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Physicochemical, bacteriological and ecotoxicological characterization of the leachate of the FkihBen Salah landfill (Morocco)

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- ✓ Mussel,
- ✓ Kaplan-Meier'smethod.

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Abstract

The composition of landfill leachates is very complex and varied according to waste composition, length of stay, age of discharge and climatic conditions. In the event of uncontrolled landfill and lack of treatments, leachates can lead to environmental pollution due to their pathogenic microbiological load and toxic substances. The objective of our work is to study the ecotoxicological effect of leachates from the Fkih Ben Salah landfill on the survival of the *Mytilusgalloprovincialis* mussel using the Kaplan-Meir method to calculate the probability of survival and duration of survival of mussels exposed to leachate from this landfill. The parameters taken into account for this study are mainly microbial load (total and fecal coliforms) and trace metals (Fe, Pb, Cu, Zn, Cd, Ni). The results of the comparison of the probability of survival of uncontaminated mussels and those treated by leachates (C1 = 5/1000 and C2 = 10/1000) show a real ecotoxicological risk on the survival of mussel studied; respectively, of the mean survival probabilities (for March, April and May 2016) of 0.8, 0.3 and 0.4.

1. Introduction

The population of the world is growing fast and would probably reach 9.2 billion inhabitants in 2075, compared to 6.4 billion today [1]. An additional 3 billion people are expected in 2030, 95% of them in developing countries [2]. Such growth raises environmental concerns in Morocco, as in other developing countries, the situation is aggravated by the rapidly changing socio-economic context, causing a significant increase in the annual production of household waste, reaching about 600,000 tons in 1960 to more than 6 million currently [3].

The major and inevitable consequence of such a landfilling is the production of a leachate that can give rise to serious ecological problems. Indeed, where the site is not equipped with a containment system, leachates rich in organic and inorganic materials, but also heavy metals can contaminate underground and surface aquatic systems [4].

Like most other Moroccan cities, the Fkih Ben Salah's household waste is evacuated, without sorting and without treatment, to an uncontrolled landfill. It is because of the wastes that these leachates are characterized by a high organic and mineral loading [5]. These effluents are likely to seep into the subsoil and contaminate the aquifer that flows into this area at a depth of about 40 meters [6]. This situation becomes more problematic when this water is consumed by human beings and living species. In addition, The anthropogenic activites disrupt the ecosystem has serious consequences for the environment and human health, driving strong social and economic impacts on communities [7].

Mussels are commonly used as bioindicators. They are ecologically relevant; they are easy to retrieve, they have a high sensitivity to many chemicals and they are sedentary organisms filtrating large amounts of water allowing them to accumulate the substances from the environment [8,9]. These responses are typical of a biomarker which is defined as "A biochemical, cellular, physiological or behavioural variation that can be measured in tissue or

body fluid samples or at the level of whole organisms that provides evidence of exposure to and/or effects of, one or more chemical pollutants (and/or radiations)" [10].
The major goal of this study isto evaluate the eco-toxicological risk associated with the presence of toxic pollutants in the young leachates of the public dump in the town of Fkih Ben Salah using M*ytilusgalloprovincialis mussel* as a bioindicator species of pollution, studying their survival with Kaplan-Meier's method [9].

2. Materials and Methods

2.1. Location and characteristics of the study zone

The public dump is located about 13 km north of the town of Fkih Ben Salah, on the national road 11 of the city of Khouribga, it occupies an area of 35.2ha (Figure 1). It is located on the phosphate plateau formed by permeable rock formation (Eocene), reaching a thickness of about 100 m and having a groundwater flowing from the north to the south in the study area at a shallow depth over ground.

