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Visualization of transparent exopolymer particles (TEP) in various source waters

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ABSTRACT

Transparent Exopolymer Particles (TEP) have been implicated as an important factor in the development of aquatic biofilm on membranes and other surfaces. We have recorded TEP and associated bacteria from four different kinds of source water (coastal seawater, freshwater lake, secondary treated wastewater and saline, high sulfide content groundwater from a deep well) as visualized by light and epifluorescent microscopy after dual-staining with Alcian Blue combined with SYBR Green. Significant concentrations of TEP were measured in all water sources. We illustrate some of the wide variety of morphological forms that these particles can assume and indicate obvious differences in the characteristics of TEP from different source waters. Visualization, together with measurement of TEP concentrations can serve as useful indicators of biofouling potential in feedwaters. These images should lead to a better appreciation of the impact of TEP in fouling of surfaces such as RO and UF membranes and the need to develop effective measures to minimize the levels of these particles in source waters.

Keywords: TEP images; Transparent exopolymer particles; Biofilm formation; Marine; freshwater; Treated wastewater; Saline well water

1. Introduction

Although oceanographers and limnologists have been aware of the ubiquitous presence of Transparent Exopolymer Particles (TEP) for almost two decades [1], the concept that these particles play a significant role in the establishment and development of aquatic biofouling in desalination and water treatment plants is relatively recent [2,3]. Strong evidence for the involvement of TEP in early biofilm formation was indicated when Bar Zeev et al. [4] found that initial attachment of these particles to clean surfaces immersed in sea water covered much greater areas than those taken up by attached individual bacteria. These investigators also showed that the levels of TEP in source water were not very effectively lowered by standard pretreatment prior to reaching RO membranes in a modern desalination plant. Other studies have also confirmed that TEP levels are not significantly lowered by pretreatment from source waters in desalination and wastewater treatment plants [5–7].

TEP are numerous in most natural waters such as pelagic and coastal sea water, lakes, reservoirs [8,9] as well as in recycled gray water [5]. As the name implies, TEP are transparent, microscopic organic particles ranging in size from about 0.4 to >200 μm. These particles are mainly composed of polysaccharides, although proteins and nucleic acids may also be present. TEP have a large, negatively charged, surface area that makes them very sticky [8] especially in saline

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waters [10]. Many TEP in natural waters are colonized by bacteria that find them a convenient and nutritional platform on which to grow [11]. These particles have been found to play important roles in the healthy ecosystem functioning of oceans and lakes [8,12,13]. There is a voluminous literature, mainly in oceanography, dealing with the physical and chemical characteristics of TEP and their involvement in a wide range of processes important to ecological function in aquatic systems [8]. Recently, a 0.05 to 0.4 μm sized fraction of acid polysaccharide staining ''colloidal'' TEP has been reported in both marine and freshwater that was even more abundant than >0.4 μm TEP [14].

TEP have also been termed ''macro-colloids'' and ''macrogels'' [15] and may be regarded as ''planktonic exopolymeric substances (EPS)'' [3,4]. These particles appear in many forms; amorphous blobs, filaments, clouds, clumps and sheets. TEP are sometimes recognizable as debris from broken plankton [9]. However, there are relatively few papers actually showing images of the very diverse morphological shapes taken by these particles. Because it now appears that TEP are intimately involved in biofilm formation on sensitive surfaces such as membranes in desalination and water treatment plants [4,7,16], we attempted to observe and characterize in the microscope some of the many different forms of TEP in typical source waters. In this paper, we show a variety of examples from a freshwater lake, coastal seawater, secondary treated wastewater and a deep (600 m) saline groundwater well. These micrograms provide vivid evidence of the various forms that TEP can assume in different aquatic environments. These images should lead to a better appreciation of the potential impact of TEP in the formation of biofilm on surfaces such as RO and UF membranes and the need to develop effective measures to minimize the levels of TEP in source waters.

