



Keywords

Rock Phosphate-Solubilizing
Bacteria,
Wheat,
Rhizosphere Soil,
Density

Received: February 17, 2017

Accepted: March 17, 2017

Published: June 6, 2017

Occurrence of Phosphate Solubilizing Bacteria in the Rhizosphere of *Triticum aestivum* L. from Meknes, Morocco

Rfaki Abderrazak, Nassiri Laila, Ibijbjen Jamal*

Soil and Environment Microbiology Unit, Faculty of Sciences, Moulay Ismail University, Meknes, Morocco

Email address

jamal_ibijbjen@yahoo.fr (I. Jamal)

*Corresponding author

Citation

Rfaki Abderrazak, Nassiri Laila, Ibijbjen Jamal. Occurrence of Phosphate Solubilizing Bacteria in the Rhizosphere of *Triticum aestivum* L. from Meknes, Morocco. *American Journal of Microbiology and Biotechnology*. Vol. 4, No. 1, 2017, pp. 1-7.

Abstract

The aim of present research is to study the relationship between density of rock phosphate-solubilizing bacteria population with physical, chemical and biological properties of the rhizosphere soil of wheat (*Triticum aestivum* L.). The native population of phosphate solubilizing bacteria (PSB) and total soil bacteria (TB) were enumerated in 132 samples of rhizosphere soil collected from fourteen agricultural areas at Meknes. The results revealed that the bacteria solubilizing rock phosphate are present in all soils analyzed in this study. The number of PSB and TB showed large variations and ranged from 12.5 – 0.002; 150 – 0.52 ($\times 10^5$ CFU/ g soil), respectively within the place of sampling. The correlations between PSB counts and TB ($r=0.82$), total nitrogen ($r=0.66$) and organic matter ($r=0.83$) were positive and significant ($p \leq 0.01$). This research extends the knowledge on rock phosphate-solubilizing bacteria population in the rhizosphere of wheat cultivated in the different regions at Meknes.

1. Introduction

It is well known as the interaction of many factors, such as the physical and chemical properties of the soil, vegetation, the rotation of crops and environmental conditions greatly influence the amount and the composition of the microflora of the soil [1, 2]. The PSB populations depend on the physical and chemical properties of the soil and agricultural practices [3]. Also, Nahas [4] reported that diversity and density of the population of PSB and their bio-activity varied from soil to another according to their nutritional status [carbon (C), nitrogen (N) and phosphorus (P)], and their own effectiveness in phosphate solubilization. Phosphorus (P) is an essential element of energy metabolism of all life forms. In fact, it is essential for living cells, as it is a main component of DNA, RNA, ATP, and phospholipids. In addition, he is consequently involved in cell division, the transmission of genetic information, the energy transfer/storage, respiration, the photosynthetic system, the formation of nodules, nitrogen fixing and the production of oils and sugars [5-8]. Moreover, all these properties make this element one of the three macronutrients necessary for the growth and development of plants, next to the nitrogen (N) and potassium (K) commonly found in chemical fertilizers [9]. Thus, the highest density of populations of PSB is located in agricultural and rangeland soils [10]. Furthermore, Katiyar and Goel [11] proved that the

abundance of PSB at soil level depended on the plant species, microbial soil composition, and the soil conditions. Studies based on molecular techniques have estimated more than 4,000 microbial species per gram of soil [12]. Number of PSB among total phosphate solubilizing microorganisms (PSM) in north Iranian soil was found as 88% and the PSB counts ranged from 0 to 10^7 cells g^{-1} soil, with 3.98% population of PSB among total bacteria [13]. The present study was aimed to looking for the density of PSB in the rhizosphere of wheat (*Triticum aestivum*) grown in different regions of Meknes and also examined the relationship between density of rock phosphate-solubilizing bacteria population with physical, chemical and biological properties of the rhizosphere soil.

2. Materials and Methods

2.1. Soil Sampling

Between April and May 2014, soil samples were collected from the rhizosphere of *T. eastivum* in fourteen agricultural areas in Meknes (Table 1). We dug approximately 5 to 10 cm around the plant and 15 to 20 cm in depth in the soil to extract the plant and its root system. Then, portions of the soil surrounding the root system were collected, the same procedure was repeated six times. Then, all of the incremental samples were mixed for a homogeneous sample representative of the study site. Next, the samples were stored immediately in a portable cooler at 4°C and brought to the laboratory for physical, chemical and microbiological analysis.

2.2. Soil Analysis

The organic matter was determined by dosage of organic carbon using the potassium dichromate oxidation method [14], the available P was determined by the colorimetric assay [15] after chemical extraction by Olsen method [16] and total nitrogen was estimated by the Kjeldahl digestion method [17]. The cation exchange capacity (CEC) was determined by Metson method [18] and the pH was measured by a pH meter equipped with glass electrode with soil / distilled water ratio (1 / 2.5).

