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Biotypology of Yeast in Soils Polluted by Hydrocarbons in Fez-Meknes Region, Morocco

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ABSTRACT

The soil, in general, is subject to contamination by various organic pollutants, including hydrocarbon discharges from storage tank spills or leaking pipes between the tanks and the hydrocarbon pumping islands punctually or accidentally. The ecological effects of this pollution on these ecosystems can have consequences on the composition and diversity of the microbial community. Yeasts are among the microorganisms considered sensitive to this contamination and reflect well the changes in environmental conditions. The objective of this work is to carry out a biotypological study of yeasts in soils contaminated by hydrocarbons. For this purpose, samples were taken from 12 sites covering a diversity of habitats in the Fez-Meknes region with very different pedological and climatic characteristics. A granulometric, physico-chemical, and biological characterization of the soil samples was carried out, and the data were evaluated using statistical methods. The microbiological characterization of the various samples revealed that the density of yeasts in the polluted soils varied from 5.2 to 32.5 x 10^4 cells g^{-1}.
Furthermore, the results showed that yeast biodiversity is significantly correlated with the organic matter, $Ca^{2+,}$ and Na^{2+} contents of the soils tested. Similarly, a correlation was found with the type of sandy soil. Of the 86 yeast strains isolated, 49% were identified as belonging to the genus *Rhodothorula* and represents the most dominant genus, followed by *Candida* (17%), *Rhodosporudium* (15%), *Pichia* (10%), *Trichosporon* (8%), and *Exophiala* (1%). This study provided a clear indication of the ecological niche of yeast in hydrocarbon-polluted soils.

Keywords: Yeasts, soils, hydrocarbons, biotypology, Morocco.

Introduction

Yeasts, a polyphyletic group of basidiomycetes and ascomycetes fungi with a unique characteristic of unicellular growth, forms one of the most important subclasses of fungi. They are unicellular, eukaryotic, non-motile, and generally more prominent than bacteria. There are approximately 100 genera, and 800 described species of yeast, and estimates suggest that these numbers represent only about 1% of the species that exist in nature, with the remainder being unculturable.[1]
The presence of yeasts in soils was shown long ago.[2] They were not considered native soil organisms, and the ability of yeasts to spread in soils has been questioned several times.[3,4] Investigations have been conducted to determine the biodiversity of yeasts in these habitats.[5]
The majority of yeast studies have been conducted *in vitro* under controlled conditions in the absence of several factors that can affect their metabolism. The influence of specific parameters on the survival of these microorganisms, namely cardinal growth temperatures, nutrient uptake, and antibiotic resistance, is necessary to gain insight into their function and role in their natural habitat.[6]

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Furthermore, pioneering work has shown the importance of the ecology of soil organisms and their functioning in their environments.[7] Although the ecological studies conducted on these yeasts were only investigations indicating the presence of cultivable yeasts on conventional isolation media, the ecology of cultivable yeast populations in the soil provides insight into the distribution and role of these microorganisms in these habitats. Indeed, soils are considered hostile ecosystems for yeasts because they are rich in nutrients and mineral and organic particles. Furthermore, these particles form heterogeneous aggregates of different sizes, which contain a complex network of pores.[8,9]
Competition for nutrients, including carbon and nitrogen sources, can affect the species composition of microbial communities. These may include yeasts and their competitors.[10] However, it may be these same elements that confer plasticity on the yeast community in terms of its ability to change its species composition, to maintain its functionality in the soil ecosystem, especially in the presence of changing environmental conditions, such as soil contamination by organic pollutants, especially hydrocarbons. This type of pollution can result from uncontrolled releases from manufacturing and refining facilities, spills during transportation, direct releases from effluent treatment plants, and runoff from land-based sources.[11–13] A biotypology study of soil yeasts and their diversity is a good indicator to evaluate the extent of ecosystem disturbance with oil pollution. The objective of this research is to enrich and deepen the biotypological knowledge of soil yeasts, especially those contaminated by organic pollutants in the region of Fez-Meknes, Morocco.

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Material and Methods

Presentation of the study area

The study area is part of the region of Fez-Meknes; it is located in the center north of Morocco and occupies a strategic geographical position.

This region was born from the regional division of 2015 from the three former regions. The territory of the region consists of disparate natural areas, which can be grouped into five geographical units belonging to the natural areas of the Rif, Pre Rif, and the Middle Atlas. The climate of the region is of Mediterranean type characterized by its cool and wet winter and its dry and hot summer. Such a climate favours, by its precipitations, the dissolution and the evacuation of elements and allows, by its temperatures, important biological activity and to accelerate chemical reactions under the action of water and solutes.

