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# Short – Term Effects of Young Landfill Leachates (LFL) on Chemical and Microbiological Properties of a Mediterranean Sandy Soil

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**Abstract**

Landfill leachates (LFL) constitute a serious environmental problem due to its high concentration of organic and inorganic compounds. However, landfill leachates can be also considered as fertilizer with respect to those substances. The present paper is an attempt to analyze the impact of application of municipal solid waste (MSW) landfill leachates on soil carbon, nitrogen and microbiological characteristics. Three doses of landfill leachates (0.5, 1 and 2%) were used corresponding to 10, 20 and 40 m3ha-1, respectively. The variation of the main physical, chemical and microbiological properties of soil was monitored. Temporary and permanent changes in several properties occurred after the application of LFL. These properties varied in sensitivity to the applied disturbance. Shortly, after the application of LFL the organic carbon and nitrogen (N) increased in soils amended. Simultaneously, an increase in the total number of soil bacteria, nitrifying populations and soil respiration (after two weeks of incubation) was occurred. But this effect disappeared after two months of treatment. The increase in microbiological activity accelerated the loss of soil organic carbon (SOC) and led to an increase of N at the end of incubation. The leachates treated soils exhibited elevated levels of electrical conductivity (EC) and lower levels of exchangeable Phosphorus (P).

**Keywords:** Landfill leachates (LFL); N dynamic; Soil organic carbon; Microbial properties; Mineralization of carbon

## Introduction

The production of solid residues is a major problem in the management and handling of urban wastes. Their accumulation in a landfill site leads to the generation of landfill leachates (LFL), by the precipitation and penetration of water into the mass of residues undergoing biodegradation [1,2]. LFL are liquid effluents. They constitute a serious environmental problem due to their high concentrations of organic and inorganic compounds. In Tunisia, 1700 tones of domestic garbage are daily collected [3]. The concentration of organic compounds (i.e., phenols, aromatic acids, chlorinated aromatic compounds and polycyclic aromatic compounds) and inorganic compounds (i.e., heavy metals) in these landfill leachates, can have deleterious effects on organisms [4], as a result of their toxic and genotoxic potential [5]. Such properties of these and other compounds present in the leachates can be aggravated by bioaccumulation through the food chain [6]. Thus, many investigators have searched for disposal and valorization potential solutions.

It has been suggested that urban landfill leachates should be used as fertilizers because of their macro- as well as micronutrient supply [7,8]. Its use for soil fertilization could, therefore, be doubly beneficial mainly in those countries having severe water deficiencies and soil organic matter and nutrients. In this perspective, several studies found positive effects of LFL irrigation on soil fertility and crops growth, showing it's fertilizing potential [9-13]. They also suggest that addition of leachates is a partial method of treating and purifying them. In theory their purification will be effected by bacterial activity, filtration, evaporation, ion exchange adsorption and other process in soils. Thus, soil provides a suitable natural environment for biodegradation of wastes and therefore serves as a sink for the adsorption and absorption of ions and as a medium for the restoration of vegetation and normal land use [14]. Sandy soils will favor the oxidation of organic matter and clay soils the removal of heavy metals. The Magnesium ammonium phosphate (MAP) precipitate obtained from LFL was applied as a fertilizer. It showed an enhanced germination and growth of four vegetables planted in sandy clay soil [15]. However, some authors reported that irrigation with leachates gradually increased soil salinity and altered the phytomass production [16]. Several scientists studied the effect of disposal of LFL by incorporation in soils predictably alters soil properties as determined by the composition of the respective waste [17]. Nutrient element and organic matter enrichment, increases in the concentration of heavy metals and sometimes drastic changes in pH have been reported [18]. Gordon et al. [19] reported a significant decrease in microbial biomass in a forest soil to which landfill leachates was applied. This may have been due to waterlogging of the soil or to toxicity to the bacteria since the leachate contained several solvents including 2 ppm of toluene. To the best of our knowledge, there is no report on the impact of the direct LFL spreading on soil carbon balance, nitrogen dynamic involving immobilization and its susceptibility to leaching and soil microbiology which are highly connected but lacking in previous works. In fact, addition of landfill leachates may have many effects in the chemical and microbiological properties of soil. These effects concern the uppermost aerated layer of the soil, in which the essential biological processes occur. Thus, the biological behavior of the top soil should be affected.