2.3. Bacterial Enumeration

To enumerate total bacteria (TB) and phosphate solubilizing bacteria (PSB) in the rhizosphere soil, about 1 g of soil was weighed and transferred into a 250 ml Erlenmeyer flask with 10 ml of a phosphate buffer solution [19]. Subsequently, the solution obtained was stirred at 150 rpm for one hour. After incubation, a series of dilutions of 10^{-2} , 10^{-3} , 10^{-4} and 10^{-5} was prepared; thereafter, 100 μ l aliquots of each dilution were plated on a suitable culture medium. For the enumeration of total bacteria (TB), the PCA medium was used (Plate Count Agar) [20]. The most suitable medium for the enumeration of PSB is the NBRIP [21], supplemented with 5 g L^{-1} rock phosphate powder from the phosphate mine

of Khouribga (RPK). Rock phosphate was composed with: O, 56.53%; F, 2.42%; Na, 1.81%; Mg, 1.94%; Al, 2.03%; P, 9.37%; S, 0.77%; Sn, 0.12%; Ca, 16.35%; Fe, 0.60% [22]. Before use, the RPK was carefully washed with the extraction solution Mehlich 3 [23], and several times with hot distilled water to remove all traces of available P; then it was autoclaved and added to the medium of sterile culture as the only source of P. The number of bacteria per gram of soil was determined by the standard method of colony forming unit (CFU) [1] after 3 days of incubation at $28 \pm 2^\circ C$. Every time, 100 μ g ml^{-1} of the cycloheximide was added to the culture medium to inhibit the growth of fungi.

2.4. Statistical Analysis

All statistical analyses were performed using the software IBM SPSS Statistics 20.0. The homogeneity of variances was tested for all variables by using the statistic of Levene. To find out if there is a significant correlation between the number of PSB and physical, chemical and biological properties of our soil samples (Total soil bacteria, pH, organic matter, available P, total nitrogen and CEC), a multiple correlation test was performed. As well, the results of all experiments were submitted to analysis of variance (ANOVA). Also, whenever the calculated F Fisher was significant ($P < 0.05$), the test of significant difference with the honesty of Tukey (HSD), was used to compare means.

3. Results and Discussion

3.1. Physical and Chemical Properties of Soil Samples

In the different sampling sites of rhizosphere soil, the results showed that the pH varies between 6.03 and 8.52 in all prospected stations (Table 1), showing a remarkable alkalinity of agricultural soils studied, which could be explained by the calcareous rock substrate constituting the plateau of Meknes-Sais. Thus, the results of organic matter in soils studied show that 79.5% of the analyzed soils are rich in the organic matter according to standards of the spatial database LUCAS [24]. This varies from 10.94 to 45.47 g.kg $^{-1}$ of rhizospheric soil (Table 1); This can be explained by the residue of plants left on the soil surface after each harvest. Furthermore, this result is a positive sign for the multiplication of microorganisms in agricultural soils in the region of Meknes, because heterotrophic organisms, such as phosphate solubilizing bacteria need a source of carbon and energy for both the synthesis of new materials of the cell and for the oxidation of carbon compounds [25].

Also, the values of the cation exchange capacity (CEC) vary between 3.96 to 81.26 cmol (+) / kg. In addition, the analysis of the results of the CEC shows that 51.13% of prospected soils reveal a great capacity for retention of the nutrient cations and only 5.7% of soils show a very low capacity for nutrient retention (Table 1). Likewise, the results of available phosphorus (P) in the studied soils show that

about 70.5% holding a high concentration, while 29.5% of soils have a low concentration according to the chemical standards of arable land in the European Union [26]. Thus, the higher content of available P (51.56 mg.kg⁻¹) is found at the station Seba Ayoun (Station (6)), while the lower content of available P (9.92 mg.kg⁻¹) is recorded in the station

Moulay Idriss Zerhoun (station (12)) (Table 1). Despite this positive result, the problem that remains is that the majority of the phosphorus added in soluble form is adsorbed by the calcium present in the complex exchange at the alkaline soils [27] and consequently becomes unavailable to plants.

Table 1. The physical and chemical characteristics of rhizosphere soil samples of *T. aestivum* collected from 14 sites in the region of Meknes and used for the enumeration of PSB.