Sampling

To cover the different soil types, soil samples were taken from different locations in the Fez-Meknes region between the periods of December 2015 to April 2016. Twelve stations were selected; their locations and coordinates are shown in Table 1 and Figure 1.

Samples were taken from a depth of 10 to 60 cm from the soil surface. Samples for physico-chemical analysis were taken with a spatula and placed in plastic bags. Samples for microbial analysis were aseptically transferred into sterile polyethylene bags and stored in a cool place at 4°C.

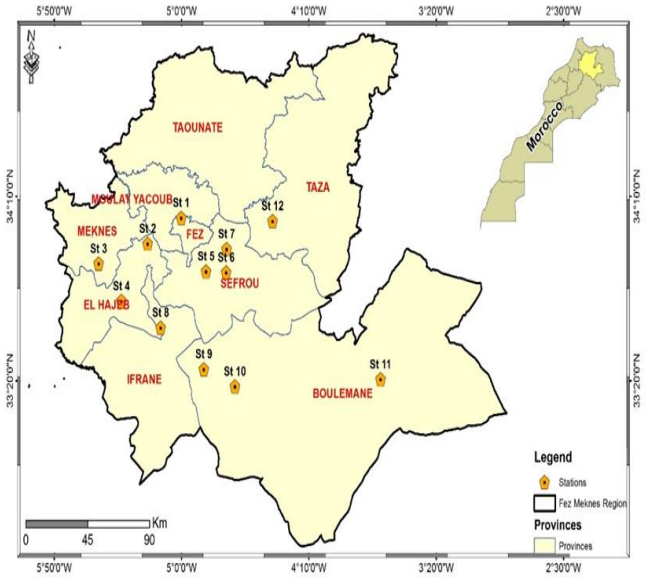

Figure 1: Geographical location of the studied stations

Table 1: Lambert coordinates of sampling stations

Stations		X	Y	Long	Latitude	Altitude
Fez	**St1**	536812.29 m	385356.78 m	-4.99950384 °	34.06655874 °	417.80 m
Ain taoujdat	**St 2**	516521.40 m	372299.99 m	5.21961543 °	33.94932340 °	466.62 m
Meknes	**St3**	486955.04 m	362381.44 m	5.53941429 °	33.85992761 °	558.21 m
El-Hajeb	**St4**	500657.74 m	343619.64 m	-5.39130449 °	33.69079909 °	872.69 m
Sefrou	**St5**	551896.96 m	358566.53 m	-4.83761684 °	33.82433591 °	819.27 m
Azzaba	**St6**	564183.82 m	358165.06 m	4.70472032 °	33.82004776 °	83.80 m
Bir tam-tam	**St7**	564346.54 m	369849.05 m	-4.70227923 °	33.92540590 °	580.83 m
Ifrane	**St8**	524585.96 m	330314.77 m	-5.13350818 °	33.57051778 °	1648.16 m
Guigou	**St9**	550800.08 m	309567.03 m	-4.85224822 °	33.38246581 °	1489.58 m
Bulman	**St10**	569872.57 m	301040.56 m	-4.64786418 °	33.30449076 °	1710.10 m
Outat el haje	**St11**	658570.94 m	305612.54 m	-3.69442105 °	33.33630542 °	796.13 m
Tahla	**St12**	592005.51 m	384107.56 m	-4.40159734 °	34.05195780 °	571.20 m

Grain size analysis of the soil samples

Grain size analysis was carried out according to the standardized protocol for grain size fractionation using the international Robinson pipette method.[14] The soil samples were dried at 60°C for 48 h, and the fine fractions (<150 µm) were separated by wet sieving. The size of the fine fraction was determined using a particle size analyzer (Sympatec, Germany).

The different fractions were individualized by destroying the organic matter that stabilizes the clays and fine silts with 30% hydrogen peroxide (H_2O_2) on a test sample of 10 g of soil. The samples were suspended in water with the addition of sodium hexametaphosphate, a powerful dispersant that neutralizes the flocculating action of colloids under the effect of mineral ions. After dispersion, the sample was left to rest to allow sedimentation of the particles.

Soil physico-chemical analyses

The physico-chemical soil parameters studied were pH, organic matter, total limestone, exchangeable bases, cation content, and ion exchange capacity.