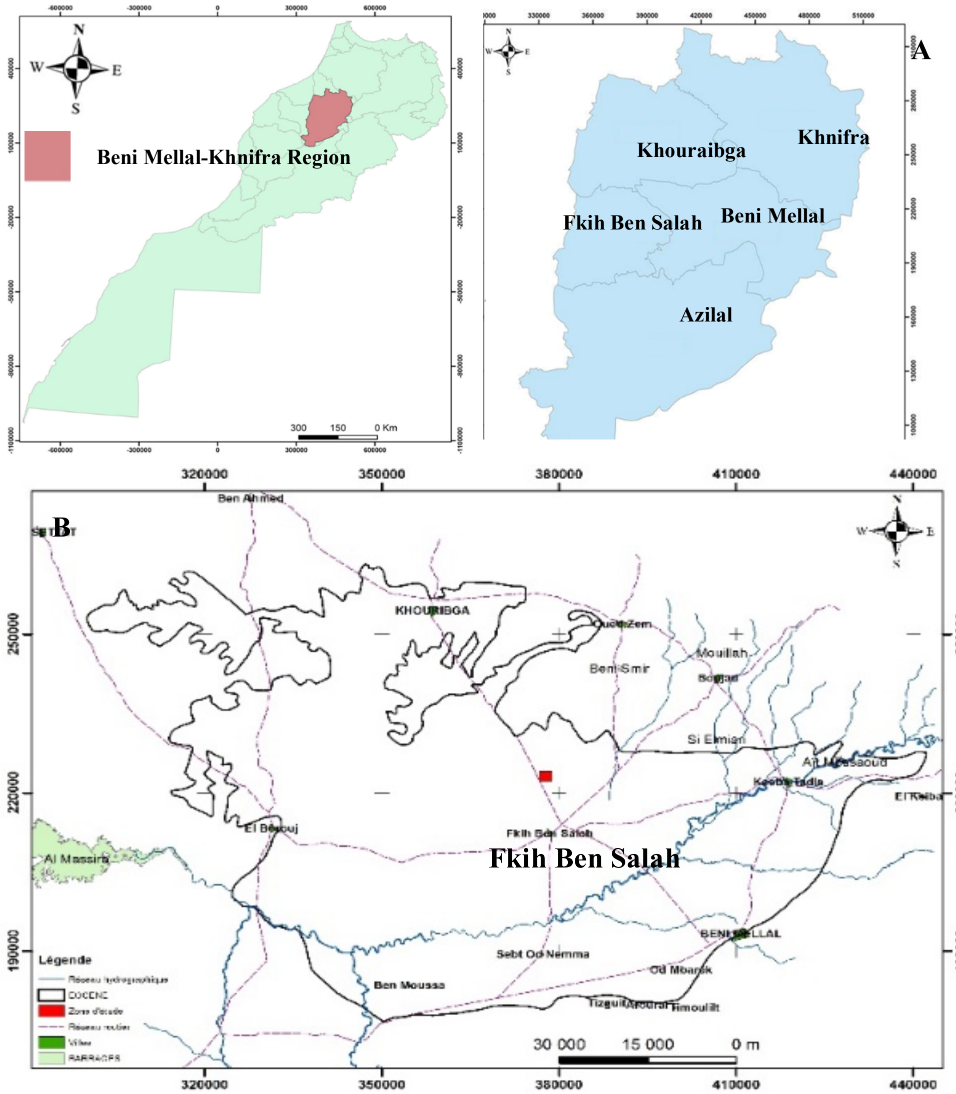

Figure 1 : Geographical location of study sites: Beni Mellal-Khnifra Region (A) and Fkih Ben Sallah town (B)
:The study zone (Landfill of the Fkih Ben Salah town)

This zone has a continental climate characterized by intense cold in winter and a very hot summer with an mean annual rainfall of 360 mm and an annual mean temperature of 25 ° C. Evaporation is intense in summer. The annual mean is on the order of 1800 mm, but it can reach 2200 mm during dry periods. The evaporation reaches its peak on July and August. The monthly mean is between 250 and 300mm [6].

2.2. Characterization of leachates from the Fkih Ben Salah landfill

2.2.1. Samples

During the year 2016, three sampling campaignsof young leachate were carried out once per month (March, April and May 2016). 5 liters of young leachate were recovered from each waste collection truck atthe end of their tours.

The samples are stored in a cooler and transferred immediately to the laboratory where they were placed in a refrigerator at 4 ° C, in the dark.

2.2.2. Physic-chemical characterization

Measurements were based on temperature (TFX 430 thermometer), electrical conductivity (LWT-01 VOLTCRAFT conductivity meter), pH (pH meter PCE-PH 22) and turbidity (turbidity meter AL400T-WL).