No.	Sampling site	Location	pH	organic matter (g.kg ⁻¹)	available P (mg.kg ⁻¹)	Total N (g.kg ⁻¹)	CEC (Cmol (+)/kg)
1	EL-Haj Kaddour (9)*	33°49'18N; 005°25'31W	7,48±0,10 ^{abc}	22,49±1,15 ^{cd}	21,54±0,56 ^e	0,61±0,02 ^{cd}	55,50±1,03 ^{ab}
2	El-Hajeb (12)	33°39'45N; 005°21'21W	8,13±0,04 ^a	45,47±0,69 ^a	40,81±0,77 ^{bc}	1,69±0,03 ^a	20,99±0,36 ^d
3	Kantina (12)	33°41'23N; 005°31'37W	8,10±0,05 ^{ab}	27,41±0,63 ^c	29,46±0,64 ^d	0,71±0,03 ^c	20,14±0,78 ^d
4	Bouderbala (12)	33°49'55N; 005°16'09W	7,98±0,06 ^{abc}	44,30±0,55 ^a	41,85±1,38 ^{bc}	0,52±0,05 ^{cde}	12,29±0,89 ^d
5	M'haya (9)	33°57'44N; 005°13'42W	8,11±0,05 ^a	14,87±0,10 ^{ef}	10,25±0,71 ^f	0,54±0,02 ^{cde}	33,93±1,61 ^c
6	Seba Ayoun (6)	33°54'27N; 005°26'35W	7,58±0,09 ^{abc}	19,79±0,15 ^{de}	51,56±0,58 ^a	1,00±0,01 ^b	18,59±0,56 ^d
7	Ait Hammad (9)	33°52'46N; 005°09'14W	6,03±0,18 ^d	43,98±0,74 ^a	37,48±1,11 ^c	1,19±0,02 ^b	17,56±0,93 ^d
8	Rass Jerry (12)	33°46'06N; 005°45'17W	7,38±0,08 ^{abc}	10,92±0,10 ^f	21,38±0,98 ^f	0,32±0,01 ^e	15,39±0,91 ^d
9	Oued Beht (6)	33°52'15N; 005°53'46W	7,62±0,02 ^{abc}	41,15±1,14 ^{ab}	31,87±0,67 ^d	1,09±0,05 ^b	56,30±1,11 ^{ab}
10	Agourai (6)	33°37'32N; 005°38'41W	7,51±0,09 ^{abc}	36,18±0,58 ^b	18,83±0,38 ^e	0,54±0,04 ^{cde}	47,89±1,43 ^b
11	Ain El Orma (9)	33°54'02N; 005°46'11W	7,06±0,06 ^c	10,94±0,78 ^f	17,39±0,85 ^e	0,38±0,03 ^{de}	61,28±1,02 ^a
12	Moulay Idriss Zerhoun (12)	34°01'48N; 005°34'33W	8,11±0,05 ^a	14,87±0,10 ^{ef}	9,92±0,31 ^f	0,52±0,03 ^{cde}	35,93±1,66 ^c
13	Ain Jemaa (6)	33°59'03N; 005°41'39W	7,53±0,06 ^{abc}	24,02±1,20 ^{cd}	11,00±0,09 ^f	0,62±0,04 ^c	11,16±0,88 ^d
14	Dar Oum Soltane (12)	33°53'56N; 005°38'50W	6,56±0,21 ^{bc}	21,81±1,44 ^{de}	45,15±1,61 ^b	0,87±0,04 ^c	12,66±0,86 ^d

The results represent the mean of (n) repetitions * ± standard deviation (SD). The different letters in the same column indicate significant differences (p < 0,05, Tukey's HSD test).

Moreover, Sharpley [28] showed that generally, a few days after fertilization, available phosphorus levels can reach similar values to those before the application. Similarly, Shen *et al.* [29] showed that only 15-25% of the synthetic phosphate fertilizer applied to overcome the deficiency of P element, remain available to plants, the rest becomes unavailable. It is for this reason that the levels of available P must be completed on most agricultural soils by adding phosphate fertilizers, which not only constitute an important part of the cost of agricultural production but also impose adverse environmental impacts on the overall health of soil and increase the degradation of terrestrial ecosystems, freshwater and marine resources [30]. In addition, in agricultural soils, the dissolution of inorganic phosphate immobilized by the complexes of exchange, is closely related to the activity of soil microorganisms [31]. So, we must think seriously of strategies involving phosphate solubilizing microorganisms to improve the performance of agricultural systems [32].

On the other hand, the results for the total nitrogen content (N), by the standards of the European Union [26], show that 89.8% of the analyzed soils are poor, which requires a reasonable nitrogen fertilization in this study area. Indeed, the highest levels of total nitrogen were found at the El-Hajeb station (Station (2)) with 1.69 g.kg⁻¹ of soil, while the lowest total N content is recorded in the Ain El Orma stations

(Station (11)) with 0.38 g.kg⁻¹ of soil (Table 1). However, the Environmental Protection requires the use of sustainable management practices, making use of some chemical inputs. Moreover, the application of fertilizers is always performed without consideration of the microorganisms present in the rhizosphere, which leads to excessive and often harmful applications.

3.2. Enumeration of PSB and Total Soil Bacteria (TB)

The quantitative composition of total bacterial populations and phosphate solubilizing, and the percentage of the BSP relative to total bacteria in the various stations prospected, are presented in Table 2. The results show that bacteria dissolving rock phosphate, are present in the 132 soils analyzed in this study. Furthermore, Levene method was used to check the homogeneity of variance of the results obtained; this test was significant, that is in other words, the variances in different groups are not equal. Then, we proceeded to the analysis of variance by the one-way ANOVA was performed on the count data of total bacteria, phosphate solubilizing bacteria and percentage of PSB relative to total bacteria (Table 2); the results indicate that there is an overall difference in the content TB, PSB and PSB percentage in different rhizospheric soils of *T. aestivum* (Table 3).