Organic matter was quantified by the Walkley-Black method. [15] The pH was measured using an electro-metric pH meter according to the NF X 31-103 standard, calibrated with two solutions of known pH (2N NaOH and 2N HCl) at 20°C, in a suspension of soil and distilled water with a solid-liquid ratio of 1:2.5.[16]

Soil humidity was determined gravimetrically with oven drying of the samples taken at 105°C. The difference between the weight before and after drying expresses the humidity content of the samples. The fresh weight (FW) and dry weight (DW) are determined with a precision balance. The moisture content (H%) of the soil was deduced by the following formula:

$$H(\%) = (FW - DW)/ DW \times 100$$

The electrical conductivity EC (µS/cm) (solid-liquid = 1:5) was measured using the YSI type conductivity meter, model 133 at 25°C. [17]

The determination of total limestone was done using the Bernard calcimeter after attacking the sample with dilute 6N hydrochloric acid described by Menzer *et al*. [18]

The total base exchange capacity is a method that consists of the analysis of the main exchangeable cations (Ca^{++}, Mg^{++}, K^{+}, Na^{+}). The determination of total cations is done by saturating the soil samples with a solution of normal ammonium acetate at pH 7, and the cations fixed by atomic absorption are determined with a flame photometer.

The result is expressed as the number of charges per 100g of soil (milliequivalents per 100g or meq/100 g).
Cation exchange capacity (CEC) is the total amount of cations that a given weight of soil (usually 100g) can adsorb onto its clay-humus complex and exchange with the soil solution under given pH conditions. In other words, it is the sum of the negative charges of the soil available for the fixation of H^+ ions as well as the basic cations Ca^{2+}, Mg^{2+}, $Na^{+,}$ and K^+. The CEC, therefore, depends on the nature of the colloids with a variable and a specific number of negative sites and, of course, on the soil pH (7). [19] It was determined by the sodium acetate method.

Isolation and identification of soil yeasts
To estimate the distribution and diversity of yeasts in the study area, the solid medium enumeration method was used. The medium used was malt-yeast-glucose-peptone agar[20] with 200 $mg.l^{-1}$ chloramphenicol added, which inhibits the growth of bacteria. The plates thus inoculated were incubated at 28±2°C for 24 to 72 hours. After incubation, the yeast colonies were counted, and the number of cells was expressed as CFU/g. Pure cultures were prepared by typical plating colonies on Malt agar slants. After incubation, the resulting pure cultures were stored at 4°C.
Pure cultures were tested for reproductive, morphological, biochemical, and physiological characteristics.[21]

Statistical analysis
Descriptive statistics were performed to interpret the results of the yeast diversity. Three replicates were performed, and the data obtained were plotted using means and standard deviations. The effects of the variables were determined by the ANOVA test followed by a Tukey's posthoc test at $p < 0.05$. The linear correlation between biodiversity and abiotic factors was obtained by Pearson's correlation coefficient at a significance level of $p < 0.05$. Principal component analyses were performed using Minitab19.1 LLC software, USA.

Results and Discussion

The present study allowed the generation of basic data on the biotypology of the cultivable yeast community isolated from hydrocarbon-polluted soils in the Fez-Meknes region.

Granulometric analysis
The granulometric analysis of all the soil samples studied showed that they are made up of clay, silt, and sand, the percentages of which were variable and were 65.43 ± 12.53%, 14.26 ± 2.92%, and 7.85 ± 2.75%, respectively. The descriptive analysis of the different granulometric fractions of the soil is shown in Table 2.
The region is characterized by the diversity of its soils, where three main types of soils can be identified: mineral soils, brown soils, shots, and vertisols. The granulometric analysis of the twelve soils studied showed the dominance of the clay fraction with a percentage that largely exceeds that of the other fractions. However, the low content of silt and sand in these soils does not favour the dissolution and leaching of hydrocarbons. The contaminants are often strongly bound to the fine fraction of the soil, which favours the dispersion of the hydrocarbon contamination.