In this paper, we explore the feasibility and potential use in remediation technologies of LFL as organic amendment for improving quality of soil .The short term effect of three application rates of LFL on physical, chemical and microbiological properties of sandy soil have been studied in an incubation experiment at laboratory scale, under controlled conditions.

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The soil used in the experiment was from Tunisia, representative of continental semi-arid to arid Mediterranean lands, with dramatic water deficiency and soil, often, poor in organic matter content. Tunisia is a high landfill leachates production country. The soil amended with LFL, was periodically analyzed for functionally related proprieties such as microbial biomass (total bacterial flora, Nitrifying bacteria), carbon mineralization. The variation of the main physical and chemical properties (total and inorganic N, pH, electrical conductivity and organic carbon) were also monitored.

## Materials and Methods

### LFL and soil origin

The soil was surface sampled from an agricultural area near Sfax, Tunisia. Crops usually cultivated in that area are Tomato, Lettuce and Cucumber. The climate of the region is typical Mediterranean, semi-arid to arid, with an average rainfall of 212 mm year$^{-1}$ and an average annual temperature of 19°C. The field-moist soil samples were sieved (<2 mm), delivered in sealed plastic bags to the laboratory under refrigerated conditions and stored at 4°C until analysis. LFL was collected from a landfill site located in Agareb at 20 km to the west of Sfax, Tunisia (34°44'N; 10°32'E). Raw landfill leachate was taken from the main collection drain currently in use. Some physical and chemical properties of the LFLs used in our study are shown in (Table 1). Each value is the mean of three replicates ± the standard deviation.

### Soil treatments

The soil used in the experiment was alkaline with a pH of 8.2. Its texture was sandy loams with 58% sand, 41% silt and 1% clay, low electrical conductivity (EC=537 dS m$^{-1}$) and low nitrogen (0.03%) and organic (0.01%) contents. Soil samples were collected from the top 20 cm of the soil in an agricultural field. Samples were mixed, air dried and sieved through a 2 mm mesh before analysis and use in the incubation experiment. Four aliquots of the soil were prepared using different concentrations of LFL (0, 0.5, 1 and 2%). These LFL doses correspond to 0, 10, 20 and 40 m$^3$ ha$^{-1}$ when considering a pluggable layer 0.2 m deep. These aliquots were placed in quadruplicate into 210 mL glass flasks, with 100 g of soil per pot, and incubated at 28°C in the dark following a complete randomized design. Soil was kept at its water holding capacity for 2, 4, 6 and 8 weeks of incubation. The samples were regularly aerated and the moisture was adjusted every three days. Each treatment was replicated five times. At 0, 2, 4, 6 and 8 weeks' incubation, five aliquots of each treatment were taken and split into sub samples for all physico chemical and biological analyses. Sub-samples for biological analyses were kept at 4°C and all determinations were done within 5 days from the soil collection. Other sub-samples were air-dried and used for physical-chemical analyses.

### Physico-chemical analyses of LFL and soil samples

Leachate samples were collected from the evaporation pond in 40 L plastic carboys. Samples were transported to the laboratory, stored at 4°C and analyzed within two days. Physical chemical characteristics of leachate samples were validated according to French standard NF XPT 90-210 [20]. The COD was estimated using the method described by Knechtel [21]. Total nitrogen contents (TKN) were measured by the Kjeldhal method using an automated apparatus (Buchi, Switzerland). Phosphorus was determined colorimetrically at 430 nm using a Shimadzu U 1000 spectrophotometer. The phosphorus content (TP) was measured colorimetrically by atomic absorption (ICE, 3000 model). The pH was measured using pH meter (INOLAB WTW720). Electrical conductivity was determined with an electronic conductivity meter

