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Comparison of the effects of fixatives on the abundance and biomass of ciliated protozoa from the solar saltwork of sfax, Tunisia

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Abstract
The planktonic ciliated protozoa of 2 Ponds of increasing salinity were investigated in the saline of Sfax, Tunisia. The samples are taken from the two basins (A5 and M2) of salinities 42 p.s.u and 216 p.s.u. At the level of these two basins, we have followed the structure and composition of the ciliary populations concerning the physic-chemical parameters of the environment, particularly the importance of salinity and the fixative's choice on the ciliated protozoa. In the Sfax saltworks, the water's salinity gradually increases from the A5 basin (S = 42p.s.u) to the M2 basin (S = 216 p.s.u). This increasing gradient in salinity has an influence on the evolution and structure of biological stands. Salinity was considered the fundamental ecological parameter for the distribution of planktonic populations in the Sfax saltworks. With regard to water temperature, water density, and MES, the latter increase with the increasing gradient of salinity. Accurate determination of the abundance of ciliates is crucial for estimating the carbon balance. However, the effect of fixatives on the abundance and biomass of ciliates was examined in cultures, lakes, and marine ciliates.

Keywords: abundance, biomass, ciliated protozoa, solar saltwork

Introduction

An extreme environment is a habitat characterized by harsh environmental conditions beyond the optimal range for humans' development, for example, pH 2 or 11, −20 °C or 113 °C, saturating salt concentrations, high radiation, 200 bars of pressure, among others. Basically, these are all inhospitable conditions for life. By definition, the organisms living in extreme environments are known as extremophiles (Rothschild and Mancinelli, 2001; Kumar *et al*., 2010) [26, 16].

A thermophile is an organism, a type of extremophile that thrives at relatively high temperatures, between 41 and 122 °C (106 and 252 °F). Many thermophiles are archaea (Chien *et al*., 1976) [10]. Psychrophiles or cryophiles (adj. psychrophilic or cryophilic) are extremophilic organisms capable of growth and reproduction in low temperatures, ranging from −20 °C to +10 °C (Siddiqui *et al*., 2013) [27]. Acidophilic and alkaliphilic species withstand environments where their pH is extreme, reaching 0 and 11, respectively (Pikuta *et al*., 2007) [25].

Halophilic extremophiles, or simply halophiles, are microorganisms that can grow and often thrive in high salt (NaCl) concentration areas. These hypersaline areas can range from the salinity equivalent to that of the ocean (~3-5%), up to ten times that, such as in the Dead Sea (31.5% average 3). Halophiles have been found belonging to each domain of life but primarily consist of archaea. They are metabolically diverse, ranging from simple fermenters to iron reducers and sulfide oxidizers (Madigan and Orent, 1999) [19]. Elloumi *et al*. (2009) [13] stated that in the salter of Sfax, there is a horizontal biological zonation; thus (i) in the range of salinities <50 p.s.u, the communities are similar to the coastal marine communities, and they present relatively small size. (ii) Between 50 and 150 p.s.u, develops significant phytoplankton biomass consisting mainly of green algae and diatoms. Heterotrophic bacteria are essential, flagellate and ciliated protozoa are present in large quantities. In this salinity range, the predation pressure by zooplankton (mainly copepods) is undoubtedly very high. (iii) Between 150 and 250 p.s.u, we are in the saline domain proper. The organisms are represented by hyperhalophilic species: the phyllopod crustacean Artemia salina, the Chlorophyceae Dunaliella salina, the ciliate Fabrea salina the Archaea. Ciliates and flagellates generally show lower abundances than in upstream basins, but ciliates' biomass can be very high due to the large size of F. salina. (iv) Above 300 p.s.u, heterotrophic bacteria and especially Archeae are very abundant. At this level, only Dunaliella salina is present and thus constitutes the only eukaryotic organism in these basins. Indeed, other algae, heterotrophic protists, and metazoa are absent in these basins.

Protists are unicellular eukaryotes that have colonized most environments: fresh stagnant or running water, brackish, salty, brine water, wastewater, sludge, and even wet or even dry earth. Moreover, this wide geographic distribution reflects their extraordinary potential for adaptation to all living conditions (free, symbiosis, commensals, Parasites). According to their shapes and modes of movement, protozoa are classified into 4 phyla: Rhizopods, Sporozoa, Flagellates, and Ciliates (Boudouresque, 2015) [6].

Ciliated protozoa are an essential link in the microbial loop and planktonic food webs. They are critical secondary producers in marine ecosystems (Brown *et al*., 2002) [7]. They constitute a trophic link between the microbial compartment and metazoa (Mostajir *et al*., 2015) [22]. Ciliates consume a good part of autotrophic and heterotrophic microbial production. They are in turn eaten by metazoa (especially copepods) and fish larvae (Petz, 1999) [24].

As already mentioned in the previous paragraph, ciliated protozoa are considered the main predators of bacteria and

phytoplankton (Elloumi, 2006) [12] and act as intermediaries between picoplankton and copepods (Calbet *et al*., 2005) [8] and of other consumers.

