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Effect of Salinity on Microbial Biomass Behaviour

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ABSTRACT

This study is to assess the microbial biomass carbon behaviour of the saline soil in Naama's Sebkha using the fumigation extraction method. The results have shown a negative correlation between the increasing salinity and the soil microbial biomass carbon. The microbial biomass carbon ranged from 117 mg/kg in the non saline soil to 22 mg/kg for the high saline soil. In terms of the soil total organic carbon, this fraction decrease with an increasing salinity forming a rate not exceeding 0.47% in the high saline soil. The incubation of the soil under an increasing salinity conditions during 12 weeks without extern energetic sources, has showed the exponential decreasing of soil microbial biomass carbon level. The estimated kinetics parameters, using the single compartment model, showed that the half-life time not exceeding 4.05 weeks for the high saline soil and an increasing of mineralization rate constant with increasing salinity.
KEYWORDS: Algeria, microbial biomass, salinity, steppe, soil, half- life time.

INTRODUCTION

The salinity is the one of soil degradation processes in arid and semi arid regions, it affects 5% of cultivated soil in the world **[1]** and about 3.2 million hectares are affected by this phenomenon in Algeria **[2]**. An important number of scientific studies have been devoted the salinity role on the physical and chemical soil propriety, on the contrary, it's effect on the microbial biomass is relatively low. The microbial biomass is defined as the fraction of the living organic matter in the soil, forming between 2-4% in term of soil total organic carbon **[3]**, composed mostly by chimio-organotrophic organisms **[4]** distributed in the form of micro-colonies within the soil aggregates **[5]**. Many studies have demonstrated the negative salinity effect on soils microbiological properties **[6,7]**, it reduces the density and microbiological soil biodiversity **[8,9,10]**, decreasing soil respiration rate and microbial biomass carbon **[11]** .Other studies **[12]** showed that in the presence of high salts concentrations, numerous physiological processes are significantly affected, which are fundamental for the survival and maintenance of microbial biodiversity; for others **[13]** the high salinity levels slows the growth of many soil bacteria, disrupt the division and cell differentiation **[14]**, inhibit the synthesis of enzymes, ribosome, proteins **[15]**, reduce the nitrogenise activity and mycorrhizal infection **[16]**, decrease bacterial colonization **[17]**, disrupt the absorption of essential nutrients such as potassium and calcium, by substitution or competition through the membrane adsorption site **[18]**. At 5% of NaCl, fungal and bacterial populations decreased significantly **[19]** such as the microbial biomass carbon **[11,20,21]**.

For resisting to the salt stress, soil microorganisms developing an osmoadaptation system based on the equilibrium between the cytoplasm osmotic pressure and the external environment. The adjustment of the intracellular osmotic pressure is realized by the accumulation of the electrolyte and carbonate compounds in the cytoplasm for controlling the internal water activities, maintaining proper cell volume and protecting the structure and activity of biological macromolecules (enzymes, proteins, nucleic acids ...) against the salinity negative effects **[22,23,24]**.

The accumulation of electrolyte is realized by the increase selective membrane permeability of some ion like K and Ca level in the cytoplasm contents compared to the Na **[25]**, other microorganisms activate the synthesis and accumulation of various organic solutes, such as sugars **[26,27]**, glycerol **[28,29]** and some amino acids (glutamate, proline, glycine) **[30,31,32]**.

This study aims to quantifying the salinity effect on microbial biomass behaviour under natural and controlled conditions.

MATERIALS AND METHODS

The influence of salinity on the microbial biomass behaviour was studied in two aspects (in natural and under controlled conditions).

In natural conditions, the study was conducted in Sebkha of Naama region located in the West of Algerian steppe; it is a closed saline depression, with the average annual rainfall not exceeding 200 mm, and the ETP exceeds 800 mm per year, the minimum temperature can reach -1°C while the maximum temperature exceeds 35°C.

The soil samples were collected from the top horizon during the month of January; the sampling was conducted on along transect with increasing salinity (from the Sebkha periphery to the high saline soil of the depression).

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The physical and chemical properties of the soil were determined by standard methods: soil texture by the international Robinson pipette method, acidity by potentiometric method, the soluble salts are extracted and analysed from the saturated paste, the cation exchange capacity (CEC) quantified using the ammonium acetate, the total limestone by calcimeter Bernard method and the organic carbon content was determined using the Anne method.

The microbial biomass carbon was estimated by the fumigation extraction method, 25g of soil samples are fumigated by chloroform vapour for 24 hours at 25°C.

The samples were extracted with 0.5M of K_2SO_4 with a soil/solution ratio (1/5) by shaking for 1 hour at 200r/min; the carbon is determined by $K_2Cr_2O_7$; the unfumigated soils samples were extracted similarly at the start of experiment.