| Analysis | Mean |
|---|---|
| pH | 6.34 (± 0.02) |
| EC (dSm$^{-1}$) | 40.6 (± 0.17) |
| Alkalinity (mg L$^{-1}$) by CaCo3 | 5435 (± 205) |
| COD (g L$^{-1}$) | 50.34 (± 2.5) |
| BOD(g L$^{-1}$) | 20.13(± 3.4) |
| BOD/ COD | 0.4 |
| TKN (mg L$^{-1}$) | 2478 (± 228) |
| $NH^{4+}$( mg L$^{-1}$) | 2400(± 55.75) |
| TP (mg L$^{-1}$) | 13.64 (± 0.4) |
| Chlorides ( mg L$^{-1}$) | 4895 (± 784) |
| Turbidity (NTU) | 4.22 (± 0.005) |
| TOC (mg L$^{-1}$) | 48000 (± 3.2) |
| OM (g L$^{-1}$ ) | 30.83 (± 0.62) |
| Sulfates (mg L$^{-1}$) | 1105 (± 66.5) |
| Ca (mg L$^{-1}$) | 4000 |
| Pb (mg L$^{-1}$) | 0.41 (± 0.1) |
| K (mg L$^{-1}$) | 2827.2 (± 174.1) |
| Mn (mg L$^{-1}$) | 5.1 (± 0.24) |
| Fe (mg L$^{-1}$) | 166.5 (± 15.7) |
| Cr (mg L$^{-1}$) | 1.81 (± 0.05) |
| Ni (mg L$^{-1}$) | 1.51 (± 0.07) |
| Cu (mg L$^{-1}$) | 0.36 (± 0.02) |
| Zn (mg L$^{-1}$) | 0.76 (± 0.06) |
| Na (mg L$^{-1}$) | 298 (± 6.03) |

EC: electrical conductivity; COD: chemical oxygen demand; OM: Organic matters; TP: Total phosphorus; TKN: total nitrogen. Data are means of three replicates with their associated standard error

**Table 1:** The physical and chemical properties of LFL.

(TACUSSEL, CD 6NG) equipped with an immersion measurement probe (cell constant $KsL_{-1}$=1 cm). The total concentrations of K, Pb, Cr, Ni, Zn, Ca, Na, Fe, Cu and Mn were determined using atomic absorption flame emission spectroscopy AAS (Thermo scientific). Prior to analysis, 20 mL of the sample was transferred into the Teflon flask and then completely dissolved in HCl–$HNO_3$ solution (30/70% in volume). After dissolution, the mixture was diluted with 100 mL of deionized water and analyzed by (AAS). The concentrations of heavy metals were also analyzed according to the standard methods for the examination of water and wastewater in order to validate/evaluate the produced results and they were found within accepted analytical error (± 7%). All chemicals used for the analytical determinations were of analytical grade. All analyses were run in tripliquate for reproducibility of data and results were the average ones. Soil pH was determined by pH meter (pH 240 L NeoMet ISTEK) using soil/water suspensions after 16 hours of maceration [22]. The EC was measured by a conductive meter (cond 720 WTW) on the liquid extract of the saturated paste after 4 hours of rest [22]. The organic matter was quantified with walkely and black methods [22]. Total N was determined using a modified kjeldhal methods [22]. Mineral N-$NO_2^-$ of the studied soils were determined on KCl (1 mol L$^{-1}$) extract by distillation and titration with HCl (0.01 mol L$^{-1}$). Exchangeable phosphorus was quantified by measuring absorbance using a spectrophotometer (SHIMADZU UV SPECTROPHOTOMETRE UV 1800 PRSA) at 840 nm according to Olsen method [23], after extraction with $Na_2CO_3$ (0.5 mol L$^{-1}$ (soil/solution, 1/20, w/v). Basal respiration was determined every three days according to Stotzky method [24] with some modifications. Moist soil samples (25 g) were incubated at 25°C in 11 airtight jars and $CO_2$ evolved over 72 h was trapped in 0.05 M NaOH solution. Carbonates were precipitated with 0.5 M $BaCL_2$ and the residual NaOH were

titrated with 0.05 M HCl using phenol phthalein indicator. All samples were corrected for the $CO_2$ content of blanks.

