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Original Research Article Charophyte community in the lowermost locality in 2 the world near the Dead Sea, Israel 3

ABSTRACT

6 Aims: First study of the lowermost locality the Neot HaKikar with charophytes in the Dead Sea region of Israel has been implemented showing the algal diversity and ecological assessment of the water object environment.

Study design: We implemented diverse bio-indication methods.

Place and Duration of Study: Institute of Evolution, University of Haifa, Israel, Central Siberian Botanical Garden of the Siberian Branch of the Russian Academy of Sciences, Russia, between January 2012 and December 2014.

Methodology: Material for this study comes from 26 samples including 9 living and 9 fixed periphyton samples, 4 fixed samples of charophytes and 4 samples of water. We used bio-indication methods for the purpose of characterizing pool water quality and ecosystem sustainability. Index saprobity S and Index of aquatic ecosystem sustainability WESI were calculated.

Results: Altogether 39 species of algae, including macro-algae Chara contraria A.Braun ex Kützing (Charales, Charophyceae), were revealed in the Neot HaKikar pool. Chara was found in significant growth in the bottom and coastal part of the studied pool. Bio-indication and chemical variables characterized the charophyte site environment as mesotrophic to eutrophic with prevailing benthic types of organisms with an autotrophic type of nutrition, which are mostly attached to the substrate and preferred standing water, medium-enriched by oxygen, with temperate temperature, medium salinity, low alkalinity, and low-to-middle organic pollution, representing the Class III of water quality. Seasonality of the algal community and water quality showed organic and other contaminants of pollution during the winter period as a result of evaporation and an atmospheric dust impact. The Charophyte community is sharply limited in its development as a result of periodical anthropogenic desiccation of the pool. We found unique properties of Chara contraria in the renewed population after two years of desiccation.

Conclusion: We can recommend the Neot HaKikar pool for the monitoring of unique natural aquatic objects in the Dead Sea area, and Chara contraria as a climatic indicator of surviving under future climate warming.

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Keywords: Charophytes, ecology, bio-indication, Dead Sea, Israel

1. INTRODUCTION 10

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12 Diversity of algae in Israel has been studied sporadically during the last century, but from 2000 on we 13 continued regular work in the rivers and other water bodies [1]. As a result, we studied known 14 localities as well as finding new localities not only for algal diversity updates but also especially for 15 revealing charophyte communities [2,3]. At the present time, we reveal 14 charophyte species (16 16 with infraspecific variety) that are known for Israel [4] from references and our own studies but only 17 now we try to describe some new undescribed localities.

18 The charophytes prefer alkaline water environments, which form on the carbonates that are widely 19 distributed in the studied region. Therefore, the Eastern Mediterranean environment gives us more 20 chances to find new, unstudied aquatic objects in which charophyte algae can be identified. The altitude gradient plays the major role in historical species diversity forming process, which is [5] 21 especially interesting in the Arava Valley, in the lowermost area of the world placed between the Red 22 23 Sea and the Dead Sea. Biodiversity of the Arava Valley refers to the Saharo-Arabian Realm with a 24 sharp arid climatic environment [6-8].

25 We assume that the diversity of this group of algae in Israel is still far from complete. Thus, the aim of 26 our work was to find new habitats of charophytes and study their community and their environments, 27 especially in the lowest place in the world that is affected by the shading of solar radiation of the dust 28 laver - more than 500 meters thick. 29

30 2. MATERIAL AND METHODS

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32 2.1. Description of study site

The Neot HaKikar pool is located in the northern part of the Arava Valley to the south of the Dead Sea at 30°57.221 N and 35°21.450 E with an altitude of 356 m below sea level (Fig. 1). The pool is permanent, about 10-12 m in diameter, about 0.5-0.7 m deep, and affiliated with the Israeli Mekorot Company as one of the freshwater sources in Kibbuz Ein Tamar. The area has a desert climate. Throughout the year, there is virtually no rainfall at Neot HaKikar. The average annual air temperature is about 24.1°C [9].