2.2.3. *Determination of Biochemical Oxygen Demand (BOD)*

The leachate samples are diluted with different amounts of dissolved oxygen-enriched dilution water containing a seed of aerobic microorganisms, with suppression of nitrification.

The samples are then incubated at 20 ° C for a fixed period of 5 days, in the dark, in fully filled and closed flasks. The determination of the dissolved oxygen concentration is carried out before and after incubation. A calculation of the mass of oxygen consumed per liter of sample is carried out for each sample.

2.2.4. Determination of the Chemical Oxygen Demand (COD)

Boiling under reflux, in the presence of mercury (II) sulphate which is a test portion in the presence of a known amount of potassium dichromate and a silver catalyst in a medium strongly acidified with sulfuric acid, for a given period of time during which part of the dichromate is reduced by the oxi-dizable materials present.

The calculation of the COD is based on the amount of reduced dichromate. COD is the concentration, in milligrams per liter, of oxygen equivalent to the amount of potassium dichromate consumed by the dissolved and suspended matter.

2.2.5. Trace metals

The metal trace elements (Fe, Pb, Cu, Zn, Cd, Ni) were analyzed by ICP-OES optical emission spectrometry after mineralization with dissolution of the samples.

2.2.6. Bacteriological characterization

The bacteriological characterization of leachates consists in the enumeration of total coliforms and faecal coliforms by the membrane filtration method. This method involves collecting, identifying and counting the bacteria in a sample on the surface of a sterile filter membrane. The leachate samples were diluted in phosphate buffer.

For total coliforms, a determined volume (100 mL)of the sample is filtered through a 0.45 μm pore size membrane filter and then incubated for 24 hours ± 2 hours at 35 ° C ± 0.5 ° C on medium m -Endo.

In the case of thermotolerant (fecal) coliforms, the method consists in filtering through 0.45 μm porosity membrane filter of a determined volume of the sample and then incubating this membrane for 24 hours ± 2 hours at 44.5 ° C ± 0.2 ° C on the m-FC medium.

2.3. Ecotoxicological characterization

2.3.1. Sample of animals

Three sampling campaigns for the *Mytilusgalloprovincialis* mussel were conducted at low tide from March to May 2016. Mussels of the 3-4 cm size class are sampled from wild mussels on the Haouzia coast at about 1 km north of the center of the city of El Jadida. The animals collected are transported in laboratories to coolers containing sea water and are placed in filtered and oxygenated seawater for a purge period of 48 (h) to ensure adaptation to laboratory conditions.

2.3.2. Preparation and contamination of animals

The thirty mussels are placed in plastic containers (37 × 27 × 9 cm) containing about 3 liters of sea water renewed every 72 hours. The oxygenation of the sea water contained in the plastic containers is ensured permanently by means of aerators [11].

The contamination of the mussels was carried out by three different concentrations of the young leachate C_1, C_2 (dilutions of the stock solution), namely 5/1000, 10/1000 respectively.

Three groups, each comprising 30 mussels, were formed; two groups are contaminated, at the time of water renewal, by concentrations C_1, C_2 respectively and a control group that receives no particular treatment.

The viability of the mussels is checked every 24 hours at the same time, noting the number of dead animals. The mussel is considered dead when its shell no longer closes after physical stimulation of its shell (touch) [12, 13].

2.3.3. Survival study (Kaplan Meier method)

This method allows the estimation of the probability of survival during a follow-up in plastic containers defined by the survival function S(t). The principle of Kaplan-Meier's estimate rests on the idea of a conditional probability (the probability of being a survivor at the end of the interval on condition that the subject was a survivor at the beginning of the interval) [14].

The fact of being alive just before the instant t makes it possible to calculate the probability of survival at this instant. The cumulative probability of survival of each individual gives the probability of survival of the group. This probability results in a curve called the survival curve of Kaplan-Meier, which illustrates the evolution of survival as a function of time, with on the ordinate the cumulative probability of survival (between 1 and 0) and on the abscissa the duration of the monitoring in units of time.

2.3.4. Statistical tests used

The log-rank test [15; 16; 17] is used for the comparison of two or more survival curves at a time. It makes it possible to compare, throughout follow-up, the risks of mortality in each group compared to the normal rate.