### Microbiological analysis of soil samples

**Total bacterial flora:** Soil samples (10 g) were suspended in 90 ml of sterile distillated water. The suspensions were stirred at 250 rpm for 30 minutes to reactivate microorganisms and liberate the cells fixed on soil particles [25]. The suspension was used for microbial count by cell enumeration assessed by the determination of the number of colony forming units (CFU), according to ISO 7218 [26]. Serial decimal dilutions of each suspension ($10^{-1}$ to $10^{-6}$) were plated in triplicate on different agar media: Plate Count Agar (PCA) medium after incubation 24 hours at 37°C, with light and dark alternation.

**Nitrifying populations:** Soil samples (10 g) were suspended in 90 ml of sterile 1 M phosphate buffer pH 7.1 - 7.4. The suspensions were stirred at 250 rpm for 30 minutes to reactivate microorganisms and liberate the cells fixed on soil particles [25]. Nitrifying populations were estimated using the most probable number (MPN) method [25]. $NH_4$ and $NO_2$ oxidizer bacteria were grown in liquid media. These samples were incubated for 6 weeks at 28°C. For the $NH_4$ oxidizers, cultures were checked visually by noting the color change from blue green to yellow, and confirmed by the Griess reaction. For the $NO_2$ oxidizers, positive tubes were revealed by the Griess reaction and confirmed by Zn powder [25]. The results are expressed using MacGrady tables, as bacteria per gram wet soil.

### Statistical analyses

All analyses were performed in tripliquates. Values of different parameters are expressed as the mean ± standard deviation. Statistical analyses were carried out with SPSS 17.0 for windows (SPSS Inc. USA). The results were analyzed by multiple ANOVA (Tukey's and Duncan's multiple range tests) to evaluate significant differences between means at the 95%.

## Results and Discussion

### Physico chemical properties of LFL

The results of the physico chemical analysis relating to the LFL are given in Table 1. The raw leachate had high COD and soluble N-$NH_4^+$ contents. The major fraction of the TKN was in ammoniacal form. Furthermore, The color of LFL was dark brown due to the presence of humic substances. These substances contain both aromatic and aliphatic compounds [27]. In addition, LFL is rich of inorganics loads such as Calcium, Sodium, Iron and Potassium. This high content of organic compounds, macro-elements and micro-elements indicated a significant fertilizing potential of the LFL that could be used advantageously in agronomy.

### Impact of LFL on soil pH and EC

LFL addition to the soil caused important and significant (<0.05) variations of its pH. This phenomenon was particularly clear at the beginning of the experiment until the second week of the incubation. The pH of soils treated was significantly lower than that of the control. This can be explained by the acidic nature of the LFL. The pH of the soils supplemented with 0.5 or 1% of acidic LFL with a pH of 6.34 was not significantly different from the control (P>0.05) (Figure 1). The LFL acidity was compensated by the soil carbonate alkalinity as given away by Sierra et al. [28]. However, the pH of soil treated with 2% LFL was significantly lower. The pH increased during the first period of incubation in the control as well as in the LFL-treated soils. Nevertheless, this change in pH was temporary since two week after having applied the LFL, the pH of treated soils returned to the same level as that of control. The increase in pH observed could be attributed to the ammonia production resulting from degradation of the organic matter contained in LFL. However, despite the acid character of this waste, at the end of the incubation period, the pH value decreased in all cases, reaching neutrality. The decrease in pH could be explained by the strong buffer capacity of the calcareous soils [29,30] and the nitrification process by which ammonia is converted to nitrites and then nitrates [31] , this reaction was accompanied by the release of H ions [32-34]. This process has also been observed during laboratory soil with pig slurry and green waste compost incubations (Figure 1).

