-0.638**</td>
<td>-0.348*</td>
<td>-0.367*</td>
<td>-0.549**</td>
<td>0.533**</td>
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<tr>
<td>T-Ph</td>
<td>-0.822**</td>
<td>-0.749**</td>
<td>-0.615**</td>
<td>-0.533**</td>
<td>-0.544**</td>
<td>-0.325*</td>
<td>-0.474**</td>
<td>-0.451**</td>
</tr>
<tr>
<td>RF</td>
<td>Cu</td>
<td>Cr</td>
<td>Cd</td>
<td>Hg</td>
<td>Zn</td>
<td>Pb</td>
<td>Mn</td>
<td>As</td>
</tr>
<tr>
<td></td>
<td>-0.810**</td>
<td>-0.529**</td>
<td>-0.601**</td>
<td>-0.603**</td>
<td>0.640**</td>
<td>-0.528**</td>
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<tr>
<td>RF</td>
<td>BOD$_5$/COD</td>
<td>BOD$_5$/NH$_4$-N</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>0.717**</td>
<td>0.624**</td>
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</table>
Table 4. Correlation coefficients (Pearson t-test, p<0.05) among toxic values with each leachate parameters tested (A) and with BOD₃/NH₄-N ratios (B) obtained in leachates collected during sampling dates/periods (N=36). (*Significant at the 0.05 level. **Significant at the 0.01 level.)

<table>
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<tr>
<th></th>
<th>Cond</th>
<th>TDS</th>
<th>NO₃⁻</th>
<th>TN</th>
<th>NH₄-N</th>
<th>BOD₃/NH₄-N</th>
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<td>24h LC₅₀ Th</td>
<td>-0.461**</td>
<td>-0.522**</td>
<td>-0.513**</td>
<td>-0.615**</td>
<td>-0.592**</td>
<td>0.320*</td>
</tr>
<tr>
<td>24h LC₅₀ Art</td>
<td>-0.525**</td>
<td>-0.544**</td>
<td>-0.533**</td>
<td>-0.579**</td>
<td>-0.458**</td>
<td>0.528**</td>
</tr>
<tr>
<td>24h LC₅₀ Br</td>
<td>-0.814**</td>
<td>-0.886**</td>
<td>-0.824**</td>
<td>-0.920**</td>
<td>-0.868**</td>
<td>0.659**</td>
</tr>
<tr>
<td>72h IC₅₀ Dun</td>
<td>-0.902**</td>
<td>-0.982**</td>
<td>-0.925**</td>
<td>-0.988**</td>
<td>-0.958**</td>
<td>0.755**</td>
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</tbody>
</table>

5. Conclusion

The current study revealed that leachate strength and toxic potency could be merely affected by seasonal alterations occurred in leachate parameters. In specific, the amount of rainfall could regulate leachate strength and toxic potency, a fact that is commonly related with the age of the landfill and leachate, its stabilization and biodegradability as well. According to the latter, the estimation of the BOD₃/D/NH₄-N ratio in leachate samples (except of the investigation of leachate parameters, that could be related with the enhancement of leachate toxicity) could be used as a low-cost effective tool in order to predict leachate strength and toxicity, at least in the case of semi-arid areas, such as the most of the Mediterranean countries.

References