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

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## 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. EXPERIMENTAL PROCEDURE

### *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 (Fig.1). 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 10ºC (on January) to 29ºC (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 (Fig. 2A,B).

### *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 (121ºC, 20 min) and maintained under conditions with minimized exposure to oxygen.

### *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 $BOD_5$/COD and $BOD_5$/ $NH_4$-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 AAnalyst 300 Atomic Absorption spectrometer (AAS), after digestion of the samples with $HNO_3$. 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 of constant levels of pH, temp, sal and DO. The results, expressed as 24h $LC_{50}$ 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 ($TU= 1/E(L)C_{50} \times 100$) 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) were investigated according to well-known protocols and guidelines [9]. Each experimental procedure was carried out in duplicate and the results expressed as $IC_{50}$ (the percentage of the leachate concentration, % v/v, that causes 50% of growth inhibition per day (24, 48 and 72h $IC_{50}$)] are the mean ± SD from 6 replicates.

**Figure 1.** Municipal landfill site of Aigeira (Peloponissos, Greece). Leachate samples.

### *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 $LC_{50}$ and $IC_{50}$ 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]).

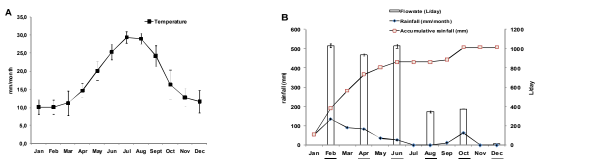


**Figure 2.** Temperature data (A), as well as rainfall data and leachate flowrate (B) during the year 2011, including the sampling dates/periods (underlined). Values are the amount of rainfall data for each month, the accumulative amount of rainfall with time, the mean ± SD temperature per month, as well as the mean ± SD value of leachate flowrate estimated during sampling dates/periods (before and within the first 5 days of each sampling date/period).

## 3. RESULTS

### 3.1 Physicochemical parameters and metal content in leachate samples

pH and DO values measured in leachate samples showed slight variations throughout the year, while Sal, Cond, TDS, COD, $NH_4$-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, $Cl^-$, $NO_3^-$, TN, $NH_4$-N, $PO_4^{+3}$-P, $P_2O_5$ 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, $SO_4^{-2}$ 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 $BOD_5$/ $NH_4$-N and $BOD_5$/COD ratios showed a gradient decrease throughout the year, with lowest values in samples from October and December (Fig. 3A,B).

**Table 1.** Physicochemical parameters (A) and metal concentrations (B) in leachate samples collected during sampling periods.