Therefore, it is essential to obtain precise estimates of the abundance and biomass of ciliates regardless of the techniques used. Direct enumeration of living ciliates is very difficult due to their small size and mobility. Therefore, to obtain a better qualitative and quantitative estimate of planktonic ciliates, the appropriate fixative must be chosen. Enumeration of ciliates in fixed samples does not imply immediate observation. The most used fixatives for ciliates are Lugol solution, Formalin, broth, glutaraldehyde, mercury chloride.

Materials and Methods

Study site

The study was conducted in the solar saltern of Sfax saltworks, an artificial parallel ecosystem to a dry Mediterranean climate; it is spatially extended over 1500 hectares, which marks a linear distance of more than 13 km. The Sfax-Thyna salt works are administratively attached to two municipalities: Sfax (400 hectares of crystallizers) and Chyna (1,100 hectares) (Chaker *et al*., 2000) [9] crossed by the wadi El Maou (Fig. 1)

Sampling

To study the effect of fixatives on the abundance and biomass of ciliated protozoa of the Sfax saline, we carried out a sampling campaign during April 2013 at the level of two contrasting salinity basins A5 and M2.

Basin A5

It is located in the front rooms, with an average salinity of around 42 p.s.u. It is a basin with a marine tendency where the water slice is small (about 50cm). M2 basin: it is located in the interior apartment, with an average salinity of around 216 p.s.u. The slice of water is about 30 cm. A 100 ml sample was fixed with different fixatives to preserve plankton.

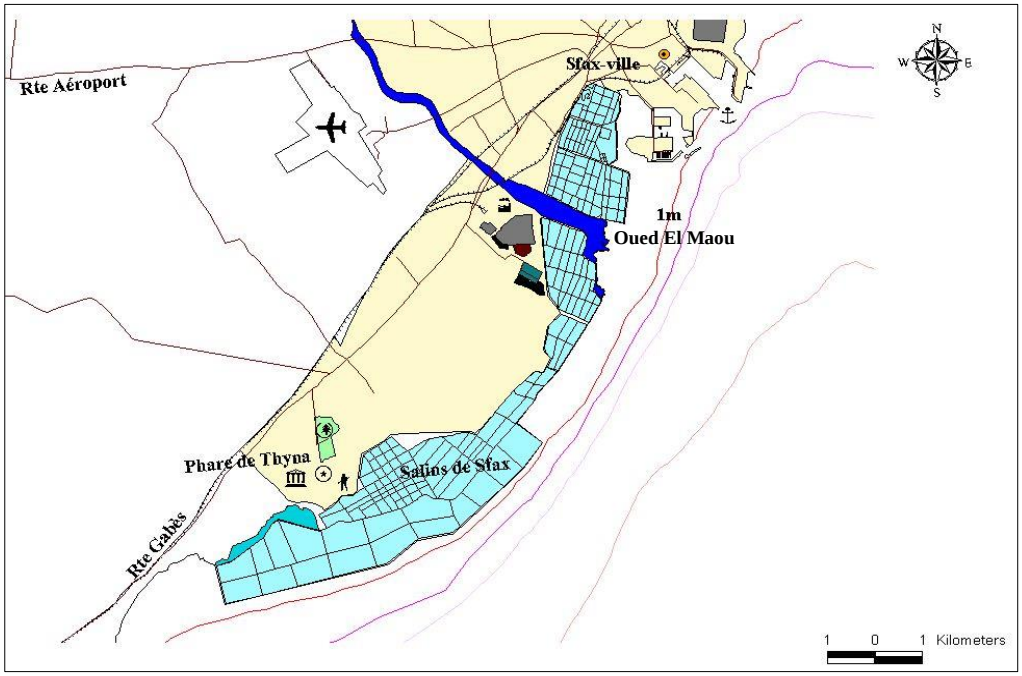


Fig 1: Location map of the Sfax saltworks

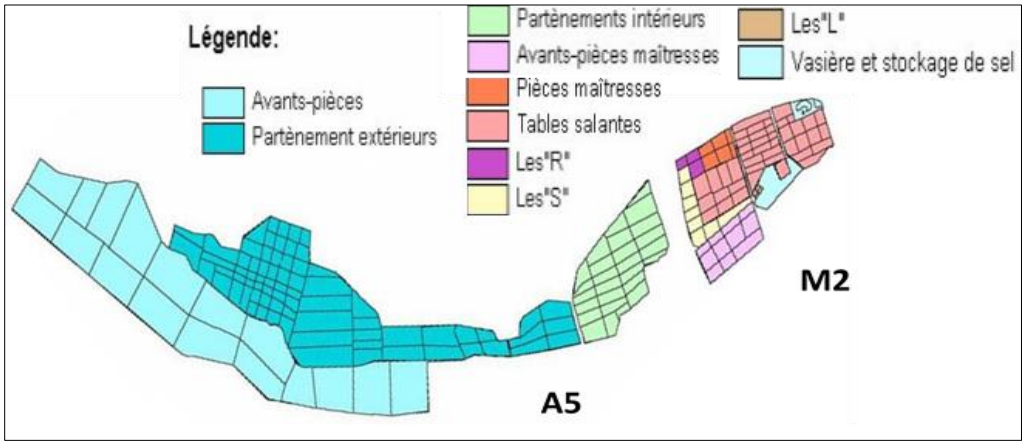


Fig 2: Location of the sampling basins (A5 and M2)

Studies of abiotic parameters

The physic-chemical parameters that were measured in the different basins: water temperature, pH, salinity, suspended matter.