Table 2. Number of total bacteria and PSB ($\times 10^5$ CFU g^{-1} of soil) in soil samples collected from the rhizosphere of *T. aestivum* cultivated in different localities of Meknes.

No.	Sampling site	TB	PSB	% PSB
1	EL-Haj Kaddour (9)*	58,10 $\pm$ 3,05 ^b	0,20 $\pm$ 0,01 ^d	0,35 $\pm$ 0,01 ^f
2	El-Hajeb (12)	50,70 $\pm$ 2,71 ^b	3,55 $\pm$ 0,19 ^a	7,00 $\pm$ 0,47 ^a
3	Kantina (12)	9,94 $\pm$ 0,61 ^d	0,13 $\pm$ 0,01 ^e	1,37 $\pm$ 0,05 ^{de}
4	Bouderbala (12)	88,00 $\pm$ 4,93 ^a	1,98 $\pm$ 0,18 ^b	2,25 $\pm$ 0,11 ^c
5	M'haya (9)	5,94 $\pm$ 0,39 ^e	0,03 $\pm$ 0,00 ^g	0,55 $\pm$ 0,01 ^{ef}
6	Seba Ayoun (6)	1,63 $\pm$ 0,12 ^h	0,04 $\pm$ 0,00 ^{fg}	2,52 $\pm$ 0,06 ^c
7	Ait Hammad (9)	37,80 $\pm$ 2,43 ^c	1,95 $\pm$ 0,15 ^b	5,15 $\pm$ 0,11 ^b
8	Rass Jerry (12)	1,77 $\pm$ 0,10 ^h	0,002 $\pm$ 0,05 ^h	0,12 $\pm$ 0,02 ^f
9	Oued Beht (6)	7,13 $\pm$ 0,44 ^e	0,48 $\pm$ 0,04 ^c	6,76 $\pm$ 0,62 ^a
10	Agourai (6)	1,96 $\pm$ 0,12 ^h	0,04 $\pm$ 0,00 ^{fg}	2,20 $\pm$ 0,06 ^{cd}
11	Ain El Orma (9)	0,98 $\pm$ 0,06 ⁱ	0,002 $\pm$ 0,00 ^h	0,23 $\pm$ 0,01 ^f
12	Moulay Idriss Zerhoun (12)	4,50 $\pm$ 0,32 ^f	0,23 $\pm$ 0,02 ^d	5,13 $\pm$ 0,23 ^b
13	Ain Jemaa (6)	1,71 $\pm$ 0,14 ^h	0,11 $\pm$ 0,00 ^e	6,31 $\pm$ 0,07 ^a
14	Dar Oum Soltane (12)	3,41 $\pm$ 0,21 ^g	0,04 $\pm$ 0,00 ^f	1,30 $\pm$ 0,43 ^e

The results represent the mean of (n) repetitions $\pm$ standard deviation (SD). The different letters in the same column indicate significant differences ($p < 0,05$, Tukey's HSD test). TB: total bacteria; PSB phosphate solubilizing bacteria; % PSB: percentage of PSB compared to TB.

Table 3. Analysis of variance for the number of total bacteria, phosphate solubilizing bacteria and for the percentage of PSB in the rhizosphere soil of *T. aestivum*.

		Sum of squares	ddl	Average square	F	Signification
TB	Intergroup	16,974	13	1,306	1142,367	0,000
	Intragroup	0,032	28	0,001		
	Total	17,006	41			
PSB	Intergroup	37,666	13	2,897	1589,456	0,000
	Intragroup	0,051	28	0,002		
	Total	37,717	41			
% PSB	Intergroup	253,085	13	19,468	230,087	0,000
	Intragroup	2,369	28	0,085		
	Total	255,454	41			

TB: total bacteria; PSB phosphate solubilizing bacteria; % PSB: percentage of PSB compared to TB.