The EC increased significantly and proportionally to LFL's doses at the starting of the incubation. Since this moment, the EC decreased until day 15 days (Figure 2). It returned to the initial level for all treated soils as well as the control at the end of experiment. There were significant positive correlations (p<0.05) between LFL doses and levels of EC, immediately after incorporation of LFL. The raise in the soil salinity could result from the main ionic species (Na, Cl and $SO_2$), which came from LFL [35]. Leachate enriched the soil with $Cl^-$ [16,34]. The evolution of EC during the incubation period suggests a biological activity inducing mineralization of organic matter. The same trend of EC evolution was shown in soils incubated with winery and distillery wastes [36].

### Impact of LFL on fertilizing properties

The studied soil was initially poor in organic matter (OM) (0.01%). LFL improved the soil organic matter's contents. LFL addition had increased significantly (p<0.05) SOC (Figure 3). At the beginning of experiment the content of organic carbon was higher in the treated soils than in the control, it reached 0.22% for the highest dose (2%). The sudden increase of SOC in the treated soils could be explained by the organic matter enrichment [19]. The values decreased at the end of incubation period as mineralisation progressed reaching minimum values for treated soils varying between 0.1% and 0.12% for the 1% and 2% doses, respectively without significant differences between treatments and the control (Figure 3). This behavior was reflected by an increase of carbon mineralisation rate at zero incubation time with a rapid decline at 10 days to the initial value to the control soil. This mineralization could be explained by the proportion of biodegradable organic matter (BOD) of LFL (BOD/COD ca. 0.4) [37]. The decrease of SOC during incubation period suggests that organic carbon, being a

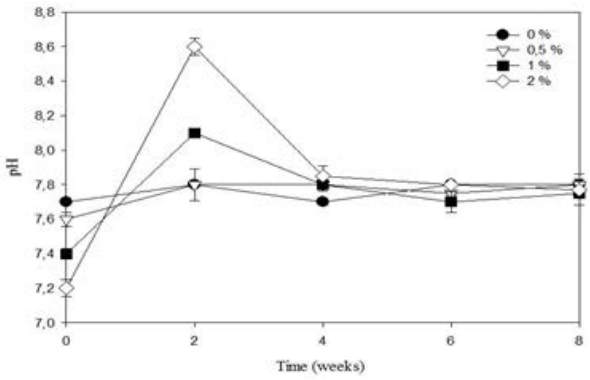


**Figure 1:** Evolution of pH during incubation time.

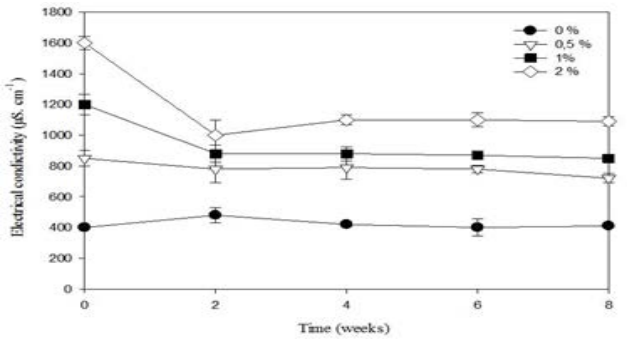


**Figure 2:** Evolution of electrical conductivity during incubation time.

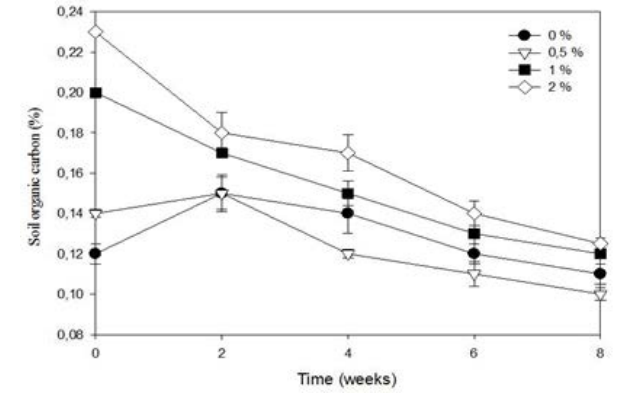


**Figure 3:** Evolution of soil organic carbon during incubation time.

source of available carbon for microbial population [30,38].