Physico-chemical characterization of soils
The pH is the first indicator of any physico-chemical evolution of soils. The measured pH values showed that the minimum pH value (7.16) was observed in the soil of the Fez station, and the maximum value was reported in the Ifrane station (8.65). The average was 7.97, with a standard deviation of 0.45, which indicates that the soil pH is neutral to slightly alkaline.
According to the values of the electrical conductivity, it was noticed that there are two groups of soil samples; the first group includes the stations of Fez, Bir tam-tam, Ifrane, Guigou, and Tahla, which present values lower than 800 µS/cm. The second group, represented by the following stations: Azzaba, El- Hajab, Taoujdat, Sefrou, Meknes, Boulemane, and Outat Elhaj, presents values higher than 1000 µS/cm. The average conductivity at the different stations studied is estimated at 1094.08± 404.855 µS/cm.
As for the temperature, the minimum value of the ground is recorded at the station of El-Hajab, which is of the order of 5°C, and the maximum value is of the order of 13°C, obtained at the station of Fez. The average is 9 ±2.13°C.
The moisture values of the soil samples ranged from a minimum value of about 21% at the Outat El-Haj station to a maximum value of about 88% at the Meknes station. The average value is 65.08%, with a standard deviation of 22.60%. Soil humidity values vary from one station to another.
According to some authors, Soil moisture constitutes only a tiny fraction (0.15%) of the water present on the earth's surface. [22] Above a certain value, it could have a threshold effect on the preservation of environmental conditions allowing the development of microorganisms and the production of enzymes.[23,24]
However, it was noted that the moisture content of the different soils studied was influenced by some physico-chemical parameters such as Ca^{2+} and the nature of the soil texture (silt and sand). This influence was confirmed by a very significant correlation between soil moisture and Ca^{2+} content (r=-0.636), silt (r= 0.517), and sand (r=-0.60) which allow water to infiltrate. They, therefore, contribute to increasing the moisture content.
There was no strong relationship between soil moisture and yeast biodiversity; however, soil moisture in this study was taken at specific times and not as an average over the season. However, it has been reported that sediment grain size largely determines the diversity and abundance of microorganisms.[25]
Furthermore, there was an almost perfect positive relationship between electrical conductivity and Ca^{2+} (r=0.619) and sand (r=0.531). Moreover, an almost perfect negative relationship existed between moisture (r=0.511) and silt (r=-0.702).
The average total limestone content in the soil was 26.99±10.81%, which means that the soil is highly calcareous according to the standards of interpretation of soil limestone content.[17] According to the results obtained, it was noted that the minimum content of limestone was recorded at the level of the soil of Fez (18.5%), and the maximum content was 50.7% in Azzaba. The soils of Azzaba, El hajab, Bir tam-tam, and Taoujdat stations had the highest total limestone content compared to the other soils, with values exceeding 25%.
With respect to the organic carbon, the results obtained showed that organic carbon contents vary between 0.34% in Guigou and 2.5% in Sefrou, with an average of 1.54±0.67%.
The analysis of exchangeable bases revealed that calcium is the most dominant element, representing an average value of about 71.6 ± 12.90 meq/100g of exchangeable cations in all soils. These soils have

Table 2: Particle size analysis of all studied soil samples

Stations	Clays (%)	Silts (%)	Sands (%)
Fez	69.11	12.00	8.09
Ain Taoujdate	71.00	12.00	6.45
Meknes	45.67	12.00	10.45
El-Hajeb	69.13	9.63	14.57
Sefrou	67.22	13.35	8.77
Azzaba	80.68	14.59	7.18
Bir Tam Tam	65.10	17.47	7.59
Ifrane	72.10	17.80	5.00
Guigou	64.78	20.01	4.00
Boulemane	69.29	14.67	6.06
Outat El-Haj	75.39	13.67	9.06
Tahla	35.69	14.00	7.06

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a high calcium content despite their decarbonization. Sodium contents are relatively high, varying between 1.2 meq/100 g in Guigou and 11.67 meq/100 g in Taoujdat with an average of 5.91 ± 3.27 meq/100 g. Despite its importance for the majority of microorganisms, including yeasts, potassium levels are low in all soils, with an average of 0.37 ± 0.20 meq/100 g. Magnesium levels are also very low, at 1.71 ± 1.33 meq/100 g. This element does not show any desaturation. Furthermore, we note that there is a very significant positive correlation between the pH and the Mg^{2+} concentrations. The correlation coefficient is 0.65.

From this, it is concluded that the exchangeable bases show acceptable exchangeable potassium (K^{+}) values for all soil samples with threshold values of 0.15-0.25 meq/100 g. On the other hand, there was a marked magnesium deficiency (Mg^{2+}) in the soil samples with values below the reference values of 1.5-3 meq/100 g.[26–28] These results are similar to those of other studies that have shown that increasing the concentration of potassium in a nutrient solution decreases magnesium absorption. [29,30] The results for the different parameters studied are shown in Table 3.