The microbial extractable carbon (E.C.) is equals the extra carbon extracted in the fumigated samples compared to the untreated control samples with chloroform.

Many studies **[33]** have confirmed that, regardless the type of soil, the extractable carbon is substantially constant proportion of the microbial biomass.

The microbial biomass carbon is calculated by the following formula:

$$C_{biomass}=2.64(C_{fumigated}-C_{unfumigated})$$

All values in this study are the average of three repetitions.

RESULTS AND DISCUSSION

1. In natural condition

The variation of the microbial biomass carbon is given in **Table n°1**. The pH and SAR shows a low variation in this study area; then, its interaction influence on the microbial biomass behaviour are mostly careless; therefore dynamics of this live fraction depends largely on the salinity effect.

Table n°1: Physicochemical characteristics of soils

	Soil texture (%)			$CaCO_3$ Total	pH	CE	CEC	Soil solution (mmol/l)				C_{org}	C_{bio}	C_{bio}/C_{org}
	Sand	Silt	Clay	(%)	1/2,5	dS/m	mmol/100g	Ca	Mg	Na	SAR	(%)	mg/kg	(%)
1	45.00	28.00	27.00	5.00	7.96	2.00	13.25	2.00	0.50	16.00	10.12	0.68	117.00	1.72
2	45.00	27.00	28.00	5.25	8.26	4.96	12.75	2.20	0.60	17.00	10.16	0.60	84.00	1.40
3	40.00	29.00	31.00	5.20	8.25	5.22	12.25	2.50	0.62	18.00	10.19	0.66	80.00	1.21
4	46.00	28.00	26.00	6.50	8.35	6.46	13.00	2.70	0.60	19.00	10.46	0.58	69.00	1.18
5	41.00	29.00	30.00	7.50	8.20	7.12	12.50	2.60	0.62	19.00	10.59	0.55	65.00	1.18
6	54.00	24.00	22.00	6.75	8.19	7.44	12.70	2.90	0.70	22.00	11.60	0.54	62.00	1.14
7	49.00	26.00	25.00	7.50	8.19	12.00	12.00	2.80	0.80	21.00	11.07	0.49	48.00	0.97
8	43.00	28.00	29.00	9.00	8.20	18.00	11.75	5.60	1.70	28.00	10.36	0.48	27.00	0.67
9	48.00	25.00	27.00	11.25	8.40	24.00	11.50	6.40	2.50	27.00	9.05	0.46	22.00	0.47

C_{org}: total soil organic carbon- C_{bio}: microbial biomass carbon- CEC: cation exchange capacity- CE: electrical conductivity- SAR: sodium adsorption ratio $Na/(Ca+Mg)^{1/2}$

The salinity increases from the periphery to the high saline soil, but the carbon (in its two forms: organic and microbial biomass) decreases in this sense, this is attributed to the adverse conditions created by salinity and high recalcitrant carbon produced by input carbon of halophyte vegetation. The organic carbon ranges from 0.68% for unsalted soil to 0.46% for high saline soil, and the microbial biomass carbon ranges from 117 mg/kg for unsalted soil to 22 mg/kg for high saline soil; in term of soil total organic carbon, this fraction varies from 1.72% for unsalted soil to 0.47% for high saline soil; these values are below than that suggested by other references **[34,35]**. From the Sebkha periphery to the high saline soil, the reduction rates reached 80%, 72% and 28% respectively for the microbial biomass carbon, the report C_{bio}/C_{org} and soil total organic carbon, which however marks a small decrease compared to other biological parameters (microbial biomass carbon and C_{bio}/C_{org}) **(table n°2)**.

Table n°2: Salinity effect on the decreasing biological parameters

Parameters	Linear equation	r^2
Microbial biomass carbon (C_{bio})	-3.807 CE+100.6	0.84
C_{bio}/C_{org}	-0.05 CE+1.588	0.91
Soil organic carbon	-0.009 CE+ 0.651	0.74

Same remarks were funded by other studies **[36]** in which have showed that when CE excess 16dS/m, the reduction of microbial biomass carbon is brutal.

The salinity effect on the microbial biomass behaviour is given in **figure n°1**.