Figure 4 depicted that the carbon mineralization rate (expressed as mg of $CO_2$ evolved per g of dry soil and per 72 h) decreased progressively with incubation time. From this figure it can be seen that the accumulated $CO_2$ evolution was higher in soils amended with 1% and 2% LFL doses than in the control throughout the incubation period ($p<0.05$). These reveal a lower microbial activity for a dose of 0.5% compared with the control soil, while the dose of 1% and 2% increased the microbial activity significantly. However, after the 2 weeks of incubation, soil respiration of the three amended soils declined and tended to be similar to that of the control soil, showing the pattern of the soil to recover its initial equilibrium status. The initial and quick carbon (C) mineralization of the residues in soils is related mainly to the amount of C present initially in soluble form, the labile C fraction. Since the decomposition proceeded, the influence of this fraction is minor due its great degradation [39,40]. This trend of $CO_2$ evolution has been also observed by Sanchez_Monedero [41], in an incubation of soil with composted sewage sludge at different stabilization degrees.

LFL treatment had significantly affected soil N content ($P<0.05$) in the soil. The total N increased significantly ($p<0.05$) and proportionally to the LFL doses immediately after their incorporation into the soil (Figure 5a). It increased from 0.3 for the control to 0.38 mg $g^{-1}$ for LFL dose of 2%. The increase of total N in the treated soils at the beginning of the experiment could be attributed to the nitrogen load of the LFL (total N up to 2478 mg $L^{-1}$). LFL contained large amounts of organic and inorganic N, which might have been retained in soil after LFL application [42,43]. The fresh input of easily available N substrates led to a rapid increase of soil respiration and accelerates organic N