Table 3: Physico-chemical analyses of the studied soils

Stations	CE (µS/cm)	CaCO3 total (%)	T(C°)	Humidity %	pH	O.M (%)	Ca^{2+}	Mg^{2+}	Na+	k+
Fez	760.00	18.50	13	55.00	7.16	1.22	67.15	1.07	10.00	0.15
Taoujdat	1523.00	35.11	11.00	55.00	7.90	1.02	97.68	2.88	11.67	0.27
Meknes	1485.00	19.90	11.00	88%	7.82	1.08	82.34	0.27	7.40	0.77
Elhajeb	1490.00	41.07	5.00	78%	8.50	2.14	89.33	3.50	8.59	0.39
Sefrou	1376.00	19.20	9.00	86.00	8.05	2.50	70.40	1.67	4.04	0.54
Azzaba	1247.00	50.70	9.00	86.00	7.90	1.94	57.24	1.84	2.03	0.12
Bir tamtam	520.00	35.30	9.00	86.00	7.69	2.05	51.33	0.80	7.96	0.23
Ifrane	518.00	20.00	9.00	86.00	8.65	0.56	63.72	3.90	3.42	0.56
Guigou	716.00	24.57	7.00	45.00	8.50	0.34	67.38	3.20	1.20	0.62
Boulemane	1378.00	19.87	7.00	45.00	7.30	1.89	70.89	0.70	6.00	0.30
Outat El Haj	1380.00	19.87	8.00	21.00	8.23	1.89	71.85	0.50	5.33	0.31
Tahla	736.00	19.87	10.00	50.00	8.03	1.89	69.89	0.20	3.22	0.20

Generic composition

The yeast population's total density in the Fez-Meknes region's studied soils was around 2.06 x 10^6 CFU/g dry soil weight with a mean of 17 x 10^4 CFU/g. The maximum was observed in Azzaba with a density of about 32.5 (10^4 CFU/g) dry soil weight, and the minimum was 5.2 x 10^4 CFU/g dry soil weight in Outat Elhaj.

The present study shows that the twelve different soils are not similar in terms of yeast density. Figure 2 shows the three groups: The first group includes Fez, Sefrou, Azzaba, and Bir Tam Tam, with an average density of 26.5 x 10^4 CFU/g. It is Followed by Ain Taoujdat, Meknes, Ifrane, and El Hajeb, with an average density of 17.8 x 10^4 CFU/g. In addition, the 3rd group includes the stations Guigou, Boulemane, Outat Elhaj with an average of 56.3 x 10^3 CFU/g.

This result shows that the yeast density depends on the soil's physical, chemical, and soil properties. This was pointed out a long time ago in a study by Pumpyanskaya [31], who showed that yeast density depends on these same soil properties.[32]

From the different colony aspects obtained on the enumeration plates, 86 yeast isolates were isolated from the twelve soil samples studied. The samples of Azzaba, Bir Tamtam, and Sefrou alone yielded a total of 58 yeast isolates. These isolates were subjected to several tests for their identification.[33]

The results of these tests showed that macroscopic observation of the colonies of the different strains grown on malt-yeast-glucose-peptone agar revealed many morphological characteristics in terms of shape, color, appearance, contour, and consistency. Microscopic examination of the cells of the different strains revealed mainly two types of shapes: round and irregular. However, it was found that strains can change their shape from round to irregular, depending on whether they are grown on liquid or solid media.

Biochemically and metabolically, the tests showed that all isolates were able to grow at temperatures between 25 and 40°C and were resistant to high osmotic pressures.

A 73% of the isolated strains showed a positive reaction for urease, and the time of urea hydrolysis depended on the strain and varied from one to three days. On the other hand, not all yeast isolates produce the acids and are unable to reduce Tetrazolium.

The assimilation test of the different types of sugar, used as a carbon source by yeast, revealed that these microorganisms could assimilate 100% glucose, sucrose, and maltose. For the other sugars (Trehalose, Manose, Arabinose, Raffinose, Xylose, and Sorbitol), the assimilation and fermentation depend on the type of strain).

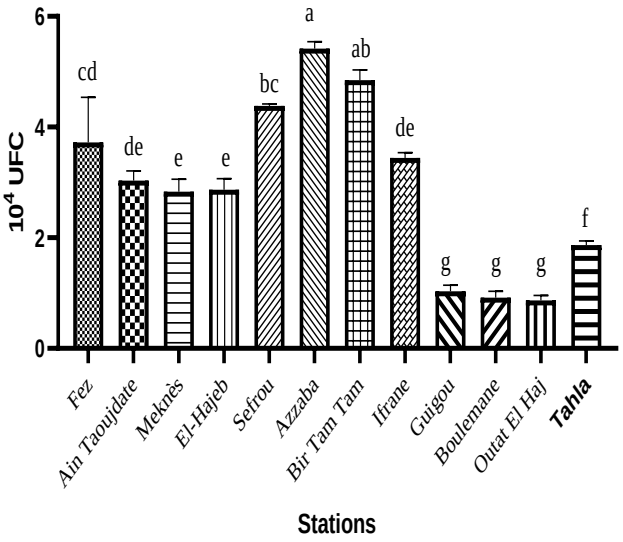

Figure 2: Yeast density at different stations in the Fez-Meknes region.

Tables 4 and 5 show the identification results of these different genera.