The long-term average annual rainfall is 53 mm [9]. The driest month is August with 0 mm. Most precipitation falls in December, with an average of 12 mm but this occurs one time per 2-4 years. The warmest month of the year is August with an average air temperature of 31.4°C. In January, the average air temperature is 15.3°C. It is the lowest average temperature of the whole year.

Periodical year-round dust storms attack the area when sunlight is rather decreased. The main air dust content is at mid-day (Fig. 2) when UV radiation is lower than in the nearby high-altitude areas. The most impacted period is at mid-day, when dust concentration in the nearby city of Beer-Sheva rises up to 2-5 mg m⁻³ in March-May, and up to 5 mg m⁻³ in January-February and May depending on the sand storm generation area [10]. The air temperature increased significantly about 10°C during the sand storm. Massive high temperature air with dust transportation covers an area of about 4,800 square kilometers in the Arava Valley [11].



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51 Fig. 1. Study site in the Neot HaKikar pool, Arava Valley, Israel



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54 Fig. 2. The Neot HaKikar area during the sandstorm, midday, in January 2012

56 2.2. Sampling and laboratory studies

57 Material for this study comes from 26 samples including 9 living and 9 fixed periphyton samples, 4 58 fixed samples of charophytes and 4 samples of water that were collected during two field trips on 59 January 16 and June 12 of 2012 in the Neot HaKikar pool.

Algological samples were collected by substrate scratching and water scooping, placed in 15 ml plastic tubes, and partly fixed with 3% neutral formaldehyde solution, as well as some (duplicates) not fixed, and transported to the laboratory in an ice box.

63 Charophytes were treated with 2-3% HCl to remove calcium carbonate. After washing several times 64 with distilled water, the material was studied with a Nikon stereomicroscope. The structure elements 65 were observed with a Nikon digital camera (DC), a DinoLight camera, and light microscopes (LM) in 66 the Institute of Evolution, University of Haifa (IEUH) and the Central Siberian Botanical Garden (NS) 67 with help using international handbooks [12,13]. Herbarium specimens were deposited in the IEUH 68 and NS.

Algae and cyanobacteria were studied with the SWIFT, NIKON, and OLYMPUS dissecting microscopes under magnifications 40x–1000x from three repetitions of each sample and were photographed with digital cameras of the Leica stereomicroscope and Nikon Eclipse light microscope. The diatoms were prepared using the peroxide technique [14] modified for glass slides [15] and were placed in the Naphrax® resin from two repetitions of each sample. Charophyte and microscopic algae abundance were assessed as abundance scores according to a 6-score scale [16] (Tab. 1).

75 **Tab. 1. Species frequencies according to 6-scores scale [1,16].** Score Visual Estimate Cell numbers of periphyton or plankton per s

Score	Visual Estimate	Cell numbers of periphyton or plankton per slide (20 x 20 mm)
1	Occasional	1-5 cells per slide
2	Rare	10-15 cells per slide
3	Common	25-30 cells per slide
4	Frequent	1 cell over a slide transect
5	Very frequent	Several cells over a slide transect
6	Abundant	One or more cells in each field of view

Temperature was measured with a thermometer. Electrical Conductivity (EC), pH, and Total Dissolved Solids (TDS) were measured with HANNA HI 9813-0. Measurements were made in five repetitions by adding the probe into the water till the reading was stabilized. Chlorides and sodium percentages were determined with "Handheld Refractometers X-Series Sodium Chloride" with three repetitions. The concentration of N-NO₃ was measured with HANNA HI 93728 with five repetitions.

81 **2.3. Bio-indication and indices calculation**

The methods and indices that can be used for bio-indication of environment quality are based on the ecological point of view to the water and biota relationships [1,16]. The mutual influence of the diversity of freshwater algae and their habitat can be determined with the help of ecological preferences of the species developing in a studied community. This is a basic principle of bioindication - compliance with the community composition to the parameters of its habitat.