The results of the enumeration reveal an abundance of total bacteria ranging between 0.98 and 88×10^5 CFU g^{-1} of rhizospheric soil, recorded respectively in the Ain El Orma stations (Station (11)) and Bouderbala (station (4)). Furthermore, the number of bacteria dissolving rock phosphate varied between 0.002 and 3.55×10^5 CFU g^{-1} soil, respectively in the two stations Ain El Orma (Station (11)) and El-Hajeb (Station (2)). The percentage of PSB to total bacteria varied between 0.12% and 7.0%, recorded successively in the Rass Jerry stations (Station (8)) and El-Hajeb (Station (2)). It, therefore appears that there is a great variation by geographic sampling site. Thus, the number of total bacteria, found in all samples analyzed, is well inserted in the theoretical and practical limits indicated in agricultural soils [33], situated between 10^4 - 10^9 CFU g^{-1} of soil. Moreover, similar results were recently reported by Fernández *et al.* [34], who found in Argentine a number between 6 - 14×10^5 CFU g^{-1} of soil in farm fields under no-till management. Similarly, Aziz *et al.* [35] found in the experimental fields in Uruguay, under continuous and rotationally cropping a number between 22 - 660×10^5 CFU g^{-1} of soil. On the contrary, Vikram *et al.* [36] found a very low abundance of TB, ranging from 0.32 to 0.95×10^5 CFU g^{-1} soil in 66 samples of agricultural soil collected from eleven districts of northern Karnataka in India. Furthermore, we observed that the number of PSB in all our samples was still

well above 2×10^2 CFU g^{-1} of soil. Which was similar to results reported by several researchers, as in Kenyan soils [1], where the number of PSB ranged from 0.38 to 9.1×10^5 CFU g^{-1} of soil. Also, a number of PSB from 6×10^5 to 22×10^5 CFU g^{-1} of soil was recorded in soils of Henan Province in China [37]. Thus, an abundance of PSB which ranged from 7.33×10^5 to 14×10^5 CFU g^{-1} of soil in Namakkal district in India [38]. Similarly, in Tachira in Venezuela soils, the number found was 8 - 28×10^5 CFU g^{-1} of soil, with a percentage of PSB compared to TB from 3% to 13% [39].

In addition, at the rhizospheric soil of *T. aestivum* cultivated in four different regions in Mali, 2.6 to 6.86×10^5 CFU g^{-1} of the soil of PSB were obtained with a percentage of PSB from 7.52% to 30.26% [40]. However, other studies have reported higher values than ours, so from those found in the soils of northern Iran, where the number of PSB was 10^7 CFU g^{-1} of soil [13], in Uruguay 0.65 - 62×10^5 CFU g^{-1} of soil [35] in different parts of Haryana in India, 3 - 67×10^5 CFU g^{-1} of soil [41].

however, very poor results were reported as the ones of Peix *et al.* [42] who found an abundance of PSB less than 10^2 CFU g^{-1} of soil in the north of Spain; likewise, Fernández *et al.* [34] in Argentina, showed a low abundance located between 0.03 and 0.08×10^5 CFU g^{-1} of soil; of such way, Vikram *et al.* [36] found an abundance ranging from 0.01 to 0.18×10^5 CFU g^{-1} of soil in northern Karnataka in India.

Moreover, these results can be explained by the rhizosphere effect of root exudates of each variety of the same plant on microbial biodiversity of the soil, which causes preferential selection [43]. In this context, it was reported recently that microbial diversity in the rhizosphere of different plant species can be attributed to the fact that the plant-microbe interaction is highly dependent on soil conditions and plant genotype [44, 45]. Furthermore, Katiyar and Goel [11] showed that the abundance of PSB in soil depended on the plant species, the microbial composition of the soil, and soil conditions.

Also, Chiarini *et al.* [46] demonstrated that depending on the species of plants, some bacterial groups present in the rhizosphere were selected. Similarly, Kundu *et al.* [41] showed significant variation in the number and biodiversity of PSB depending on the species of plants and place of sampling. Again, Reyes *et al.* [47] reported that in different species of plants in the same soil type, cohabited different populations of PSB. Contrariwise, Fernández *et al.* [34] in Argentina showed that the number of PSB per gram of soil was not statistically different between the sampled sites, soil management programs or the seasons. Equally, Kucey [48] found no relationship between the type of vegetation and the number or percentage of BSP in all 17 tested soils in southern Alberta at Canada.

3.3. The Effect of Soil Properties on the PSB Population

The results of multiple correlation (Table 4) between the physical, chemical and biological properties of our soil samples (TB, pH, organic matter, total nitrogen, available P and CEC) and the number of PSB, demonstrate the existence of a significant positive correlation ($p < 0.01$) between the total number of bacteria ($r = 0.82$), organic matter ($r = 0.84$), total nitrogen ($r = 0.66$) and PSB population. Contrariwise, pH, available phosphorus and the CEC are not correlated ($p < 0.01$) with the PSB population. This agrees with the observations made by Vikram *et al.* [36] indicating the existence of a positive correlation between the total number

of bacteria ($r = 0.80$, $p < 0.01$), the organic carbon ($r = 0.40$; $p < 0.01$), the available nitrogen (N) ($r = 0.4$; $p < 0.05$) and the number of PSB, on the contrary the pH and available phosphorus showed no significant correlation with the number of PSB. Similarly, Ndung'u-Magiroy *et al.* [1] showed a positive and highly significant ($p < 0.001$) correlation between PSB and PSM populations ($r = 0.98$), exchangeable Ca ($r = 0.93$), exchangeable Mg ($r = 0.92$) and organic C ($r = 0.76$), while pH and extractable P did not correlate with the PSB population. However, Yahya and Al-Azawi [10] found no significant correlation between the number of PSB and organic matter in Iraqi soil. Thus, in our case, there is not a highly significant correlation between available P and the number of PSB. Likewise, no relationship was reported between the P content and the number of BSP in soils [1, 4, 10, 36, 48]. Contrarily, Fernández *et al.* [34] found that a change of 25 times of available P was associated with a less than 1.2 times change in the population of PSB. Similarly, Hu *et al.* [37] found a difference in the abundance of PSB in parcels under different fertilizer regimes and concluded that the supply of P increased the size of the population of the PSB.