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The soil variability in the Fes-Meknes region has led to functional diversity among yeasts, and this variability results in diversity across taxonomic entities.[34]

Soil yeasts are taxonomically diverse and possess extraordinary adaptations that allow them to survive in a wide range of environmental conditions. In this study, the composition of the soil yeast community is diverse and consists of genera: *Rhodothorula* (49%), *Candida* (17%), *Rhodosporudium* (15%), *Pichia* (10%), *Trichosporon* (8%), and *Exophiala* (1%) (Figure 3).

Basidiomycetes dominate these soils, among the most numerous fungal operational taxonomic units *Rhodotorula*, *Trichosporon*, and *Rhodosporudium*. These genera are among the most frequently reported soil species. [2,32,35]

The genus *Rhodotorula* showed a ubiquitous distribution, as it is the dominant genus in most soils of the Fez-Meknes region. This genus can be used as an indicator of environmental quality, mainly as a bioindicator of heavy metal disturbances in nutrient-poor soils. [36] It is associated with the genera *Pichia* and *Candida*, of which several studies have reported the presence of these ascomycete and basidiomycete genera in hydrocarbon-polluted soils. [37] Competition for nutrients such as a carbon source impacts the species composition of soil microbial communities. *Candida tropicalis* emerged as a dominant n-alkane-using microbe when soil microcosms containing inocula from oil-contaminated soils were treated with a mixture of n-alkanes. [38]

Exophiala, which is considered "black yeast," has also been isolated from hydrocarbon-polluted soils in small quantities.[39] This low amount of black yeast has been interpreted as evidence of the minor importance of this group of yeasts in soil functioning.[40–42] However, there is little information on exophytic species in nature. The almost perfect positive relationship between *Exophiala* and $CaCO_3$ (r=0.6577) as these yeasts are considered extremophiles. [43,44] Further examination of soil samples with a wide range of media may generate unique species and add to the list of yeasts that may participate in the active soil consortium. Similar studies have found a high proportion of potentially new species in mid-Atlantic hydrothermal fields and acid mine wastewater systems.[45]

In this study, the correlations obtained between yeast presence, soil type, EC, humidity, and nutrient availability explain the dominant yeast species' distribution and the interactions between these yeasts. These results are not similar to those of Starkey and Henrici.[46]

The principal component analysis was carried out with all the variables to determine the correlations between the different abiotic and biotic parameters. The two PCAs present the correlations between the measured parameters and the axes (C1 and C2), respectively. The two axes explain 51.29% of the information contained in the data matrix.

Table 4: Physiological tests of the genera

Strains	***Rhodothorula***	***Candida***	***Rhodosporudium***	***Pichia***	***Trichosporon***	***Exophiala***
Urease	++	--	++	--	++	++
Acid production	--	--	--	- -	--	--
No vitamin	++	++	++	++	++	++
Amide formation	--		--	--	+/-	-/+
HCl	--	--	--	--	--	--
Resistance to 5% Glu-10% NaCl	+	--	--	--	--	--
25°C	++	++	++	++	++	++
28°C	++	++	++	++	++	++
30°C	++	++	++	++	++	++
40°C	++	++	++	++	++	++

Table 5: Assimilation and Fermentation of sugars by genera

Strain sugars	***Rhodothorula***		***Candida***		***Rhodosporudium***		***Pichia***		***Trichosporon***		***Exophiala***	
	Assimilation %	Fermentation %	Assimilation %	fermentation %	assimilation%	fermentation %	assimilation%	fermentation %	assimilation %	fermentation %	Assimilation %	fermentation%
Glucose	100	0	100	100	100	0	100	100	100	0	100	0
Saccharose	100	0	100	85	100	0	100	86	100	0	100	0
Maltose	100	0	100	86	100	0	100	84	100	0	100	0
Trehalose	11	0	12	2	9	0	7	1	5	0	7	0
Manose	11	0	0	0	10	0	2	0	15	0	12	0
Arabinose	12	0	0	0	12	0	0	0	14	0	12	0
Raffinose	4	0	8	0.75	2	0	6	0.25	2	0	3	0
Xylose	14	0	14	2	10	0	15	1	6	0	10	0
Sorbitol	10	0	11	8	11	0	9	7	9	0	10	0

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On the first PCA of a matrix "stations-biodiversity-physico-chemical characteristics," it appears clear that the negative correlation is strong between the stations El-Hajeb, Ain Taoujdat, and Fez, which is represented in the first axis are related to the nature of the substrate. On the other hand, the Ifrane and Guigou stations are positively correlated to the C1 axis, which is explained by the similarity of the generic composition of yeast in these two stations (*Pichia*, *Trichosporon*, *Candida*, and *Rhodosporudium*) (Figure 4).