87 Our ecological analysis has revealed a grouping of freshwater algae indicators to pH, salinity, and 88 saprobity as well as for other habitat conditions [1,16]. Each group was separately assessed in 89 respect to its significance for bio-indication. Those species that predictably responded to 90 environmental variables can be used as bio-indicators reflecting the response of aquatic ecosystems 91 to eutrophication, pH levels, salinity, organic pollutions, nutrition type, and trophic level.

Index saprobity S was calculated according to [17] using the species-specific saprobity index s of
 revealed taxa and its abundance scores (Tab. 1).

The calculated integral index of aquatic ecosystem sustainability (Aquatic Ecosystem State Index, WESI) is based on the water-quality classes [1,16] (Tab. 2) reflecting self-purification capacities for each of the sampling stations. Index WESI was calculated according to [1,16] as (1):

97 WESI = Rank S / Rank N-NO₃. (1)

Where: Rank S – rank of water quality on the Sladeček's indices of saprobity; Rank N-NO₃ – rank of
 water quality on the nitric-nitrogen concentration (Tab. 2).

100 If WESI is equal to or larger than 1, the photosynthetic level is positively correlated with the level of 101 nitrate concentration. If the WESI is less than 1, the photosynthesis is suppressed presumably 102 according to toxic disturbances [1,16].

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104 Tab. 2. Ecological water quality classification [1,16].

Water quality Class	Rank	NO_3^- mg N L ⁻¹	Index saprobity S
I - very pure	1	< 0.05	<0.5
II - pure	2	0.05-0.20	0.5-1.0
II - pure	3	0.21-0.50	1.0-1.5
III - moderate	4	0.51-1.00	1.5-2.0
III - moderate	5	1.01-1.50	2.0-2.5
IV - polluted	6	1.51-2.00	2.5-3.0
IV - polluted	7	2.01-2.50	3.0-3.5
V - very polluted	8	2.51-4.00	3.5-4.0
V - very polluted	9	> 4.00	>4.0

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106 **2.4. Taxonomic analysis and classification**

For taxonomic identification, the handbook series was used [13, 18-25]. Modern species names in our
work come from algaebase.org [26] employing the common system nomenclature derived from T.
Cavalier-Smith [27].

110 3. RESULTS

112 **3.1.** Chemical composition of the pool water

113 Chemical variables were measured two times in winter and summer seasons (Tab. 3). Environment 114 variables are fluctuated in small ranges and reflected fresh to brackish, low alkaline, temperate 115 temperature, and low polluted waters [1,16]. Index of saprobity S fluctuated in small ranges and 116 reflects low levels of organic pollution, Class III of water quality. Water salinity rather fluctuated 117 between winter and summer. Remarkably, water conductivity, TDS, and salinity are higher in summer 118 whereas nitrates increase in winter.

Variables	Aver_summer	stdev	Aver_winter	stdev
Conductivity, mS cm ⁻¹	6.75	0.11	6.37	0.46
N-NO ₃ , mg L ⁻¹	0.00	0.00	0.41	0.43
рН	7.13	0.20	7.14	0.30
Total Dissolved Solids (TDS), g L ⁻¹	1.73	0.00	1.21	0.28
Т, С°	31.70	0.66	25.67	1.96
CI, %	0.51	0.11	0.28	0.10
Na, %	0.51	0.11	0.28	0.10
No. of Species	24.00	1.15	24.00	1.00
Index saprobity S	1.80	0.21	1.79	0.18
Index WESI	1.00	0.00	1.21	0.46

119 **Tab. 3. Chemical and biological variables in the Neot HaKikar pool in 2012**.

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122 3.2. Diversity and ecology of algae

We revealed 39 species of algae (Tab. 4) diversity which were rather constant during the sampling dates in summer (23) and winter (25) communities. The majority of species in winter was diatoms (15) whereas summer community enriched by greens also (10 and 10 respectively).