Furthermore, we observed that the soil with a high organic matter and total nitrogen content contain more PSB and TB than other soils. This could be explained by the heterotrophic nutrition mode of PSB, requiring exogenous sources of organic matter to solubilize phosphate, and also for the synthesis of the new components of the cell and the oxidation of carbon compounds to elaborate cellular energy [25].

Also, John *et al.* [49] proved that the high content of soil organic matter decomposing, improving the most active and prolific soil microbes. More, Bashan *et al.* [50] revealed the existence of a positive correlation between the nitrogen content and organic matter with the bacterial viability in the soil, against they found no effect of pH of the soil, the concentration of phosphorus, potassium, the electrical conductivity and the C / N ratio, on the bacterial viability in the soil.

Table 4. Multiple correlations between the number of PSB and the physical and chemical characteristics of rhizosphere soil samples of *T. aestivum*.

	PSB	TB	pH	OM	P	N	CEC
PSB	1						
TB	0,821**	1					
pH	0,130	0,113	1				
OM	0,837**	0,662**	-0,035	1			
P	0,389*	0,338*	-0,250	0,491**	1		
N	0,664**	0,428**	-0,090	0,649**	0,614**	1	
CEC	-0,221	-0,123	0,069	-0,140	-0,416**	-0,202	1

** Indicates a significant correlation at ($p < 0.01$).

PSB: phosphate solubilizing bacteria; TB: total bacteria; pH: pH of soil; MO: organic matter; P: available phosphorus; N: total nitrogen; CEC: cation exchange capacity.

4. Conclusion

The results obtained show that the PSB are present in all our samples and the number of them was always well above 2×10^2 CFU g^{-1} of soil. Also, we found the existence of a

positively significant correlation ($p < 0.01$) between the number of PSB and the number of TB, organic matter and total nitrogen. In contrast, pH, CEC and available phosphorus, are not correlated with the population of PSB. However, the protection of the environment requires the use

of sustainable management practices, making use of fewer chemical inputs and taking into account the microorganisms present at the rhizosphere, which leads to more sustainable and eco-efficient applications.