In the case where survivors do not follow the principle of proportional hazards (survival curves intersect), the Gehan Wilcoxon test [18] is used to check if there is a statistically significant difference between the groups studied, survival curves and the probabilities of survival are realized by the software NCSS.

3. Results

3.1. Physico-chemical characterization

The recorded pH values indicate the acidic character of leachates from the Fkih Ben Salah landfill. These results (pH between 3.37 ± 0.30 and 4.13 ± 0.32) adequately reflect the biological evolution phase as leachate juveniles and not yet stabilized (Table 1).

The BOD_5 / COD ratio is an indicator of the biodegradability of organic matter and the maturation of leachates [19; 20]. In our study, this ratio varied between (0.352 ± 0.082) and (0.414 ± 0.036) where the biological activity corresponds to the acidic phase of aerobic degradation, this ratio reaches the value of 0.83, Whereas the aged leachates or stabilized are characterized by a rather low organic load and are generally refractory to biodegradation [21].

Table 1: Physico-chemical characterization of raw leachate from the Fkih Ben Salah landfill in March, April and May 2016 (Mean ± SD).

	T °C	pH	BOD_5 (mg O_2/L)	COD (mg O_2/L)	BOD_5/COD
March-16	21.83± 0.76	4.13± 0.32	7704 ± 368.60	18657.67 ± 792.78	0.414 ± 0.036
April-16	25.07± 0.12	3.37± 0.30	6670.00 ±1089.99	19232.33 ±2377.41	0.352 ± 0.082
May-16	30.00± 1.00	3.47± 0.25	9419.67± 439.15	25397.67 ± 470.06	0.371 ± 0.010

The study of the concentration variation of the trace metals analyzed revealed high levels in these leachates. The iron threshold is high in relation to other metals and varies between (85.55 ± 7.08 and 100.64 ± 1.93) (mg / l). Indeed, the concentrations of the other metallic elements studied, in particular those of cadmium and lead, have recorded relatively low values. Therefore, these trace metals can exert an inhibitory effect on microbial development. Moreover, these trace elements present a risk of major contamination for groundwater due to the shallow depth of the water table circulating in the area of the landfill (Table 2).

Table 2: Trace metal content of raw leachate from the Fkih Ben Salah landfill in March, April and May 2016 (Mean ± SD).

	Cadmium (mg/l)	**Copper (mg/l)**	**Zinc (mg/l)**	**Lead (mg/l)**	**Iron (mg/l)**
March-16	0.04 ± 0.005	5.89 ± 0.68	8.84 ± 0.46	2.98 ± 0.83	85.55 ±7.08
April-16	0.03 ± 0.00	7.27 ± 1.33	8.28 ± 1.03	2.98 ± 0.61	92.68 ± 4.81
May-16	0.05 ± 0.01	4.35 ± 0.45	8.44 ± 0.41	2.54 ± 0.49	100.64 ± 1.93

3.2. Bacteriological characterization (Table 3)

The bacteriological analysis of the leachate shows the existence of high levels of coliforms and total germs. The mean maximum values of total and faecal coliforms are respectively 7.89 ± 0.56 and 0.23 ± 0.05 ($\times 10^6$ CFU / ml). This makes these leachates extremely toxic and thus a source of permanent contamination for the environment. These results are more or less similar to those found in the leachates of the Fes discharge by [22], ie $7.8.10^5$CFU for total coliforms and $2.3.10^4$CFU for fecal coliforms.

Table 3: The characterization of Bacteriological of the raw leachate from the Fkih Ben Salah landfill in March, April and May 2016 (Mean ± SD).

	Total Coliforms ($\times 10^6$ CFU/ml)	Fecal coliforms ($\times 10^6$ CFU/ml)
March-16	6.67 ± 0.734	0.15 ± 0.01
April-16	7.89 ± 0.56	0.23 ± 0.05
May-16	7,58 ± 1,32	0,14 ± 0,02

3.3. Eco-toxicological characterization

Exposure of mussels to toxicants into the water can be performed through direct contact of the organisms with contaminated water. . In this context, bivalves, such as the genus Mytilus, are a good choice [23]. They are sedentary organisms filtrating large amounts of water allowing them to accumulate the substances from the environment. They also satisfy the other conditions to be bioindicators and these are: suitable dimensions, easy dentification and collection of organisms, abundance in the ecosystem and accumulation of the elements to a degree suitable to measure [24, 25].