Temperature (° C)

Water temperature is a significant factor in aquatic ecosystems in general and in saline in particular. It was measured in the field using a thermometer graduated in (° C) at the level of the surface water layers of the four basins in our study.

pH

The pH or the Hydrogen Potential measures the acidity and basicity of water. In aquatic ecosystems, the pH depends on the amount of dissolved CO2. It is a partial indicator of photosynthetic activity. The pH of the water was measured using a pH meter upon return to the laboratory.

Salinity (p.s.u)

The salinity of the brines was assessed using a refractometer. You put a drop of the sample on the refractometer, so it displays the corresponding salinity.

Suspended matter

This parameter makes it possible to evaluate the water load in mineral and organic particles and in microorganisms. The estimate of the brines' suspended matter was assessed by weighing the material retained on a Whatman GF / C filter, dried in an oven, of porosity 0.45 µm after vacuum filtration of a volume of 'water ranging from 50 ml for B1 to 100 ml for M1. MES expressed in (mg / l) are measured by the difference in mass of the filter before and after filtration according to the following formula:

$$MES = (M_1 - M_0) \times V \times 1000$$

Where

M_0 = initial mass of the filter disc (mg)

M_1= final mass (filtering disc + retained material) (mg)

V= volume of the sample to be analyzed (ml)

Study of biotic parameters: Study of ciliated protists

Preservation of samples

Ciliated protozoa are generally sensitive to contact with the fixatives used. In our study, we used different fixatives which are commonly used to preserve ciliates, namely:

1. **Lugol:** is a fixative widely used and widely recommended for preserving ciliated and flagellated protozoa (Leakey *et al*., 1994). Lugol or iodized potassium iodide solution is a solution prepared according to the method of Throndsen (1978) [30], composed of 100g of potassium iodide (KI), which has been dissolved in 1L of distilled water, and 50g of iodine (I2) (crystalline) which was dissolved in 100ml of glacial acetic acid. The two solutions are mixed, and the precipitates are removed. This solution has a mahogany brown color.
2. **The Formalin:** is a colorless organic compound of the family of chemical formula aldehydes (CH2O)
3. **Bouin solution:** is a bright yellow staining solution composed of a saturated aqueous solution of picric acid (75%), formaldehyde (Formalin 20%) and 5% glacial acetic acid.
4. **Protargol:** developed by Montagnes and Lynn (1987) [21], is a promising method combining the identification and enumeration of ciliates.
5. **Paraformaldehyde:** used for fixing planktonic ciliates (Sime-Ngando and Grolier, 1991) [28].

Taxonomic identification and enumeration

The density of ciliates was Determined selon the method of Utermöhl (1958) using the inverted microscope (Hasle, 1978; Gifford and Caron, 2000) [14].

A volume of 10 to 20 ml of the sample is left to sediment for 2 hours, qui est.

Then Observed under an inverted microscope Leica deviation (magnification: 400 ×).

Ciliates are identified at the level of the genus or even at the level of the species. To have the total number of cells N containing in 1 L: N=n×1000/v

N: total density of ciliates is expressed in cells / 1.

n: number of cells counted in 10ml volume.

V: volume of the sedimentation tank (10 ml)

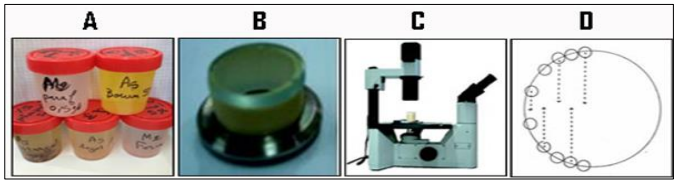


Fig 3: Study Techniques ciliates. A: Samples ciliates fixed. B: Sedimentation chamber of 10 ml. C: Inverted microscope type "Leica. » D: Uthermöhl counting method.

Estimation of biovolumes and biomass

For each ciliate species, an average biovolume is estimated by measuring around fifty cells and assimilating them to simple geometric shapes.

A minimum of 30 to 50 individuals is considered for these measurements. For the estimation of biomass, the conversion factor is chosen according to the fixative.

Analysis of variance (ANOVA)

Analysis of variance (ANOVA: Analysis Of Variance) is a statistical technique used to compare the means of two or more populations. ANOVA is used when the groups analyzed are distinguished by one of several qualitative factors.