| | | Feb | Apr | Jun | Aug | Oct | Dec |
|---|---|---|---|---|---|---|---|
| A | pH | 8.25±0.05 abcd | 8.38±0.02 aefgh | 8.74±0.04 beijk | 8.67±0.06 cfilm | 8.19±0.005 gjln | 8.11±0.009 dhkmn |
| | Sal (‰) | 3.11±0.09 abcd | 2.36±0.04 aefg | 2.41±0.09 bhij | 3.10±0.09 ehkl | 7.15±0.112 cfik | 7.05±0.05 dgjl |
| | Cond (mS/cm) | 3.75±0.05 abcde | 3.95±0.04 afghi | 4.55±0.01 bfjkl | 5.91±0.07 cgjmn | 12.49±0.03 dhkmo | 8.65±0.04 eilno |
| | DO (ppm) | 0.23±0.02 ab | 0.18±0.02 cd | 0.28±0.02 ef | 0.18±0.02 gh | 0.06±0.04 aceg | 0.48±0.13 bdfh |
| | TDS (g/L) | 3.02±0.07 abcde | 2.29±0.01 afghi | 2.41±0.03 bfjkl | 3.09±0.01 cgjmn | 6.92±0.02 dhkm | 6.98±0.02 eiln |
| | TSS | 170±15.49 ab | 160±12.65 cd | 352.5±69.7 acefg | 493.3±57.5 bdehi | 191.7±34.3 fh | 125.0±19.49 gi |
| | VSS | 130±28.43 ab | 154.3±19.44 cd | 321±93.98 acefg | 484±47.02 bdehi | 182.7±35.13 fh | 118.3±28.34 gi |
| | COD(g/L) | 2.245±0.10 ab | 2.592±0.36 cd | 2.110±0.008 efg | 2.692±0.24 ehi | 3.810±0.28 acfhj | 6.040±0.39 bdgij |
| | $BOD_5$ (g/L) | 0.69±0.06 a | 1.925±0.78 abcd | 1.388±0.33 e | 0.525±0.02 be | 1.170±0.12 c | 0.984±0.41 d |
| | Cl (g/L) | 0.708±0.07 abcde | 1.378±0.41 afghi | 2.026±0.19 bfjk | 2.200±0.22 cglm | 2.868±0.31 dhjln | 3.495±0.32 eikmn |
| | $NO_3$ | 10.7±0.2 ab | 7.4±0.1 cd | 9.6±0.4 ef | 11±0.2 gh | 25.8±1.3 aceg | 23.7±6.9 bdfh |
| | TN (g/L) | 0.337±0.02 ab | 0.328±0.04 cd | 0.322±0.05 ef | 0.296±0.04 gh | 1.207±0.07 acegi | 1.107±0.002 bdfhi |
| | $NH_4$-N (g/L) | 0.171±0.003 ab | 0.163±0.006 cd | 0.144±0.003 ef | 0.082±0.008 gh | 1.308±0.01 acegi | 1.744±0.14 bdfhi |
| | $SO_4^{-2}$ | 232±85.30 a | 231±77.90 b | 308±1.80 c | 330±1.30 abd | 195±4.00 cde | 293±3.10 e |
| | $PO_4^{+3}$-P | 2.9±0.3 abcde | 9±1.6 afg | 7.7±0.2 bhi | 8.5±0.4 cjk | 15.6±0.6 dfhjl | 12.6±0.3 egikl |
| | $P_2O_5$ | 6.2±0.0 abcde | 20.7±3.7 afgh | 12.2±3.9 bfijk | 19.6±0.9 cilmn | 35.7±1.4 dgjl | 28.7±0.8 ehkmn |
| | T-Ph | 7.1±1.2 abcd | 7.0±0.2 efgh | 15.1±0.5 aeij | 15.7±1.4 bfkl | 21±0.8 cgik | 21.1±4.9 dhjl |
| | $BOD_5$/COD | 0,31±0,03 abcd | 0,72±0,20 aefg | 0,66±0,15 bhij | 0,20±0,03 ceh | 0,31±0,05 fik | 0,16±0,06 dgjk |
| | $BOD_5$/ $NH_4$-N | 4,05±0,36 abcd | 11,93±5,22 aefg | 9,59±2,23 bhij | 6,46±0,77 ehkl | 0,89±0,08 cfik | 0,58±0,29 dgjl |
| B | Cu | 0.68± 0.14 abcde | 0.13± 0.021 afgh | 0.15± 0.03 bijk | 0.36± 0.05 cfi | 0.41± 0.11 dgj | 0.39± 0.07 ehk |
| | Cr | 0.91± 0.28 abc | 0.48± 0.04 defg | 1.58± 0.19 dh | 2.28± 0.67 ae | 2.87± 0.66 bfh | 2.32± 0.69 cg |
| | Cd | 0.03± 0.01 abc | 0.04± 0.01 de | 0.14± 0.04 ad | 0.13± 0.04 b | 0.17± 0.06 ce | 0.10± 0.08 |
| | Hg | 0.001± 0.00 abcd | 0.003± 0.002 e | 0.006± 0.00 a | 0.009± 0.003 be | 0.008± 0.004 c | 0.007± 0.005 d |
| | Zn | 0.27± 0.10 abc | 0.82± 0.20 ade | 1.39± 0.37 bdfgh | 0.81± 0.36 cfi | 0.16± 0.03 egij | 0.64± 0.11 hj |
| | Pb | 0.88± 0.50 abc | 0.75± 0.22 defg | 2.04± 0.31 adhi | 1.88± 0.62 behj | 2.91± 0.27 cfjk | 1.52± 0.23 gik |
| | Mn | 1.56± 0.19 abcde | 1.02± 0.14 afgh | 0.81± 0.05 bij | 0.41± 0.03 cfik | 0.46± 0.09 dgjl | 0.78± 0.06 ehkl |
| | As | 0.002± 0.00 ab | 0.02± 0.01 c | 0.01± 0.003 d | 0.04± 0.02 e | 0.05± 0.03 af | 0.11± 0.05 bcdef |