Tab. 4. Algal diversity with abundance scores and species ecological preferences (according to V. Sladeček [16], and H. Van Dam [28]) in the Neot HaKikar pool in January (Win) and June (Sum) 2012, and in A. Ehrlich [29] (Hist).

Таха	Hi st	Win	Sum	S	Hab	т	Reo	рН	Sal	Sap	D	Aut- Het	Tro	pH range
Cyanobacteria														
Anabaena sp.	-	3	-	-	-	-	-	-	-	-	-	-	-	-
Anabaenopsis sp.	-	-	1	-	-	-	-	-	-	-	-	-	-	-
Chroococcus turgidus (Kützing) Nägeli	-	1	2	0.8	P- B,S	-	aer	alf	hl	0	-	-	-	-
pusillum (Van Goor) Komárek Limnococcus limneticus (Lemmermann)	-	1	-	1.8	Ρ	-	-	-	-	b	-	-	me	-
Komárková, Jezberová, O.Komárek & Zapomelová <i>Microcoleus</i> <i>autumnalis</i>	+	-	3	1.5	Ρ	-	-	-		o-b	-	-	o-m	-
(Gomont) Strunecky, Komárek & J.R.Johansen	-	-	1	2.3	B,S	-	st-str	-	-	b	-	-	-	-
sancta Kützing ex Gomont	-	4	-	2.7	P-B, S	eterm	st-str, aer	-	i	b-a	-	-	me	-

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(Grunow)	-	3	-	1.3	В	-	str	alf	hl	0	-	ats	е	-
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ex W.Smith														
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(Ehrenberg) Otto	-	5	'	-	U	wann	30	inu		-	63		-	7.0-3.0
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Muller Seminavis strigosa (Hustedt) Danieledis & Economou-Amilli	+	-	-	-	D									
Muller Seminavis strigosa (Hustedt) Danieledis & Economou-Amilli	+	-	-	-	D									
Mulier Seminavis strigosa (Hustedt) Danieledis & Economou-Amilli Surirella	+	-	-	•	D									
Muller Seminavis strigosa (Hustedt) Danieledis & Economou-Amilli Surirella angustata	+	-	-	- 1.7	В	-	-	alf	i	b	-	-	-	-
Muller Seminavis strigosa (Hustedt) Danieledis & Economou-Amilli Surirella angustata Kützing	+	- 1		- 1.7	в	-	-	alf	i	b	-	-	-	
Mulier Seminavis strigosa (Hustedt) Danieledis & Economou-Amilli Surirella angustata Kützing Trvblionella	+	- 1	-	- 1.7	В	-	-	alf	i	b	-	-	-	-
Mulier Seminavis strigosa (Hustedt) Danieledis & Economou-Amilli Surirella angustata Kützing Tryblionella bungarice	+	- 1	-	- 1.7	В	-	-	alf	i	b	-	-	-	-
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Mulier Seminavis strigosa (Hustedt) Danieledis & Economou-Amilli Surirella angustata Kützing Tryblionella hungarica (Grunow) Frenguelli Euglenozoo	+ - +	- 1 -	-	- 1.7 2.9	B P-B	-	-	alf alf	i mh	b a-b	- sp	- ate	- e	-
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Mulier Seminavis Strigosa (Hustedt) Danieledis & Economou-Amilli Surirella angustata Kützing Tryblionella hungarica (Grunow) Frenguelli Euglenozoa Trachelomonas	+ - +	- 1 -	-	- 1.7 2.9	B P-B	-	-	alf alf	i mh	b a-b	- sp	- ate	- e	-
Mulier Seminavis strigosa (Hustedt) Danieledis & Economou-Amilli Surirella angustata Kützing Tryblionella hungarica (Grunow) Frenguelli Euglenozoa Trachelomonas volvocina	+ - +	- 1 - 1	-	- 1.7 2.9 2.0	B P-B	- -	- - st-str	alf alf	i mh	b a-b	- sp	- ate	- e	4.4-8.4
Mulier Seminavis Strigosa (Hustedt) Danieledis & Economou-Amilli Surirella angustata Kützing Tryblionella hungarica (Grunow) Frenguelli Euglenozoa Trachelomonas volvocina (Ebrenberg)	+ - +	- 1 -	-	- 1.7 2.9 2.0	B P-B B	- - eterm	- - st-str	alf alf ind	i mh	b a-b b	- sp -	- ate	- e	- - 4.4-8.4