Results

Study of abiotic parameters

The values of the various physicochemical parameters measured for the two study basins (A5 and M2) are presented in Table 1.

Temperature (°C)

In the present study, the spatial variation in water temperature shows thermal variability depending on the basin (Table 1). Generally, the highest values are recorded at the level of the inner apartments M2 ponds.

Salinity (PSU)

During our study, we recorded salinity values that are different between the A5 and M2 basins. Salinity values vary between 42 p.s.u at the A5 p.s.u basin and 216 at the M2 basin (Table1).

pH

The pH values are between 8.4 at the level of basin A5 and 7.8 at the basin M2. We note that the pH decreases with increasing salinity.

Suspended solids MES (mg.l-1)

The suspended solids contents gradually increase from basin A5 to basin M2, which follows the increasing gradient of salinity (Table 1). SS concentrations are higher in hypersaline pools (M2), which appear more turbid.

Table 1: The physic-chemical parameters measured in the A5 and M2 basins of the Sfax saltworks

Parameters	Sites	
	A5	M2
Temperature (°C)	16	18
Salinity (PSU)	42	216
pH	8,4	7,8
MES (mg.l^{-1})	1800	11200

Study the effect of the biotic parameters of fixatives on qualitative and quantitative estimation of ciliates

Qualitative study

This study is interested in fixatives (Lugol, Formol, Bouin, Paraformaldehyde, and Protargol) at different concentrations (final CC ° = 0.5; 1; 5%) richness, densities, and biomasses of protozoa. Ciliates at the level of two contrasting salinity basins of the Sfax saltworks (A5=42p.s.u) et (M2=216 p.s.u). Fixation with Formalin (final CC ° = 1%) reveals 9 taxa of ciliates in the A5 basin and 5 taxa in the M2 basin (Fig. 4). The qualitative list of the different genera and species identified for each fixative is presented in Tables 2 and 3. At the basin A5, the lowest number of taxa (a single species) is recorded with paraformaldehyde (final CC ° 5%).

So, at the level of the hypersaline M2 pelvis, with Protargol (final CC ° = 5%) no ciliate species is recorded and with Lugol (final CC ° = 0.5%) and paraformaldehyde (final CC ° 1 and 5%) only one species is counted (fig.4). Despite the contrasting salinity of the two study basins, the highest taxon was noted with Formalin (CC° final=1%). At the level of basin A5, with formalin (final CC ° = 5%) and paraformaldehyde (final CC ° = 0.5%) we found the highest number of taxa (8 taxa).

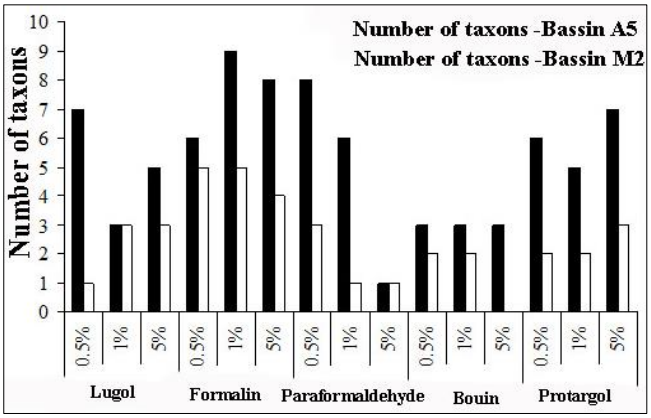


Fig 4: Number of taxa of ciliated protozoa according to fixative

The qualitative list of species identified in basin A5 is presented in Table 3.

Whatever the fixative and the concentrations used, it is noted that the Spirotrichea class largely dominates the ciliary communities with 7 species. This class is subdivided into three subclasses: S / C Oligotrichia with 3 species (Strombidinopsis acuminatum, Strombidium sp. And Loboea strobila), S / C Choreotrichia with 3 species (Tintinnopsis beroidea, Leegardiella sol, and Lohmanniella oviformis), and S / C Hypotrichia with a single species (Euplotes Charon).

At the sit of bassin A5 in addition to Spirotrichea, we recorded two other classes namely: (i) the class of prostate with two species (Urotricha sp. and Holophrya sp.) (Ii) The class of Litostomatea (Mesodinium rubrum and Enchelyodon sp.) (Table 2). Only the species "Strombidium sp. is found regardless of the fixative used and the concentration used. But with Formalin (final CC ° 1%), we find the highest density of this species with 1.4 × 105 cells. L-1. On the other hand, the lowest density is recorded with protargol (final CC ° 5%) with 500 cells. L^{-1}.