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.

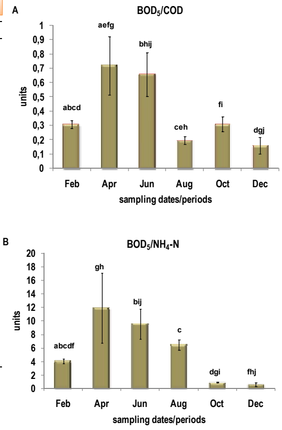


**Figure 3.** Estimation of $BOD_5$/COD (A) and $BOD_5$/$NH_4$–N ratio (B) in leachate samples collected during sampling dates/periods. Values (expressed as units) are the average ratio estimated from each parameter tested ($BOD_5$, COD and $NH_4$–N) in each case. Values that share the same letter indicate significant difference from each other (Bonferroni test, p < 0.05).

### 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 (Fig. 4A-D). Sensitivity of all species to leachate toxic effects, as obtained by the average $LC_{50}$ or $IC_{50}$ mean values from each species tested, was ranked as *Thamnocephalus platyurus*> *Dunaliella tertiolecta*> *Brachionus plicatilis*≥ *Artemia franciscana* (Fig. 5).

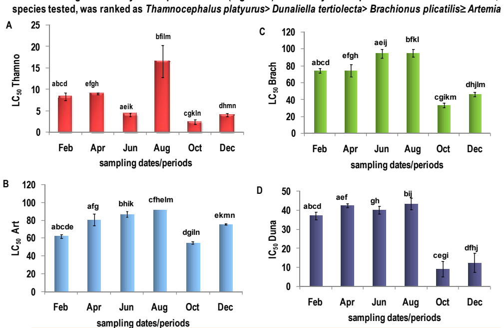


**Figure 4.** Determination of 24 h LC50 in (A) *Thamnocephalus platyurus*, (B) *Artemia franciscana*, (C) *Brachionus plicatilis* and/or 72 h IC50 values in (D) *Dunaliella tertiolecta* after exposure to leachate collected during sampling dates/periods. 24 h LC50 values (expressed as %, v/v) are mean ± SD from 6 independent measurements, while 72 h IC50 values are mean ± SD from 3 independent measurements (each measurement was performed in duplicate). Values that share the same letter indicate significant difference from each other (Bonferroni test, p < 0.05)..

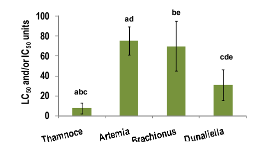


**Figure 5.** Sensitivity of species tested. Values are the average of LC50 or IC50 mean values obtained by each toxicity test throughout the year (N = 6, in terms of sampling dates/periods). Values that share the same letter indicate significant difference from each other (Mann–Whitney u-test, p < 0.05).

### 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 2). 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. 6). 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, $PO_4^{+3}$, $P_2O_5$ 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 $P_2O_5$ 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 3).