References

- [1] Ndung'u-Magiroyi KW, Herrmann L, Okalebo JR, Othieno CO, Pypers P, Lesueur D (2012). Occurrence and genetic diversity of phosphate-solubilizing bacteria in soils of differing chemical characteristics in Kenya. *Ann. Microbiol.* 62:897–904. doi:10.1007/s13213-011-0326-2.
- [2] Jha DK, Sharma GD, Mishra RR (1992). Ecology of soil micro-flora and mycorrhizal symbionts. *Biol. Fert. Soils.* 12:272–278.
- [3] Kim KY, Jordan D, McDonald GA (1998). Effect of phosphate-solubilizing bacteria and vesicular-arbuscular mycorrhizae on tomato growth and soil microbial activity. *Biol. Fert. Soils.* 26:79-87.
- [4] Nahas E (2007). Phosphate solubilizing microorganisms: effects of carbon, nitrogen and phosphorus. In: Velazquez E, Rodriguez- Barrueco C (eds) *First Int Meet microbial phosphate solubilization*. Springer SBM, Dordrecht, pp 111–115.
- [5] Saber K, Nahla L, Ahmed D, Chedly A (2005). Effect of P on nodule formation and N fixation in bean. *Agron. Sustain. Dev.* 25:389–393.
- [6] Zaidi A, Khan MS, Ahemad M, Oves M (2009). Plant growth promotion by phosphate solubilizing bacteria. *Acta. Microbiol. Immunol. Hung.* 56:263–284.
- [7] Eftekhari G, Fallah AR, Akbari GA, Mohaddesi A, Allahdadi I (2010). Effect of phosphate solubilizing bacteria and phosphate fertilizer on rice growth parameters. *Iranian J. Soil Res. (Soil Water Sci).* 23:2.
- [8] Elser JJ (2012). Phosphorus: a limiting nutrient for humanity. *Curr. Opin. Biotechnol.* 23:833–838.
- [9] Liu Y, Chen J (2008). Phosphorus Cycle. In: S. E. Jørgensen and B. Fath (ed), *Encyclopedia of ecology*. Academic Press, Oxford, pp. 2715-2724.
- [10] Yahya AI, Al-Azawi SK (1989). Occurrence of phosphate-solubilizing bacteria in some Iraqi soils. *Plant Soil.* 117:135–141.
- [11] Katiyar V, Goel R (2003). Solubilization of inorganic phosphate and plant growth promotion by cold tolerant mutants of *Pseudomonas fluorescens*. *Microbiol. Res.* 158:163–168.
- [12] Montesinos E (2003). Plant-associated microorganisms: a view from the scope of microbiology. *Int. Microbiol.* 6:221–223.
- [13] Fallah A, Kargar A (2006). Abundance and distribution of phosphate solubilizing bacteria and fungi in some soil samples from North of Iran. In: 18th Congress of Soil Science. Available at: <http://www.ddd.go.th/18wcsc/techprogram/P19283.HTM>. Accessed 15 Aug 2013
- [14] Walkley A, Black A (1934). An examination of the Degtjareff method for determining soil organic matter and proposed modification of the chromic acid titration method. *Soil Sci.* 37: 29-37.
- [15] Murphy J, Riley JP (1962). A modified single solution method for the determination of phosphate in natural waters. *Anal. Chim. Acta.* 27:31–36.
- [16] Olsen SR, Cole CV, Watanabe FS, Dean LA (1954). Estimation of available phosphorus in soils by extraction with sodium bicarbonate. In: USDA Circular 939:1-19. Gov. Printing Office, Washington.
- [17] Trivedy RK, Goel PK, and Trisal CL (1998). *Practical methods in Ecology and Environmental media*, Series in methodology-2. Enviro Media publication. Postbox-60. Karad, 415110, India.
- [18] Metson AJ (1956). Methods of chemical analysis for soil survey samples. *NZ Soil Bur Bull* n°12.
- [19] Araújo WL, Lima AOS, Azevedo JL, Marcon J, Sobral JK, Lakava PL (2002). editors. *Manual isolation of endophytic microorganisms*. Department of Genetics - College of Agriculture "Luiz de Queiroz" - Piracicaba ESALQ – USP, Portuguese.
- [20] Atlas RM (2004) *Handbook of microbiological media*. CRC Press, Boca Raton, Florida.
- [21] Nautiyal CS (1999). An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. *FEMS Microbiol Lett.* 170:265–270.
- [22] Hamdali H, Hafidi M, Virolle MJ, Ouhdouch Y (2008). Rock phosphate solubilizing Actinomycetes: Screening for plant growth promoting activities. *World J. Microbiol. Biotechnol.* 24:2565-2575
- [23] Mehlich A (1984). Mehlich-3 soil test extractant: A modification of Mehlich 2 extractant. *Communications in Soil Science and Plant Analysis*, 15, 1409-1416.
- [24] Tóth G, Jones A, Montanarella L (Eds.) (2013). *LUCAS Topsoil Survey. Methodology, Data and Results*. JRC Technical Reports. Publications Office of the European Union, EUR26102–Scientific and Technical Research Series, Luxembourg, p. 141. doi:10.2788/97922.
- [25] Moat A G, Foster JW (1988). *Microbial Physiology*, 2nd edn. Wiley, New York, 597 pp.
- [26] Tóth G, Guicharnaud RA, Tóth, B, Hermann T (2014). Phosphorus levels in croplands of the European Union with implications for P fertilizer use. *Eur. J. Agron.* 55:42–52. doi:10.1016/j.eja.2013.12.008
- [27] Giroux M, Cantin J, Rivest M, Tremblay G (2002). L'évolution des teneurs en phosphore dans les sols selon leur fertilité, leur richesse en phosphore et les types de sol. Colloque sur le phosphore: une gestion éclairée. Drummonville, 6 Novembre.
- [28] Sharpley AN (1985). Phosphorus cycling in unfertilized and fertilized agricultural soils. *Soil. Sci. Soc. Am. J.* 49, 905–911.
- [29] Shen J, Yuan L, Zhang J, Li H, Bai Z, Chen X, Zhang W, Zhang F (2011). Phosphorus dynamics: from soil to plant. *Plant Physiol.* 156:997–1005.
- [30] Tilman D, Fargione J, Wolff B, D'Antonio C, Dobson A, Howarth R, Schindler D, Schlesinger WH, Simberloff D, Wackhamer D (2001). Forecasting agriculturally driven global environmental change. *Science.* 292:281–284.