Table 2: Effect of fixatives on the qualitative estimate of the ciliary species in the basin A5 Species identified by the fixative (-) species unidentified by the fixator (+)

Fixatives	Lugol		Formalin			Paraformaldehyde					Protargol		Bouin		
Concentrations (%)	0.5	1	5	0.5	1	5	0.5	1	5	0.5	1	5	0.5	1	5
Spirotrichea class															
S/C Oligotrichia															
Strombidinopsis acuminatum	+	-	-	-	+	+	+	-	-	-	-	-	+	+	+
Strombidium sp.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Loboea strobila	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-
S/C Choreotrichia															
Tintinnopsis beroidea	+	-	-	+	-	+	+	+	-	+	+	+	+	+	+
Leegardiella sol	+	-	-	+	+	+	+	+	-	-	-	-	+	-	+
Lohmanniella oviformis	-	-	+	+	+	+	+	+	-	-	-	-	-	-	-
S/C Hypotrichia															
Euplotes charon	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-
Class of prostomatea															
Urotricha sp.	+	-	+	+	+	+	+	+	-	+	+	-	-	+	+

Holophrya sp.	+	-	+	+	+	+	+	+	-	-	-	+	+	+	+
Litostomatea class															
Mesodinium rubrum	-	-	+	-	+	-	+	-	-	-	-	-	+	-	+
Enchelyodon sp.	-	-	-	-	+	-	-	-	-	-	-	+	-	-	+

Quantitative study

The use of the five fixatives at different concentrations has shown that there are effects on specific richness and cell densities and biomasses.

ANOVA analysis of variance showed significant differences in the density of ciliated protozoa between the five fixators (ANOVA, F = 10.72; p <0.01) (ANOVA, F = 5.03 p <0.05) at basin level A5 and M2 respectively. Similarly, for the biomasses, significant differences were recorded in the density of ciliated protozoa between the five fixators at the level of the A5 basin (ANOVA, F = 25.60; P <0, 001) and the M2 bassin (ANOVA, F = 8.15; P <0.01). Whatever the fixative used, there are cell losses and changes in cell volumes, affecting the densities and biomasses of the ciliates.

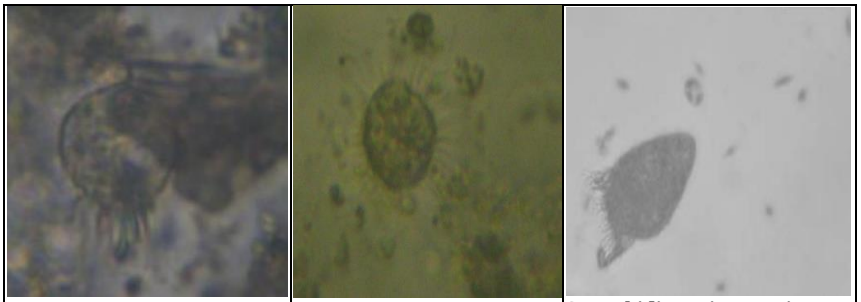
Table 3: Average lengths and average biovolumes of the different ciliary species fixed by five fixators.

Fixatives	**Lugol**		**Formalin**		**Paraformaldehyde**		**Protargol**		**Bouin**	
	L (µm)	**Biovolume ($\times 10^3$ µm^3)**	**L (µm)**	**Biovolume ($\times 10^3$ µm^3)**	**L (µm)**	**Biovolume ($\times 10^3$ µm^3)**	**L (µm)**	**Biovolume ($\times 10^3$ µm^3)**	**L (µm)**	**Biovolume ($\times 10^3$ µm^3)**
Tintinnopsis beroidea	83	33	100	43	66	25	88	35	73	27
Strombidinopsis acuminatum	58	27	66	43	58	21	0	0	65	24
Leegardiella sol	30	5	25	7	28	4	0	0	18	3
Lohmanniella oviformis	19	2	24	2	18	2	0	0	0	0
Loboea strobila	53	11	63	35	0	0	55	20	0	0
Strombidium sp.	25	3	30	5	21	3	24	4	23	4
Euplotes charon	66	42	60	68	0	0	0	0	0	00
Mesodinium rubrum	28	2	30	2	20	1	0	0	25	2
Enchelyoden sp.	0	0	53	7	0	0	0	0	0	0
Urotricha sp.	19	3	23	3	20	2	20	2	19	2
Holophrya sp.	30	11	31	12	25	7	33	11	25	4
Fabrea salina	125	242	150	373	105	139	135	286	98	184
Metaurostylopsis salina	65	14	80	31	0	0	0	0	0	0
Nassula sp.	0	0	98	306	0	0	0	0	0	0
Trachelus ovum	0	0	113	65	0	0	0	0	60	39

The density of the total ciliates in the A5 basin varies from 0.7 × 103 cells. L-1 for samples fixed with Protargol (final CC ° 5%) and 146.4 × 103 cells. L-1 for samples fixed with Formalin (final CC ° 1%) (Fig. 6). In terms of biomass, at the level of basin A5, the highest biomass is obtained for the samples fixed by Formalin (final CC ° 1%) with 104.1 µgCl-1-1). However, the lowest biomass is recorded with paraformaldehyde 5% and Protargol 1 and 5%, where the biomasses do not exceed 1.2 µgC.l-1 (Fig. 6). At the level of the M2 basin, the density of ciliated protozoa is higher (4.8 × 10^3 cells.l-1) with the samples fixed with Formalin (final CC ° 1%), and the density is of the order of zero with Protargol (final CC ° 5%) (Fig. 7).