The Sefrou and Azzaba stations are positively correlated with the C2 axis, which is explained by the dominance of the yeast genera (*Rhodotorula*, *Candida*, and *Exophiala*) in these two stations whereas the other stations are rather distributed on the negative ordinate (Figure 4).

The stations were classified according to their biotic and abiotic similarity by hierarchical clustering using hierarchical ascending classification (HAC). According to the dendrogram, two clusters of stations were identified (Figure 5).

The linear correlations obtained for the 20 variables studied are represented in Figure 6, where the critical values of Pearson's r correlation coefficient are indicated for $p<0.05$.

The Bravais-Pearson correlation test showed that the genus *Rhodotorula*, the most dominant in the soil of the Fez-Meknes region with an average of 8.4 x 10^4 cfu/g, presents an almost perfect negative relation with *Trichosporon* (r= -0.608) (p=0.02). *Rhodotorula* is present in nearly all stations; It is very dominant in the sample of Azzaba with a value of about $2.2*10^5$ cfu/g. On the other hand, the population was very low or null in Ifrane and Guigou.

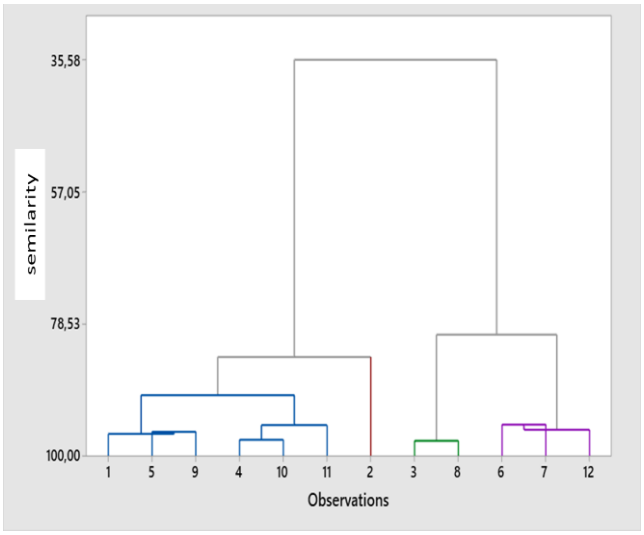

Figure 5: Ascending dendrogram showing the four groups obtained. **1:** Ain Taoujdate. **2:** El-Hajeb. **3:** Meknes. 4: Boulemane. **5:** Outat El-Haj. **6:** Sefrou. **7:** Azzaba. **8:** Bir Tam-Tam. **9:** Ifrane. **10:** Fez. **11:** Guiguou. **12:** Tahla

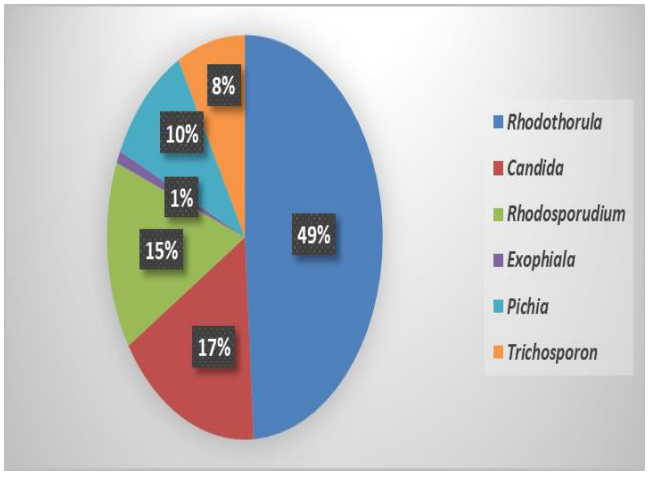

Figure 3: Generic composition of yeasts from the Fez-Meknes region.

There was a very significant positive correlation between *Pichia and Trichosporon* (r= 0.866) (p=0.000). These two genera present a maximum value at the level of the soil of the station of Ifrane $6.7*10^5$ UFC/g and $4*10^4$ UFC/g, respectively. The correlation coefficient r reveals a positive direction, i.e., the two genera develop concomitantly and have the same preferences (Figure 6).

Pichia showed a significant negative correlation with E.C (r=-0.720; p=0.008), this genus needs the mineral salts that are essential for growth in the soil. *Trichosporon* shows a positive correlation with silt (r=0.67; p=0.01), and a negative relationship with M.O (r=-0.57, p=0.04) and C.E (r=-0.58; p=0.04).