- [31] Richardson AE (2001). Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants. *Aust. J. Plant Physiol.* 28:897-906.
- [32] Zaidi A, Khan MS, and Ahmad E (2014). *Microphos: Principles, Production and Application Strategies* In Khan MS, Zaidi A, Musarrat J (ed.) *Phosphate Solubilizing Microorganisms: Principles and Application of Microphos Technology*. Springer International Publishing Switzerland, pp 1-30.
- [33] Kennedy AC (1999). Bacterial diversity in agroecosystems. *Agric. Ecosyst. Environ.* 74:65–76.
- [34] Fernández LA, Agaras B, Wall LG, Valverde C (2015). Abundance and ribotypes of phosphate-solubilizing bacteria in Argentinean agricultural soils under no-till management. *Ann. Microbiol.* 65: 1667–1678. doi:10.1007/s13213-014-1006-9
- [35] Azziz G, Bajsa N, Haghighi T, Taulé C, Valverde Á, Igual JM, Arias A (2012). Abundance, diversity and prospecting of culturable phosphate solubilizing bacteria on soils under crop–pasture rotations in a no-tillage regime in Uruguay. *Appl. Soil. Ecol.* 61:320–6.
- [36] Vikram A, Alagawadi AR, Hamzehzarghani H, Krishnaraj PU (2007). Factors related to the occurrence of phosphate solubilizing bacteria and their isolation in Vertisols. *Int. J. Agric. Res.* 2 (7):571–580.
- [37] Hu J, Lin X, Wang J, Chu H, Yin R, Zhang J (2009). Population size and specific potential of P-mineralizing and -solubilizing bacteria under long-term P-deficiency fertilization in a sandy loam soil. *Pedobiologia.* 53(1):49–58. doi:10.1016/j.pedobi.2009.02.002
- [38] Ponmurugan P, Gopi C (2006). Distribution pattern and screening of phosphate solubilizing bacteria isolated from different food and forage crops. *J. Agron.* 5:600–604.
- [39] Reyes I, Valery A, Valdiz Z (2006). Phosphate-solubilizing microorganisms isolated from rhizosphere and bulk soil of colonizer plants at an abandoned rock phosphate mine. *Plant Soil.* 287:69–75.
- [40] Babana AH (2003). Mise au point d'un inoculant biologique pour le blé irrigué du Mali. Published PhD thesis. Faculté des sciences de l'agriculture et de l'alimentation de l'Université Laval Québec.
- [41] Kundu B, Nehra K, Yadav R, Tomar M (2009). Biodiversity of phosphate solubilizing bacteria in rhizosphere of chickpea, mustard and wheat grown in different regions of Haryana, Indian. *J. Microbiol.* 49, 120–127.
- [42] Peix A, Rivas-Boyer AA, Mateos PF, Rodríguez-Barrueco C, Martínez-Molina E, Velázquez E (2001). Growth promotion of chickpea and barley by a phosphate solubilizing strain of *Mesorhizobium mediterraneum* under growth chamber conditions. *Soil Biol. Biochem.* 33:103–110. doi:10.1016/S0038-0717(00)00120-6
- [43] Costa R, Götz M, Mroczek N, Lottmann J, Berg G, Smalla K (2006). Effects of site and plant species on rhizosphere community structure as revealed by molecular analysis of microbial guilds. *FEMS Microbiol. Ecol.* 56:236–249. doi:10.1111/j.1574-6941.2005.00026.x
- [44] Belimov AA, Puhalsky IV, Safronova VI, Shaposhnikov AI, Vishnyakova MA, Semenova EV, Nadezda YZ, Natalya MM, Walter W, Igor AT (2015). Role of plant genotype and soil conditions in symbiotic plant-microbe interactions for adaptation of plants to cadmium-polluted soils. *Water Air Soil Pollut.* 226:1–15. doi: 10.1007/s11270-015-2537-9
- [45] Schreiter S, Sandmann M, Smalla K, and Grosch R (2014). Soil type dependent rhizosphere competence and biocontrol of two bacterial inoculant strains and their effects on the rhizosphere microbial community of field-grown lettuce. *PLoS ONE* 9:e103726. doi: 10.1371/journal.pone.0103726
- [46] Chiarini L, Bevivino A, Dalmastrì C, Nacamulli C and Tabacchioni S (1998). Influence of plant development, cultivar and soil type on microbial colonization of maize roots. *Appl. Soil Ecol.* 8:11–18.
- [47] Reyes I, Bernier L, Simard R R, Tanguay P and Antoun H (1999). Characteristics of phosphate solubilization by an isolate of a tropical *Penicillium rugulosum* and two UV-induced mutants. *FEMS Microbiol. Ecol.* 28:291–295.
- [48] Kucey RMN (1983). Phosphate solubilizing bacteria and fungi in various cultivated and virgin Alberta soils. *Can. J. Soil Sci.* 63:671–678.
- [49] John L, Herms D, Stinner B, Hostink H (2001). Mulch effect on soil microbial activity, nutrient cycling, and plant growth in ornamental landscape. *Ornamental Plant Annual Report and Research Reviews*. The Ohio State University, Columbus
- [50] Bashan Y, Puente ME, Rodriquez MN, Toledo G, Holguin G, Ferrera-Cerrato R, Pedrin S (1995). Survival of *Azorhizobium brasilense* in the bulk soil and rhizosphere of 23 soil types. *Appl. Environ. Microbiol.* 61:1938–1945.