Likewise, the highest biomass (193.3 µgCl^{-1}) is recorded with the samples fixed with Formalin (final CC ° 1%) (Fig.7). The ciliates' mean densities show that Formalin represents the highest densities at the basins A5 and M2 with 130.4 × 103 cells.l-1 and 3.8 × 10^3 cells.l-1 respectively.

Followed by the Lugol with 98 × 103 cells. L-1 for the A5 basin and 2.3 × 10^3 cells. L-1 for the M2 bassin. Paraformaldehyde and brine represent much lower densities than Formalin and Lugol so the densities do not exceed 39 × 103 cells.l-1 for A5 and 3.1 × 103 cells.l-1 for M2. However, Protargol shows the lowest densities for A5 and M2 which are 1.9 × 10^3 cells.l-1 and 0.27 × 103 cells.l-1 respectively.



Strombidium sp. ***Strobilidum neptuni*** ***Strombidinopsis acuminatum***

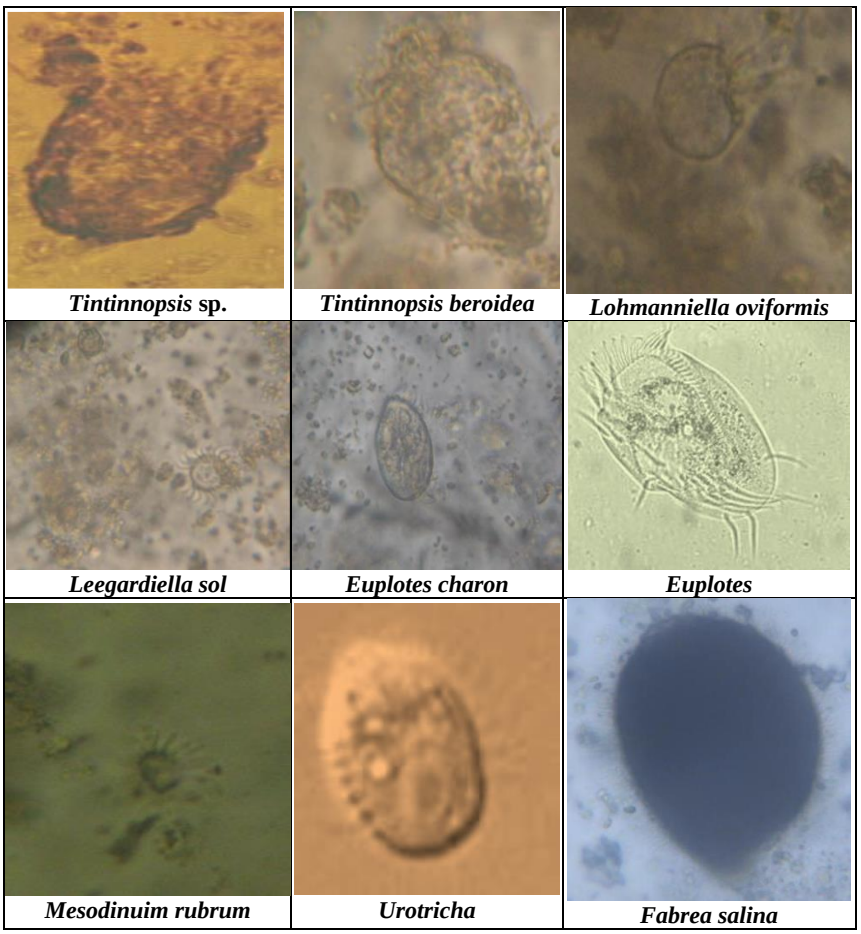


Fig 5: some species of ciliates in the basins (A5 and M2) of the Sfax saltworks

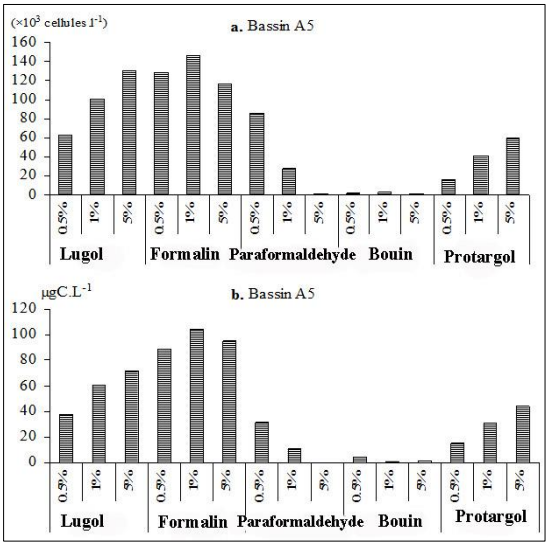


Fig 6: Variation of the densities (a) and biomasses (b) of the total ciliates as a function of the fixatives at the level of the A5 bassin