Table 4: Probability of survival of different groups of mussels studied during the months of March, April and May 2016.

March-16			
Number of survivors (n)	Survival time (days)	Probability of survival S(t)	Standard deviation of S(t)
	Control (T)		
27	15.0+	0.90	0.06
	C_1 **(5/1000)**		
14	15.0+	0.42	0.09
	C_2 **(5/1000)**		
16	15.0+	0.50	0.09
April-16			
Number of survivors (n)	Survival time (days)	Probability of survival S(t)	Standard deviation of S(t)
	Control (T)		
24	15.0+	0.83	0.07
	C_1 **(5/1000)**		
11	15.0+	0.37	0.09
	C_2 **(5/1000)**		
12	15.0+	0.40	0.09
May-16			
Number of survivors (n)	Survival time (days)	Probability of survival S(t)	Standard deviation of S(t)
	Control (T)		
23	15.0+	0.77	0.08
	C_1 **(5/1000)**		
8	15.0+	0.27	0.08
	C_2 **(5/1000)**		
12	15.0+	0.40	0.09

Figure 2 show the survival functions of the three groups of mussels studied based on the use of the Kaplan Meier method. The table 4 shows the calculation of the probability of survival of the two groups of the mussel *Mytilusgalloprovincialis* contaminated, respectively by the concentrations $C_1 = 5/1000$, $C_2 = 10/1000$ and a control group that did not undergo any contamination. During the months of March, April and May 2016, each group of mussels has a probability of survival after 15 days of follow-up (Table 4).The survival functions of the three groups of mussels studied based on the use of the Kaplan Meier method are shown in figure 2.