Ehrenberg

Chlorophyta														
Eudorina elegans Ehrenberg Gemellicystis	-	-	2	2.3	Ρ	-	st-str	-	i	b	-	-	-	-
imperfecta (Korsh.) Lund	-	1	-	1.2	-	-	-	-	-	-	-	-	-	-
apiculatus (West & G.S.West) Chodat	-	1	1	-	Ρ	-	st-str	-	-		-	-	-	-
Charophyta														
Chara contraria A.Braun ex Kützing Cosmarium	-	3	2	1.1	B	-	st-str	alf	i	o-b	-	-	-	-
laeve Rabenhorst	-	6	6	1.9	P-B, aer	-	st-str	ind	i	o-a	-	-	me	5.4-9.4

Note: S (S): species-specific index saprobity. Ecological types (Hab): P, planktonic; B, benthic; P–B, planktonic-benthic, S, soil. Temperature (T): temp, temperate waters inhabitant; eterm, eurythermic inhabitant; warm, warm-water inhabitant; H₂S, anoxia indicators. Streaming and Oxygenation (Reo): str, streaming waters inhabitant; st-str, low streaming waters inhabitant; at, standing waters inhabitant; aer, aerophytic inhabitant. pH (pH): ind, indifferent; alf, alkaliphil; acf, acidophil; alb, alkalibiont. Halobity (Sal): i, oligohalobious-indifferent; hl, oligohalobious-halophilous; mh, mesohalobious; ph, polyhalobious. Saprobity (D): es, eurysaprob; sx, saproxen; sp, saprophil. Saprobity (Sap): o, oligosaprob; o-a, oligo-alpha-mesosaprob; b-p, betameso-polysaprob a-b, alpha-beta-mesosaprob; b-a, beta-alpha-mesosaprob; o-b, oligo-beta-mesosaprob; b-p, betameso-polysaprob a-b, alpha-beta-mesosaprob; b-a, beta-alpha-mesosaprob; x, xenosaprob. S: species-specific Index saprobity according [17]. Nitrogen uptake metabolism (Aut-Het) [28]: ats, nitrogen-autotrophic taxa, tolerating very small concentrations of organically bound nitrogen; ate, nitrogen-autotrophic taxa, tolerating elevated concentrations of organically bound nitrogen; hne, facultatively nitrogen-heterotrophic taxa, needing periodically elevated concentrations of organically bound nitrogen; hce, nitrogen-heterotrophic taxa, needing elevated concentrations of organically bound nitrogen; hce, nitrogen-heterotrophic taxa, needing elevated concentrations of organically bound nitrogen; hce, nitrogen-heterotrophic taxa, needing elevated concentrations of organically bound nitrogen; hce, nitrogen-heterotrophic taxa, needing elevated concentrations of organically bound nitrogen. Trophic state (Tro) [28]: me, meso-eutraphentic; e, eutraphentic; m, mesotraphentic; ot, oligotraphentic; o-m, oligo-mesotraphentic; he, hypereutraphentic.

129

One of the species that were found in the Neot Hakikar was the macrophyte alga *Chara contraria* (Fig. 3). Structural elements and the thallus habitat showed that our samples fell within the typical
 diagnosis ranks [13].