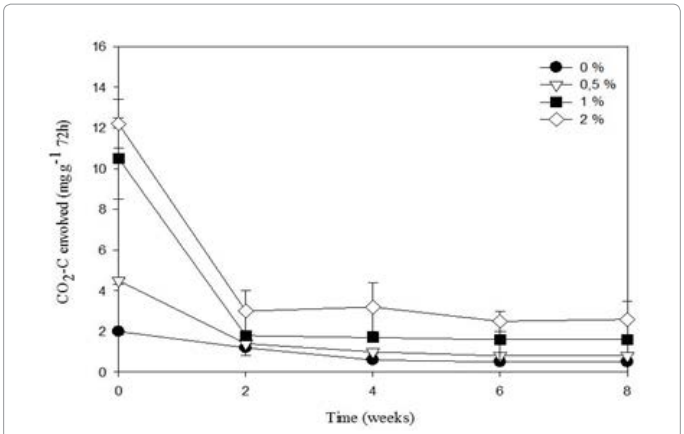


**Figure 4:** Evolution of $CO_2$- C during incubation time.

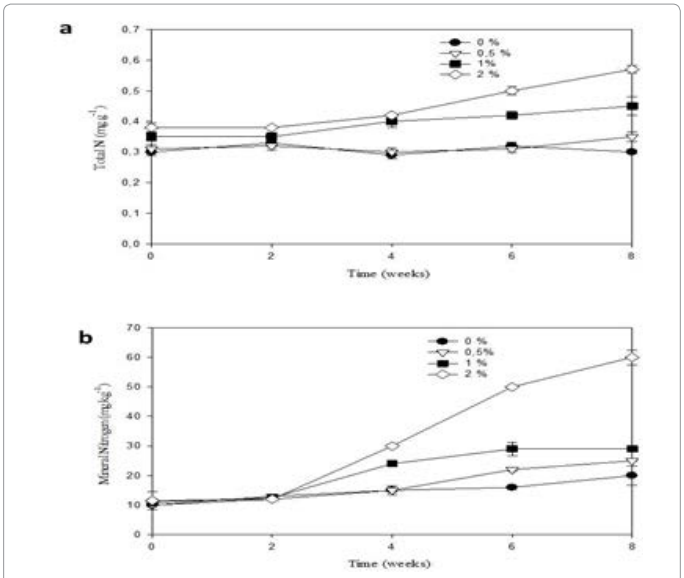


**Figure 5:** Evolution of total N (a) and inorganic N soil content (b) during incubation time.

decomposition. This could be attributed to the high contents of organic nitrogen and the light texture of the sandy soil, which characterized by a good aeration and permeability [44]. Hernandez et al. [45] showed that sandy soil favouring more than clayey loam and loams soils mineralisation processes. A consistent increase of total N and inorganic N content was measured after 4 weeks and only in the soils treated with 1 and 2% doses of LFL (Figure 5b), probably due to these doses showed the highest initial TKN contents, being the N concentration of the residue the most influencing factor in the dynamics of soil mineral N [40,46]. Indeed, LFL addition increased the soil N content and led to a progressive enrichment of this fraction at the end of experiment. Similar results with regard to increases in N were published by Cheng [42] for soil samples of plants irrigated with leachates.

Leachates are usually low in phosphorus, having the highest total phosphorus content of only 30.3 mg $L^{-1}$. The major form that existed in the leachate was orthophosphate ($PO_4^{3-}$). Phosphorus is essentially immobile in soil and the landfill body [35]. Immediately, after the addition of LFL, the exchangeable P increased in the treated soils compared to the control ($p<0.05$). While, no accumulation of exchangeable P in leachates treated soils was found in the present

study at the end of experiment which might be due to the low level of P contained in the leachates [33] (Figure 6).

## Impact of LFL on microbial properties

Analysis of variance showed that LFL addition led to a significant change of the number of total bacteria (Figure 7a) and nitrifying populations (Figure 7b) ($p<0.05$). Immediately after LFL addition the numbers of both properties were similar to the control soil. The control soil was very poor in organic nitrogen (0.03%) so, the number of nitrifiers was feeble [47]. However, after two weeks of incubation the LFL addition enlarged, in a meaningful manner, their number. Results show that total bacteria and nitrifying populations increased reaching maximum values for treated soils. This increase was more remarkable in soils receiving 1% and 2%.The total bacteria varying between $2.65 \times 10^5$ CFU $g^{-1}$ and $3 \times 10^5$ CFU $g^{-1}$ for the 1% and 2% doses respectively. The impact of the LFL addition on soil microflora could be explained by the temporary enrichment of soil with a readily available carbon (C) source. The fresh input of LFL resulted in high levels of available C and N led to a rapid increase of soil respiration and an increase of microbial biomass [38]. As a consequence, LFL amendments generally enhance the development of the total bacteria and increase the global activity of the soils. These changes reflect organic matter inputs to the soils, the efficiency of conversion to microbial C, loss of carbon from the soil, and the stabilization of organic C by soil fractions [48]. After two weeks of incubation, the numbers of total bacteria and nitrifying population decreased as incubation continued and remained at an average level of $5 \times 10^4$ CFU $g^{-1}$ for total bacteria and $5 \times 10^3$ bacteria $g^{-1}$ for nitrifying populations.

The results of this study showed that the fertilization potential of landfill leachates seems to be substantial especially for the supply of N and C. The presence of carbon increased the microbial activity for organic nitrogen breakdown and the decomposition rate of organic nitrogen. LFL leachates supplementation had no durable impact on the pH, but they increase the EC of all amended soils and this could the major concern regarding the use of the studied doses of LFL.