During this study, Protargol appeared to be the most destructive fixative for planktonic ciliates in the Sfax saltworks' basins. In terms of biomass, the same finding as for densities. Thus, with Formalin, the highest average biomass is recorded with 96.02 µgC.l-1 and 148 µgC.l-1 for A5 and M2, respectively. Protargol represents the lowest biomasses with 2.05 µgC.l-1 at the level of basin A5 and 5.4 µgC.l-1 at the basin M2. We can say that CC ° final 1% formalin is a good fixative for the ciliates of the Sfax salter, while Protargol (final CC ° 5%) is not suitable for fixing the ciliates of the saline.

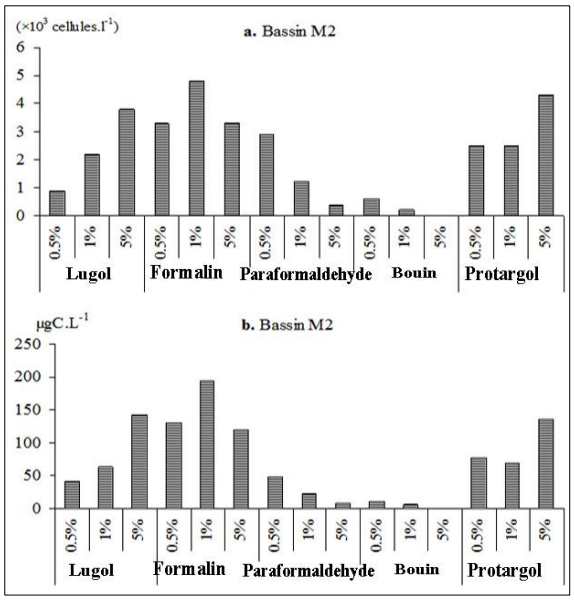


Fig 7: Variation of densities (a) and biomasses (b) of total ciliates as a function of fixatives at pelvis level M2.

Figures 8 and 9 show that the five fixators affect the density and biomass of different ciliated protozoa classes. There is a difference in the relative abundances of the other ciliate types. At the level of the A5 basin, whatever the fixative and the concentration used, the Spirotrichea class dominates it always exceeds 79% of the total ciliates. Except with Protargol CC ° final 1%, the Spirotriches do not exceed 35.5% of the total ciliates, and it is the prostate (Urotricha sp.) Which dominates (64% of the total ciliates) (Fig. 8a and 8b) In terms of biomass, the Spirotrichea class still dominates with more than 80%.

Most ciliary species are strongly influenced by the fixations in biovolume (Table 6), which explains the variations in the relative abundances of biomasses compared to the densities (Fig. 8a and 8b).

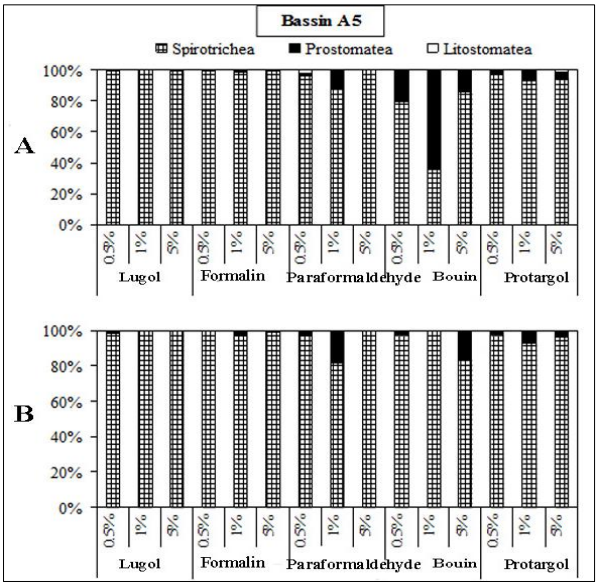


Fig 8: Variations in the relative abundances of densities (a) and biomasses (b) of the different ciliary classes at the level of the A5 bassin

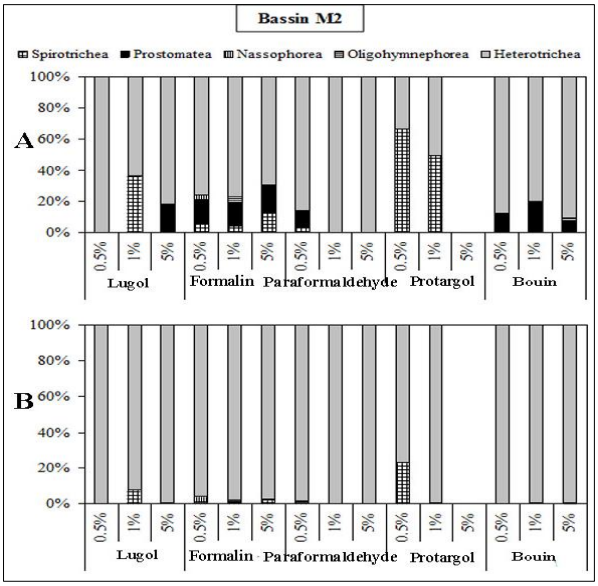


Fig 9: Variation of the relative abundances of densities (a) and biomasses (b) of the different ciliary classes at the level of the M2 basin