The strongly incrusted fragile thalli were 4–8 cm in length (Fig. 3). The stem cortex was diplostichous, tylacanthous or nearly isostichous. The spine-cells were solitary, papillose. The short ellipsoid stipulodes were in double rows. The branchlets were up to 12 mm in length and consisted of 2-3 corticate segments and ecorticate segment mostly broken off within studied specimens. The branchlet cortex was diplostichous and complete (the five tubes were seen). The bract-cells were unilateral, the posteriors were rudimental and the anteriors were 0.8-1.2-times longer than the oogonium. The gametangia were solitary and conjoined. The black ripe oospores were present in studied specimens.



Fig. 3. *Chara contraria*: 1 – axis with stipulodes, base of whorl, axial cortex, and oogonia; 2, 3 –
 oogonia; 3 – axis with branchlets and oogonia. Scale bar 1 mm

143

144 **3.3.** Bio-indication of the studied pool environment

145 Bio-indication methods characterized pool water quality and ecosystem sustainability on the basis of 146 the identified species (Tab. 4). The majority of taxa represent the inhabitants of benthos (37), which 147 corresponds to the small size of the pool. Temperature indicators represent not only temperate 148 species (5) but also warm water inhabitants (4). One species of cyanobacteria Pseudanabaena 149 redeckei is an indicator of anoxia and sulfides, which come from the bottom sediments as a result of 150 organic matter degradation. As a whole, the pool community contains more species that are indicative 151 of medium oxygen enrichments (11). A wide range of groups, from acidophilic to alkalibionthic, 152 represent indicators of water pH, and alkaliphilic species strongly prevailed (20). It is remarkable that 153 the spectrum of salinity indicators shift to the high-salinity group such as mesohalobes (8) and even 154 polyhalobes (1), but the group of oligonalobious-indifferent species number prevailed (17). Indicators 155 of organic pollution in [17,30] demonstrated wide ranks of species from 11 groups of saprobity from 156 which indicators of Class II and III prevailed. Watanabe's system indicators (D) were represented by 157 diatoms only and reflect medium organic enrichments. Indicators of nutrition type [28] that preferred 158 the revealed algal species shows a shift to the autotrophic groups that used photosynthetic ways of 159 protein synthesis. As a result, the trophic indicators [28] reflecting that pool environment 160 corresponded to a eutrophic ecosystem state.

161 A comparison of the seasonal dynamics shows increases in high-temperature groups in winter (Fig. 162 4.1). Substrate preferences (Fig. 4.2), salinity (Fig. 4.3) and pH indicators (Fig. 4.4), were distributed over the same groups in similar proportions in both seasons. Nutrition type indicators mostly show species of autotrophic nutrition in winter and species with heterotrophic ability in summer (Fig. 4.5). Indicators of trophic states were more diverse in summer and reflect mesotrophic and eutrophic states of the pool in both seasons (Fig. 4.6). Only one species, *Pseudanabaena redeckei*, was an indicator of anoxia and sulfides in both seasons (Tab. 4).



Fig. 4. Bio-indication of the Neot HaKikar pool. Ecological group in each histogram are follow in the indication variable increasing.

We use Tab. 4 with Index saprobity S values that were calculated on the basis of species abundance scores (Tab. 1) and species-specific index s after the Sládeček [17,30] model, and nitrate concentration (Tab. 3) data for ecosystem state index WESI calculation. Despite the Index Saprobity S values that show low-organic matter concentration, the index WESI fluctuated from 1.00 to 1.33, which can characterize the studied site's ecosystem as high capacity to self-purification.

176 3. DISCUSSION

177

178 The studied site (Neot HaKikar) is unique, not only because it is the lowermost locality in the world but 179 also because it is subjected to the impact of sand storms. As a result, the sunlight intensity decreases 180 for 20-25 percent [31] from Beer-Sheva at about 300 m above sea level down to Neot HaKikarat 181 about 356 m below sea level, altogether about 650 m of air thickness.