2. Materials and methods

Water samples were collected over a period of two years from the following sites: 1. Lake Kinneret, freshwater. 2. Palmachim Desalination Facility intake, coastal seawater, 3. Shafdan Wastewater Treatment Plant, secondary treated sewage water, and, 4. Ketziot Desalination Plant, saline groundwater from a 600 m deep well in the Negev region of Israel.

For microscope observation, water samples (10 to 100 ml, depending on source) were vacuum filtered (under maximum 100 mm of Hg) onto 25 mm, 0.4 μm Nuclepore filters. The samples were first filtered down to a volume of 5 ml. In order to stain bacterial cells, 50 μL of 1:100000 diluted SYBR Green 1 (Invitrogen) were added for 5 min. Filtration of the samples was then continued down to ~0.3 ml. Then 0.5 ml of 0.02% Alcian Blue (AB) in 0.2% acetic acid was added to stain for acid polysaccharides and filtration immediately resumed. After rinsing with 5 ml of 0.2 μm filtered distilled water (or 0.2 μm filtered seawater or groundwater for marine and groundwater samples, respectively), the filters were transferred to Cytoclear microscope slides (GE Clearing Slides, GE Osmonics Labstore). The coating on these glass slides enables direct microscope viewing of material collected on the Nuclepore filters without interference by the polycarbonate filter matrix. A small drop of immersion oil was placed on the filter surface which was then covered with a glass cover slip that was carefully pressed from above.

The slides were observed under regular light illumination for TEP (blue staining with AB) and with epifluorescent U.V. illumination for bacteria after SYBR Green (SG) staining. For visualization of TEP and associated bacteria we used Image Pro software combined with an Applitec SuperCam camera.

Concentrations of TEP in the water samples were measured using the method of Passow and Alldredge [17]. Note that these concentrations are conventionally given in units of μgGX L^{-1} (Gum Xanthan, taken as a model particulate polysaccharide) and not in conventional units (e.g. moles L^{-1} or mgC L^{-1}). However, these units have been widely used to quantify TEP and allow comparison of relative amounts of these particles in various aquatic environments.

3. Results and discussion

3.1. TEP concentrations in various source waters

In Table 1 we show the ranges of TEP concentrations that were measured in various source waters. The highest concentrations of TEP were measured in the secondary treated sewage water as could be expected, given the high dissolved and particulate organic matter levels in this source. Kennedy et al. [5] reported a much lower TEP level in secondary wastewater effluent in a Dutch treatment plant, perhaps reflecting colder operating conditions and different treatment procedures. At present, we have no information concerning the sources of TEP in these secondary treated effluent waters, these could be formed via abiotic processes (e.g. coagulation from colloidal precursors) and/or through microbial mediated processes (bacterial or algal mucus proliferation).

The TEP concentrations measured in freshwater Lake Kinneret during this study were in the same general range as have been previously reported for this lake [9]. In contrast to Villacorte et al. [7], we found

Table 1
Average and range of TEP concentrations in different source waters*

	TEP (μg GX/L)		
	Average	Range	*n*
Freshwater (L. Kinneret)	1,605	759–2385	16
Coastal Seawater	491	80–1003	18
Treated Wastewater	2,265	746–4157	35
Saline groundwater	288	132–417	16

*Measurements during 2008-2009.

higher TEP concentrations on average in freshwater than in seawater. However, in our case, the freshwater source was from a mesotrophic-eutrophic lake with relatively high phytoplankton biomass and microbial activity, while the seawater was coastal water from the oligotrophic Levantine Basin with lower TEP levels than North Sea water. A recent study of TEP in the Levantine Basin measured TEP concentrations ranging from 19 to 600 μgGX L^{-1} (Bar Zeev, pers. com.).