**Table 2.** 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).. *. Significant at the 0.05 level. **. Significant at the 0.01 level.

| | FR | Sal | Cond | TDS | TSS | VSS | COD | $BOD_5$ |
|---|---|---|---|---|---|---|---|---|
| RF | 0.807** | -0.625** | -0.633** | -0.638** | -0.348* | -0.367* | -0.549** | 0.533** |

| | T-Ph | $Cl^-$ | $NO_3^-$ | TN | $NH_4$-N | $SO_4^{-2}$ | $PO_4^{+3}$-P | $P_2O_5$ |
|---|---|---|---|---|---|---|---|---|
| RF | -0.822** | -0.749** | -0.615** | -0.533** | -0.544** | -0.325* | -0.474** | -0.451** |

| | Cu | Cr | Cd | Hg | Zn | Pb | Mn | As |
|---|---|---|---|---|---|---|---|---|
| RF | - | -0.810** | -0.529** | -0.601** | - | -0.603** | 0.640** | -0.528** |

| | $BOD_5$/COD | $BOD_5$/$NH_4$-N |
|---|---|---|
| RF | 0.717** | 0.624** |

**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).. *. Significant at the 0.05 level. **. Significant at the 0.01 level.

| A | Cond | TDS | COD | T-Ph | $Cl^-$ | $NO_3^-$ | TN | $NH_4$-N | $PO_4^{+3}$-P | $P_2O_5$ | As |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 24h $LC_{50}$ Th | *-0.461*** | *-0.522*** | -0.376* | -0.368* | -0.363* | *-0.513*** | *-0.615*** | *-0.592*** | -0.422* | -0.329* | - |
| 24h $LC_{50}$ Art | *-0.525*** | *-0.544*** | - | - | - | *-0.533*** | *-0.579*** | *-0.458*** | - | - | - |
| 24h $LC_{50}$ Br | *-0.814*** | *-0.886*** | -0.698** | -0.496** | -0.534** | *-0.824*** | *-0.920*** | *-0.868*** | -0.700** | -0.751** | -0.525** |
| 72h $IC_{50}$ Dun | *-0.902*** | *-0.982*** | -0.800** | -0.721** | -0.734** | *-0.925*** | *-0.988*** | *-0.958*** | -0.765** | -0.767** | -0.615** |

| B | $BOD_5$/$NH_4$-N |
|---|---|
| 24h $LC_{50}$ Th | *0.320** |
| 24h $LC_{50}$ Art | *0.528*** |
| 24h $LC_{50}$ Br | *0.659*** |
| 72h $IC_{50}$ Dun | *0.755*** |

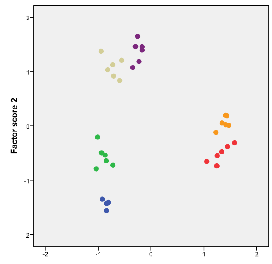


**Figure 6.** Scatter plot of the first two PC extracted factor scores for the rainfall related leachate physicochemical parameters. Symbols with different colors indicate grouped-variables for each sampling date/period.

## 4. Discussion

According to the results of the present study, leachate toxic potency showed species- and time-dependent alterations, thus reflecting changes of its strength. The occurrence of seasonal alterations of leachate parameters, such as Cond, TDS, and N-derived parameters are significantly related with the observed toxic effects in all species tested, thus indicating the deleterious effects of leachate. Similar findings were also observed by other studies, concerning the important role of leachate physicochemical parameters in the enhancement of its toxicity [10, 11]. Alterations of the aforementioned parameters were significantly related with the amount of rainfall, thus the rainfall regime could affect leachate's strength and toxic potency. Indeed, the low amount of rainfall could attenuate degradation process, while increased amount of rainfall could lead to intensive degradation of solid residues. The fact that during "wet" months the leachate is diluted and seems less polluted indicates that the local rainfall regime could regulate the "age" of the leachate and its concomitant degree of stabilization and biodegradability, commonly indicated by the $BOD_5$/$NH_4$–N ratio, as well as its toxic potency. According to the latter, the estimation of a battery of leachate physicochemical parameters, such as Cond, TDS, NH4–N and the respective $BOD_5$/$NH_4$–N ratio, could be used as a low-cost effective tool in order to estimate leachate strength and toxicity, at least in the case of semi-arid areas, such as the most of the Mediterranean countries.

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