182 Environmental variables fluctuated in small ranges, which reflect the relative stability of the 183 environment. Tab. 3 shows that summer chemical variables (excluding nitrates) are higher than winter 184 variables. We assume that this finding could be the result of evaporation under high temperatures in 185 summer that impacted the first stage of the dissolved solids [1,8,16]. Nitrate concentration that usually 186 correlated with organic pollution is higher in winter, which can be a result of dust from sand storms that contained organically enriched particles and chlorides [10,11,32,33]. Nitrate formation in the Mediterranean region air correlated with sea-salt particle enrichments during the dust storm and increased with UV radiation up to five times [34]. As a whole, this dynamic of chemical variables and salinity and nitrates in particular can be the result of the impact of a sharp arid climate that has been revealed in the water bodies of the Arava Valley [35,36], Central Asia [36], and Southern Ukraine [37].

192 Diversity of algae in the Neot HaKikar was studied from 1995 by A. Ehrlich [29] and represented by 20 193 taxa of diatoms only. We revealed 39 species in which diatoms prevail but other species are 194 represented by cyanobacteria, euglenoids, and green and charophyte algae. Only five species from 195 Ehrlich's list and our finding overlapped. Moreover, we did not find the 15 species that were 196 represented in Ehrlich's book [29], but found 33 more species of cyanobacteria, euglenoids, green 197 algae, and the very abundant charophyte Cosmarium than Ehrlich [29] listed. Altogether, the algal 198 species list now includes fifty-four species in which diatoms prevail (Tab. 4). Species abundance 199 fluctuated between seasons and in divisional level taxa. Therefore, the most abundant species 200 included cyanobacteria (Pseudanabaena redeckei) and diatoms (Mastogloia braunii) that were 201 abundant in winter, and charophytes (Cosmarium laeve) were well developed in both seasons. Chara contraria was not abundant but were represented in both summer and winter communities. 202

203 Chara contraria is widely distributed in the Mediterranean countries and in some similar dry climate 204 regions and is cosmopolitan [38]. Species distribution is known from the sea level up to 2000 m a.s.l. 205 in Central Europe [39]. It seems that the lowermost altitude for charophytes had not been assessed at 206 all. Previously, we had shown that the species of the genus Chara in Israeli populations are well 207 separated from one another according to Amplified fragment length polymorphism (AFLP) analysis 208 [40] including Chara contraria. The Chara contraria community was dominated by diatoms (Tab. 4) 209 that attach to macro-alga as well as to stones and plants in the pool bottom. Unfortunately, periodic 210 reconstructing of the area causes the pool to dry up. During several field trips in 2002-2014 we 211 couldn't find the pool, but it was periodically renewed. As a result of periodic desiccation, the 212 charophyte plants died, but they can be renewed after one-two years after a dry period. We assume 213 that survival of Chara contraria in this dry land site can be possible with oospores stored in the pool 214 sediments. It is very important to note that the studied populations of Chara demonstrated high 215 tolerance to desertification despite the ecological consequences of climate change [32] in the region 216 [41]. Our exploration of Israeli charophytes shows that oospores can survive for at least two years that 217 we know of [3] but possibly even longer.

Halobity groups' alignment with brackish-water indicators suggests long-term effects of salinity increases in the long process of diversity developing in the pool in excessively arid environments. As has been revealed [31,43], the natural water streams in the Arava Valley, and the Neot HaKikar in particular, have a permanent trend in decreasing in water content under the impact of an arid climate. On the other hand, the source of salinity in the Arava Valley comes from atmospheric dust, which combines with nitrates during the dust storms [34].

As can be seen in Tab. 4, the water quality defined by bio-indication is the same that is shown by the chemical components of the water (Tab. 3). In conclusion, we can characterize the studied pool as mesotrophic to eutrophic with prevailing benthic types of organisms with an autotrophic type of nutrition, which are mostly attached to substrates and prefer standing water with medium oxygen enrichment water, temperate temperature, moderate salinity, low alkalinity, and low-to-middle organic pollution.