The presence of measurable quantities of TEP in the deep-lying saline groundwater was unexpected although these waters are known to harbor sulfur and iron bacteria (Nechushtai, pers. com.). Possibly these microorganisms were the source of the TEP observed but this has not been investigated. The present data appear to be the first report of TEP presence and concentrations in this kind of source water.

As can be seen in Table 1, we measured considerable variation in the TEP concentrations during the period of this study within each of the water sources examined.

3.2. TEP Image Gallery

3.2.1. Lake Kinneret; freshwater (Fig.1)

High concentrations of TEP were found in lake water at all seasons and at all depths in a previous extensive and detailed study by Berman and Viner-Mozzini [9]. In near surface and coastal samples taken during 2008 and 2009, TEP concentrations ranged from 759 to 2,385 μgGX L^{-1}. Lake water samples examined in the microscope showed a very wide diversity of morphological forms of TEP (Fig. 1). Often these particles had an amorphous, cloud-like appearance (Fig. 1a). AB stained particles could sometimes be clearly identified as originating from particulate detritus, disintegrated algal cells or zooplankton body parts (Figs. 1c,e). Frequently TEP appeared as sheets of mucilaginous-like material either surrounding or still partially attached to algae and cyanobacteria (Fig. 1g). Most, but not all, of the TEP had considerable numbers of bacteria associated with them (Figs. 1b,d,f,h). We also observed clusters of bacteria with no correspondence to TEP or other visible particles (not shown). Between 40% to 80% of the TEP (seen as AB-stained particles) also showed a dim green fluorescence when viewed under UV. This fluorescence probably derived from nucleic acid molecules either incorporated within the TEP or adsorbed to the surface of the particles.

3.2.2. Palmachim Desalination Plant intake; Coastal Mediterranean Sea water (Fig. 2)

Although the Eastern Mediterranean Sea is extremely oligotrophic, the coastal waters have somewhat higher nutrient levels. In comparison with the freshwater samples no distinctly ''marine'' morphological forms of TEP were observed but we noted a tendency for a much higher proportion of filament-like AB stained particles (Fig. 2 a,b,d). Here too, TEP was often attached to algal cells (Fig. 2d,g). Not surprisingly, there were considerably fewer bacteria visible in marine samples and these tended to be smaller and more heterogeneously scattered than in lake water. A lower proportion of marine TEP had bacteria associated with them. Nevertheless, some marine TEP were densely populated by bacteria (Fig. 2f) and, more frequently than in lake water samples, we observed dense bacterial clumps or clusters unrelated to any AB staining particles (Fig.2h). Some marine TEP were clearly derived from algal cells and very occasionally we found detritus of larger organisms (e.g. copepod body parts) that partially stained with AB (not shown). However, the precise origin of most marine TEP could not be ascertained.

3.2.3. Shafdan Wastewater Treatment Plant after secondary treatment (Fig. 3)

As expected, the highest concentrations of TEP in our study were recorded in the Wastewater Treatment Plant. Here many of the particles were only partially stained with AB, many showed a brownish colored, solid sections (Fig. 3 a,g) which may have due to some inorganic component such as silica grains, possibly deriving from the sand filters through which these waters had passed in pretreatment. Almost all AB staining particles in these samples were heavily populated by bacteria and most also showed dim overall SG staining indicating the presence of surface-adsorbed nucleic acids (Fig 3b,d,h). Some of the TEP appeared to originate from the mucilage surrounding cyanobacterial (Fig 3e,f) or algal cells (Fig. 3g,h).