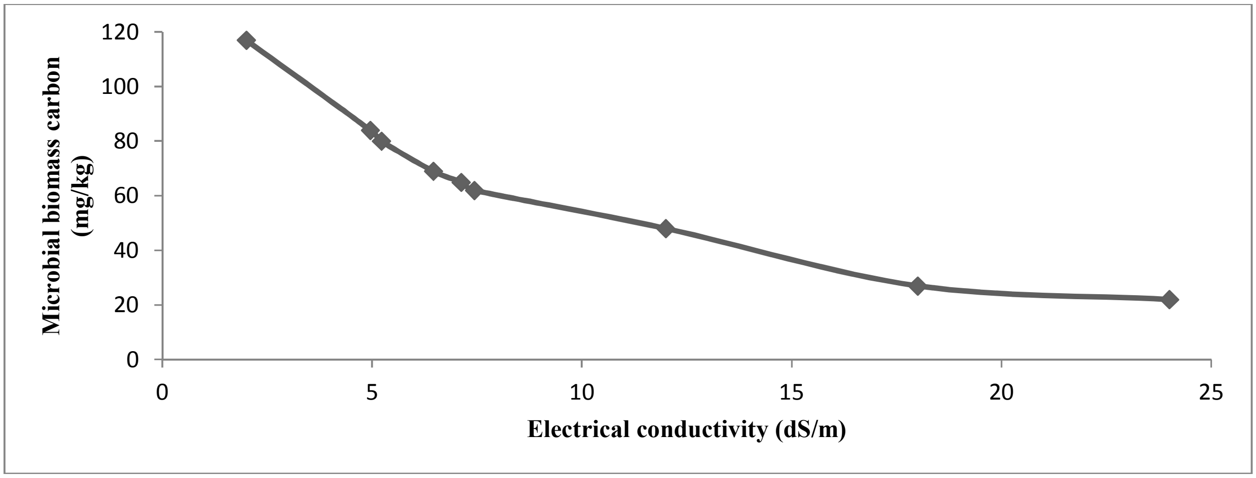

Figure nº1: Salinity effect on the microbial biomass

The curve shows the adverse effect of salinity on the soil microbial biomass carbon content, this reduction takes an asymptotic form when the salinity excess 15dS/m. It's a minimum level of microbial biomass that can host soil when habitat conditions became unfavourable; this fraction lives in dormancy and is undemanding in growth factors **[35]**.

Under arid conditions and when the electrical conductivity is less than 5dS/m, the estimation of this dormancy fraction by different adjustment equations gives an average value of 120 mg/kg, forming 1 to 2% in a terms of soil organic carbon **[10]**; however, in this degraded soil by an excess salinity (EC exceeds 12dS/m), these parameters are lower (C_{bio} does not exceed 50 mg/kg and the report C_{bio}/C_{org} is less than 1%) (**table nº1**). These values are under then found in other studies **[34]** in which they have fixed the thresholds values of organic carbon and microbial biomass carbon in degraded saline soil respectively at 0.6% and 100mg/kg.

2. In controlled condition

The salinity tolerance of soil microbial biomass is estimated from incubation the unsalted soil samples under an increasing stress salinity. The physicochemical characteristics of the incubated sample are given in **table nº3**.

Table nº3: Physicochemical characteristics of the incubated soil

Soil texture (%)			$CaCO_3$	pH	CE	CEC	Soil solution (mmol/l)				C_{org}	C_{bio}	C_{bio}/C_{org}
Sand	Silt	Clay	Total (%)	1/2,5	dS/m	mmol/100g	Ca	Mg	Na	SAR	(%)	mg/kg	(%)
45.00	28.00	27.00	5.00	7.96	2.00	13.25	2.00	0.50	16.00	10.12	0.68	117.00	1.72

The experimental protocol consists to subjecting the sample to an increasing salt stress under controlled conditions (80% of retention capacity moisture and a temperature of 28ºC). Salt stress is prepared from a mixture of NaCl and $CaCl_2$ solution with a fixed pH to 8 and SAR to 10. The incubation lasted 12 weeks, the sampling dates are fixed after 0,1,1,1,2,2,3 and 2 weeks or 12 weeks in terms of cumulative time, the carbon assessment of microbial biomass carbon was estimated by fumigation extraction method.

The effect of salinity stress on soil microbial biomass evolution during the incubation time, is given in **figure nº2**.