Few species of filamentous cyanobacteria and euglenoids (Tab. 4) can confirm that the charophyte site is impacted by organic and other contaminants mostly in winter. This situation is similar to that of the Upper Jordan River previously examined by us [42], where the pollution coming from the catchment area pollutes the water more in winter than in summer. The source of nitrates in the Arava Valley is from atmospheric dust, which brings saline particles during the dust storms on which nitrates forming from the atmospheric gases are helped by UV radiation [34].

We can assume that there are only a few polluting factors that influence the water quality at Neot HaKikar pool and its algal community. Because the pool is under Mekorot company protection, there is no strong pollution impact. But we can see that the anthropogenic influence comes from periodic reconstruction of the pool area, which we in particular observed during 2012-2013. Unfortunately, the studied pool is still under climatic impact also, which provokes increases in salinity and nitrates[34,41], and therefore algal species richness will change [44,45].

On the other hand, this area is under decreasing sunlight during the sandstorms that periodically come from the Sahara Desert, from the Arabian Desert across the Negev Desert [10]. Massive dust transportation not only covers large deserted areas [11], such as the Arava Valley, but also decreases in sunlight intensity during the day. It is especially important in the lowermost area near the Dead Sea in which light intensity decreased 25% [31] as a result of the dust layer thickness, which is more than 250 m.

As a protected mechanism, algal cells formed special compounds [46] as a response to the UVradiation impact [47] on the one hand, and negatively reacted to sunlight inhibition. Increasing UVradiation effects include inhibition of photosynthesis, inhibition of growth, and DNA damage. As a result, algae have developed a mechanism of avoidance as well as adaptation to light intensity fluctuation during its evolutionary process. It especially relates to the charophyte species definition.

253 Well known is that Chara vulgaris and C. contraria are two cosmopolite species that are sometimes 254 difficult to distinguish one from another [48]. Moreover, these species often occupied the same 255 habitat, as we revealed in the Negev Desert stream Ein Avdat [32,40]. Because each charophyte 256 species evolved in the presence of UV radiation, a multitude of adaptive strategies have been 257 developed, which allowed them to exist under sunlight exposure (C. vulgaris) or in less exposed 258 places (C. contraria) [13], and the repair of DNA damage as a result of developing a major 259 mechanism of UV adaptation [47]. As at was found in our research for the Avdat stream, with the 260 AFLP analysis the charophyte populations is divided into clusters corresponding to the levels of light 261 intensity over the shadow gradient in this deep canyon in the Central Negev. Therefore, we can 262 assume that environmental preferences of both morphologically similar species of Chara are 263 entrenched in the process of evolution as a result of repairing injured DNA by ultraviolet radiation and 264 subsequent consolidation of other features. As a result, we are seeing the shade-tolerant C. contraria 265 in the Arava Valley inhabiting the lowest place in the world that is affected by the shading of the dust 266 layer more than 500 meters thick.

267 5. CONCLUSION

268

269 The new unique locality, the Neot HaKikar pool in the Arava Valley, is a protected area near the Dead 270 Sea and is the lowermost habitat of charophytes in the world that is located at an altitude of about 350 271 m below sea level. The pool's environment can be characterized as natural, brackish, low alkaline 272 with low-to-middle organic polluted waters that are inhabited by 39 (54 with references [29]) algal 273 species from which the charophyte Chara contraria and diatoms dominated. The charophyte C. 274 contraria, which is distributed all over the world, after one-two years of periodic desiccation can be 275 renewed with the help of oospores buried in bottom sediments. This unique property of C. contraria 276 can help charophytes survive in the Eastern Mediterranean region, which is under desertification with 277 the impact of periodic sandstorms that decrease photosynthetic radiation intensity about 25% as a 278 result of high dust concentration and regional climate change.

Therefore, the Neot HaKikar pool as a unique charophytes habitat, ecosystem of which have high capacity to self-purification, can be protected for anthropogenic reconstruction, as well as its water quality and algal communities can be studied and monitored for more detailed characteristics of diversity that we have presented here mostly for the first time, and in indicating various climate change parameters.

284

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