Discussion

The water temperature increases from basin A5 to basin M2; this could be explained by the decrease in the water section from basin A5 to basin M2 with the absence of vertical thermal stratification (Elloumi *et al*., 2006, 2008; Abid *et al*., 2002, 2008; 2009), same findings as Lei *et al*. (2009) in the salt marshes of the Yellow Sea (Pacific Ocean).

The salinity values increase in parallel with the increase in temperature, which varies between 42 p.s.u at the basin A5 and 216 p.s.u at the level of basin M2. The pH decreases with increasing salinity. These results are consistent with those found by Lei *et al*. (2009) in the salt marshes of the Yellow Sea. The pH is a parameter influenced by biological activity (Bayoudh, 2011). The development of macroalgae and phanerogams undoubtedly contributes to water alkalinization through photosynthesis (Bayoudh, 2011). During this phenomenon, phytoplankton consumes a significant amount of CO2 dissolved in the environment, which increases the pH (Elloumi, 2006; Guermazi, 2008; Abid, 2009). The enrichments of water in nutrients and pollutants of natural or anthropogenic origin can change the pH (Ama *et al*., 2009) Fixation with Formalin (final CC ° = 1%) reveals nine taxa of ciliates at the level of the A5 basin and 5 taxa at the level of the M2 bassin. The number of taxa decreases with increasing salinity (Elloumi *et al*., 2006). Whatever the fixative and the concentrations used, it is noted that the Spirotrichea class largely dominates the ciliary communities at the level of the A5 basin. However, the Heretrotrichea class proliferates in the M2 bassin. These results are already mentioned by Elloumi *et al*. (2006, 2009).

In the current investigation, it was noticed that most ciliary species are strongly influenced by fixators, which suggests that the impact of fixation on ciliate biovolumes was due to changes in cell volumes (Bouyoudh, 2011). Variations in the relative abundances of ciliary populations showed that the Spirotrichea class dominates in the A5 basin. In terms of biomass, the Spirotrichea class still dominates in the A5 basin with more than 80%. At the level of the M2 bassin, the Heterotrichea (Fabrea salina) proliferate. Fabrea salina, a valid halophilic species, can grow in a salinity range varying from 65 p.s.u to 190 p.s.u and seems intolerant to low salinity (Lei *et al*., 2009). Fabrea salina represents the most essential biovolume of ciliates, which is of the order of 373 × 103 µm3 with samples fixed in Formalin. The latter can cause significant cell loss (Stoecker *et al*., 1994), causes minor cell shrinkage allowing ciliates to be determined at least at the genus level (Modigh *et al*., 2005). Cellular shrinkage of ciliated protozoa may be related to the fixative nature and concentration (Lei *et al*., 2009). According to Stoecker *et al*. (1994), it appears that the effect of fixatives was not the same for all morpho types and classes. For example, cells of conical morphology such as (Loboea strobila, Strombidium sp.) Showed the most significant shrinkage in Lugol solution compared to Formalin. Another experiment conducted by Leakey *et al*. (1994) showed that the average cell volume of Balanion sp. Strombidium epidermal and Tintinnopsisnana was more prominent in samples fixed in Formalin, followed by glutaraldehyde, Lugol, and Bouin. These experiments corroborate our results that the mean volume of the different ciliary species is more significant in the samples fixed in Formalin, followed by Lugol and Bouin.

Whatever the fixative used, there are cell losses and changes due to cell volumes, affecting ciliates' densities and biomasses

(Bayoudh, 2011). Besides, each fixator has these drawbacks. Lugol's dark brown color obscures morphological details (Gifford *et al.*, 2000), causing difficulties in microscopic analyzes, which creates a confounding factor (Pace and Orcutt, 1981). In fact, to avoid dark coloring, Formalin has been more convenient (Modigh *et al*., 2005). Another disadvantage of the binding of plankton samples by the Lugol solution is the formation of aggregates. The overlap of ciliates with dinoflagellates and diatoms prevents species identification (Modigh *et al*., 2005)

Brine fluid is particularly inadequate for ciliated protozoa's attachment (Lei *et al*., 2009; Bayoudh, 2011). In the Sfax saline basins, Bayoudh (2011) showed that with Bouin's liquid, the quantitative losses are higher than Formalin and Lugol. During this study, Protargol appeared to be the most destructive fixative for planktonic ciliates in the Sfax saltworks' basins.

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