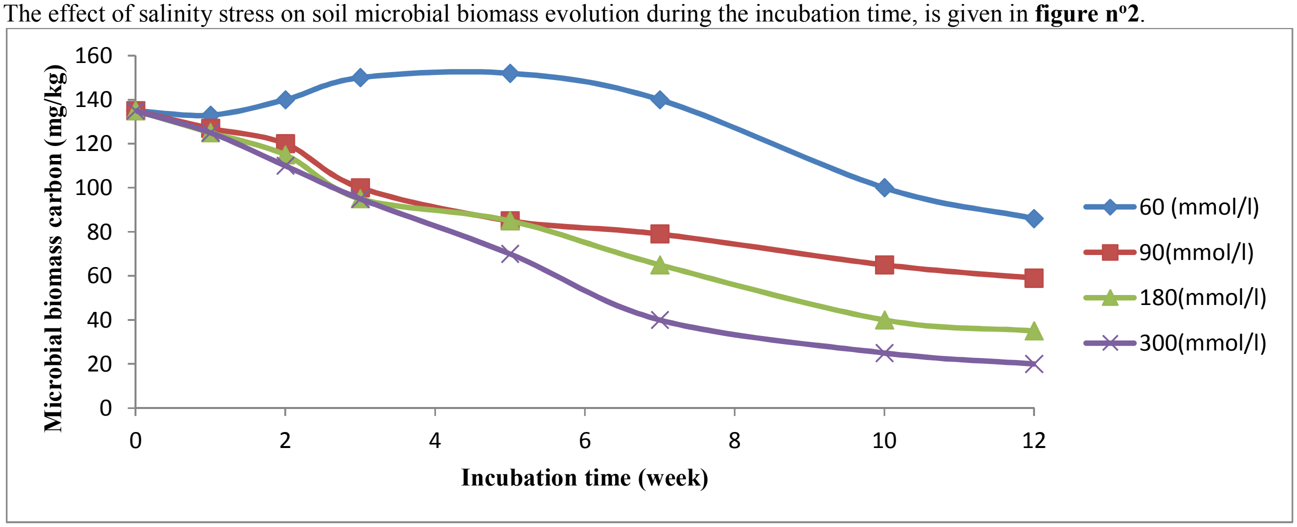

Figure nº2: Microbial biomass carbon evolution during the incubation time

Except for the sample subjecting by the concentration of 60mmol/l, the other curves demonstrate a decrease in level of microbial biomass carbon, this reduction is much more pronounced when the salt concentration exceeds 90mmol/l.

When the salt concentration is less than 60mmol/l, the microbial biomass marks a relatively regular behaviour, where we registered the usual four phases of microbial evolution (lag phase, growth phase, stationary phase and death phase), this is consistent with some studies, which have shown that the microorganisms of non saline soils are more tolerant to the brutal change in salinity than the microorganisms of saline soils **[18,37,38]** and also, the high role of Na on the aggregate dispersion and solubilisation of organic matter, making it more accessible to the microbiological activity; the same observations were showed by other authors **[39,40]**. On contrary, the effect of increasing salinity stress disturbs the usual microbial evolution, causing the soil microbial biomass reduction from the fellow of experimental phase, dominated by a high mortality rate, this reduction is not only due to reduces of the substrate but also to the microbial biomass characteristics that cannot tolerate this increased salinity concentration, which means that this microbial biomass is not very halo tolerant. Despite the adjustment mechanism of the intracellular osmotic pressure by a vacuole accumulation of organic or mineral solute, the mortality rates increase with increasing salinity to reach 85% for the sample of 300mmol/l. This rate is consistent with that found by Karem and al. (2016) **[41]** who observed a sharp reduction in microbial biomass carbon when the EC exceeds 16dS/m.

The adjustment of the experimental data to a single compartment model which describes the mineralization kinetics of the microbial biomass carbon as the result of degradation of one compartment B_m following a 1 order kinetics mineralization; this model write as following formula: $C_{bio} = B_m.e^{-kt}$.
C_{bio}: microbial biomass carbon remaining after time t (mg/kg)
B_m: microbial biomass carbon potentially mineralizable (mg/kg)
K: mineralization speed constant ($week^{-1}$)
T: incubation time (week)
The kinetic parameters of this model are shown in **table nº4.**

Table nº4: Kinetic parameters of the model (p=0.05)

Sample	r^2	B_m (mg/Kg)	K ($week^{-1}$)	Ln2/K (week)
60 (mmol/l)	0.57	152.90	0.03	23.00
90 (mmol/l)	0.87	131.40	0.07	9.85
180 (mmol/l)	0.98	141.00	0.11	6.27
300 (mmol/l)	0.98	148.20	0.17	4.05

Except for the first testing, which the experimental data are dispersed a wing to the resumption of the microbial biomass activity, the applied model give a satisfactory results for estimating the kinetics parameters. Therefore, the half-life time ranges from 9.85 weeks in middling saline sample to 4.05 weeks in high saline sample (300mml/l), the mineralization rate increase with increasing salinity, which passes from 0.07 $week^{-1}$ in middling saline sample to 0.17 $week^{-1}$ in high saline sample. These values are highest than showed by others studies **[42,43,44,45];** that confirms the biological resources of this saline ecosystem trends to irreversible degradation.

CONCLUSION

This study has demonstrated the negative influence of salinity on soil microbial biomass behaviour. Under high salinity, the microbial biomass carbon constitutes less 1% in terms of soil organic carbon which shows a high sensibility with the salinity variation compared to others soil parameters, this parameter may be a threshold value, used like a potential biomarker of the degraded soil by salinity. In this ecosystem the limit value of salinity tolerance is around to 90mmol/l, these value can used like critical salinity concentration for improvement the degraded saline soils; above this level, the microbial biomass shows a strong decrease and take the minimum values; this fraction is undemanding in growth factors and lives in dormancy inside of soil aggregate with a low residence time in soil.

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