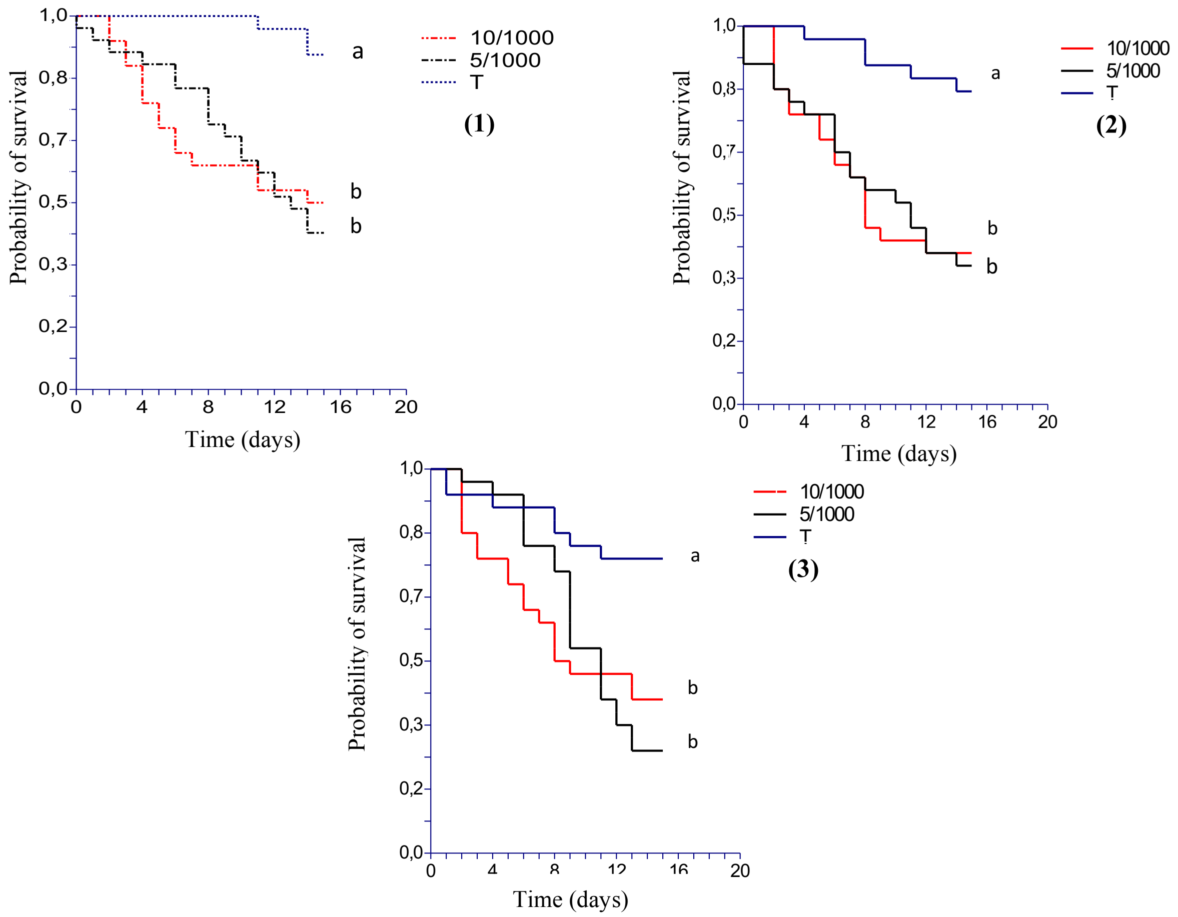

Figure 2: Survival curves of the *Mytilusgalloprovincialis*mussel of the groups studied in March (1), April (2) and May 2016 (3). (curves with the same letter are not statistically different)

As shown in the figure 2, the probability of survival is inversely proportional to the mortality rate. The group with a high mortality rate always has a lower probability of survival than another group with a lower mortality rate. In March 2016, the probability of survival is 0.9 for the control (T) group of 0.50 for the mussels contaminated by the concentration C_2 (10/1000),of 0.42 for the mussels which are contaminated by the concentration C_1 (5/1000). The comparison between the survival curves revealed a statistically significant difference between the control group and the other two groups.In April 2016, the probability of survival is 0.83 for control group mussels, 0.40 for mussels contaminated with concentration C_2 and 0.37 for mussels contaminated by concentration C_1. The only statistically significant difference is recorded the control group on the one hand and the other two on the other groups.With respect to follow-up during May 2016, the control group had the highest survival probability of 0.77. It is followed by the group of mussels contaminated with concentration C2 with a probability of 0.40. The group of mussels contaminated by the C1 concentration is ranked third with a probability of 0.27. The statistically significant differences recorded in this month are identical to those found in the first two months.

Mussels that have been contaminated by the two concentrations (C_1, C_2) of the leachate and whose survival probabilities are the shortest, are exposed to a complex mixture of pollutants. They are forced to spend a great deal of their energy to ensure the detoxification process and maintain homeostasis at the expense of organism growth and production. The presence of pollutants can also reduce their ability to survive. In addition, the results of the response of mussels contaminated by the different leachate concentrations are consistent with several studies that have shown that animals are influenced by the effect of heavy metals [26-29].

The variation in the survival probabilities of mussels in our study (between 0.7 and 0.9 for untreated mussels and between 0.2 and 0.5 for leachate contaminated mussels) is almost the same as the change in (Cd, Co and Zn) have a very low probability of survival (between 0.2, and 0.6) compared to mussels collected from less polluted areas of Oualidia (between 0.7 and 0.9). Moreover, the accumulation of microorganisms in bivalve's due to their physiological filtration activity can significantly affect their filtration capacity by causing physiological disturbances of the mussels [30].

Conclusions

The physicochemical and microbiological characterization of the young leachate generated by waste from the town of Fkih Ben Salah has shown that leachate is an important metal and microbial pollutant (total and fecal germs) that can contaminate the water table circulating at shallow depths about 40 m, measure 2012 [6].
The present study revealed also an ecotoxicological risk due to the presence of high trace metal concentrations and a significant load of pathogenic germs, significantly affecting the survival times of mussels exposed to young leachates in the town of Fkih Ben Salah.
The mussels as bioindicators organisms has been well used in monitoring studies all over the world, as a result of the continuously anthropogenic-induced impacts on the environmental health status. Moreover, the Kaplan Meir method are considered as reliable tools for the monitoring of the aquatic and terrestrial ecosystems

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