On the one hand, the genus Candida is dominant in the soil of Ifrane station with a value of 8.105 cfu/g. On the other hand, it is absent at El-Hajeb station. The *Candida* genus correlates positively with *Exophiala* (r= 0.689; p= 0.01), humidity (r= 0.84; p=0.000) and an almost perfect negative correlation with Ca^{2+} (p=0.029; r=-0.628).

The genus *Exophiala* appeared about $3.6*10^4 \pm 7.2*10^3$ CFU/g. The maximum value of the biomass is recorded in Azzaba ($2*10^4$ CFU/g). Furthermore, an almost perfect positive relationship between *Exophiala* and CaCO3 (r=0.6577) and moisture (r=0.666; p=0.01).

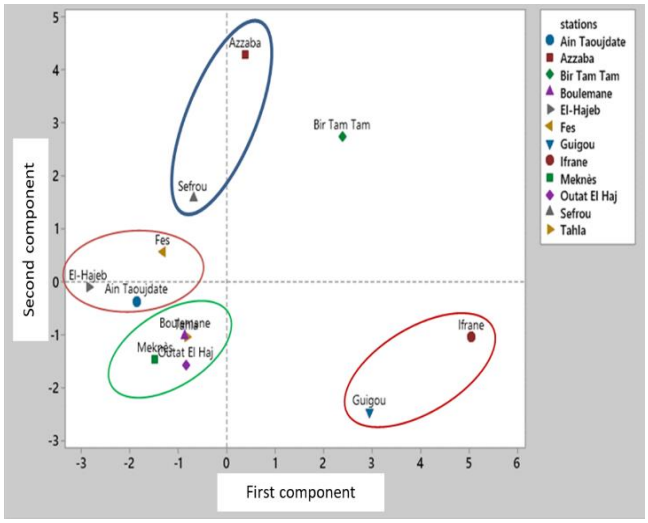

Figure 1: Principal component analysis in the C1-C2 plane showing the stations

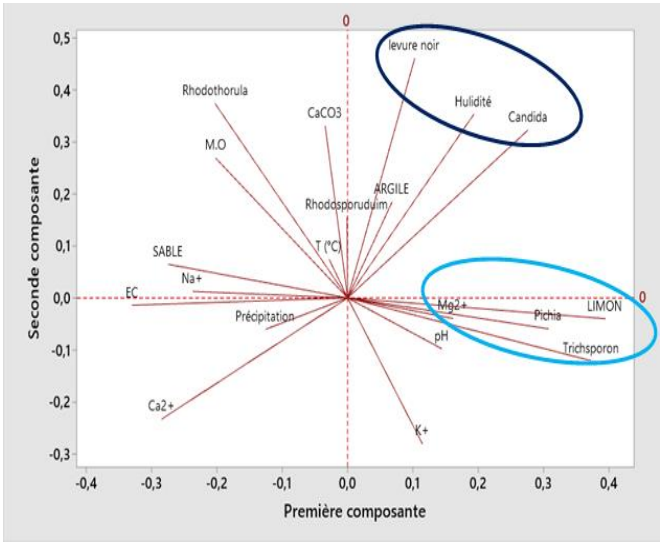

Figure 6: Principal component analysis in the C1-C2 plane presenting the correlations of the physico-chemical parameters of the soil and the yeast genera

Biostatistical analysis of all the factors studied resulted in a negative correlation between the genus *Trichosporon* and soil organic carbon content (r=-0.57). In contrast, other research has found a positive correlation between soil yeast population and soil organic carbon content. [47] This correlation between the genus *Trichosporon* and organic matter is explained by the involvement of *Trichosporon* in the degradation of organic matter. These results are consistent with some studies showing indirectly that the yeast *Trichosporon* increases during the decomposition of forest litter.[48]

Conclusion

This work carried out a biotypological study of the cultivable yeast community of hydrocarbon-polluted soils in the Fez-Meknes region, Morocco. To the best of our knowledge, this is the first detailed research on biotypology of yeast in soils polluted by hydrocarbons in Fez-Meknes region. The composition of the yeast community in the soils consists f six genera: *Rhodothorula, Candida, Rhodosporudium, Pichia, Trichosporon,* and *Exophiala*. Furthermore, the results showed that yeast biodiversity is significantly correlated with the tested soils' organic matter, $Ca^{2+,}$ and Na^{2+} contents. Similarly, a correlation was found with the type of sandy soil. The environmental dynamics of these soils seem to keep the yeast communities stable and robust, hardly influenced by changes in soil physico-chemical factors. This study provided an accurate indication of the ecological niche, which, however, awaits further exploration.

Conflict of Interests

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that they will bear any liability for claims relating to the content of this article.

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