## Conclusion

LFL constitutes a serious environmental problem. Several physico chemical and biological processes to reduce their contaminant effects have been proposed. Many studies have established that this wastewater have a high fertilizer value when applied to the soil. Soils in semi-arid and arid areas are known to have low organic matter levels, a low fertility and a high exposure to degradation, desertification and pollution. Nowadays, organic wastes of various origins and nature are widely used as amendments to increase SOC and N.

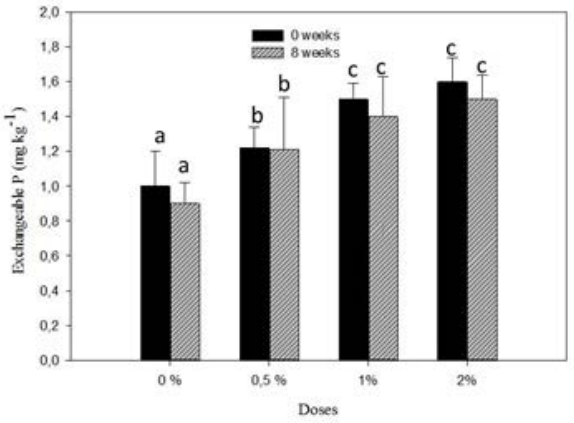


**Figure 6:** Evolution of exchangeable P during incubation time.

**Note:** For each sampling date and type of soil, mean values denoted by the same letter are not statistically different according to Turkey's test at $P<0.05$.

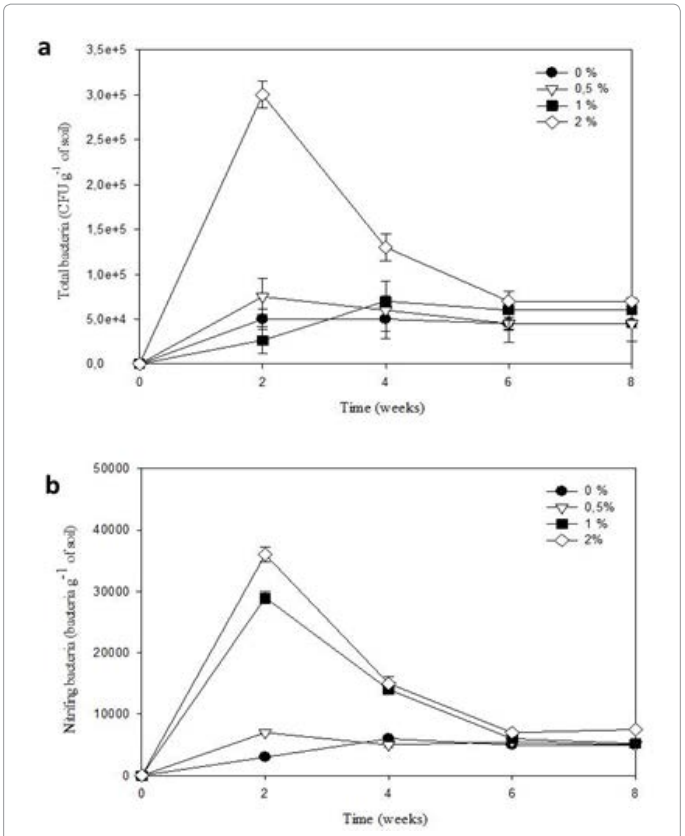


**Figure 7:** Evolution of total bacteria (a) and Nitrifying bacteria (b) during incubation time.

In conclusion, it is evident that this study has shown temporary and permanent changes of several chemical and microbiological soil properties occurred following LFL application, showing that the properties have variable sensitivity to the applied disturbance and that soil has an intrinsic buffering capacity to resist the applied perturbation. Although the experiments, as those presented here, are limited by the laboratory, controlled conditions adopted, they may be suitable for assessing the temporary response of soil to an applied disturbance. Furthermore, these investigations may be helpful guidelines for further studies to validate and extrapolate the data to natural situations.

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