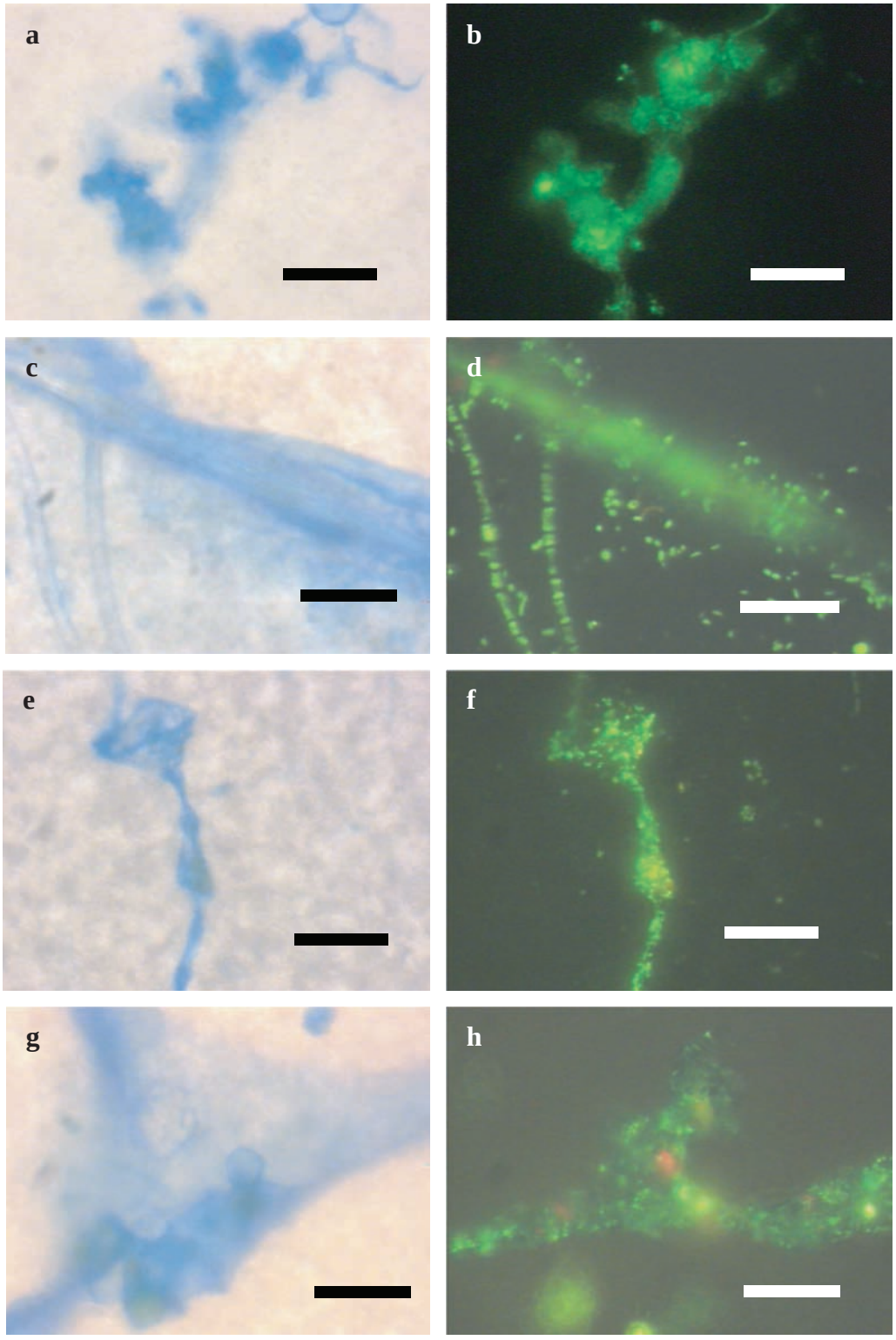

Fig. 1. TEP in Freshwater (Lake Kinneret). TEP particles when stained with Alcian Blue dye are colored blue under regular microscope illumination (left panels). When the same microscope fields are viewed with ultra-violet epifluorescent illumination (right panels), bacteria staining with SYBR Green appear as bright green spots or rods. The faint green staining of some, but not all, TEP is probably due to nucleic acids adsorbed to these particles. Red staining objects are chlorophyll containing algal cells. See text for further details. Scale bars = 10 microns.

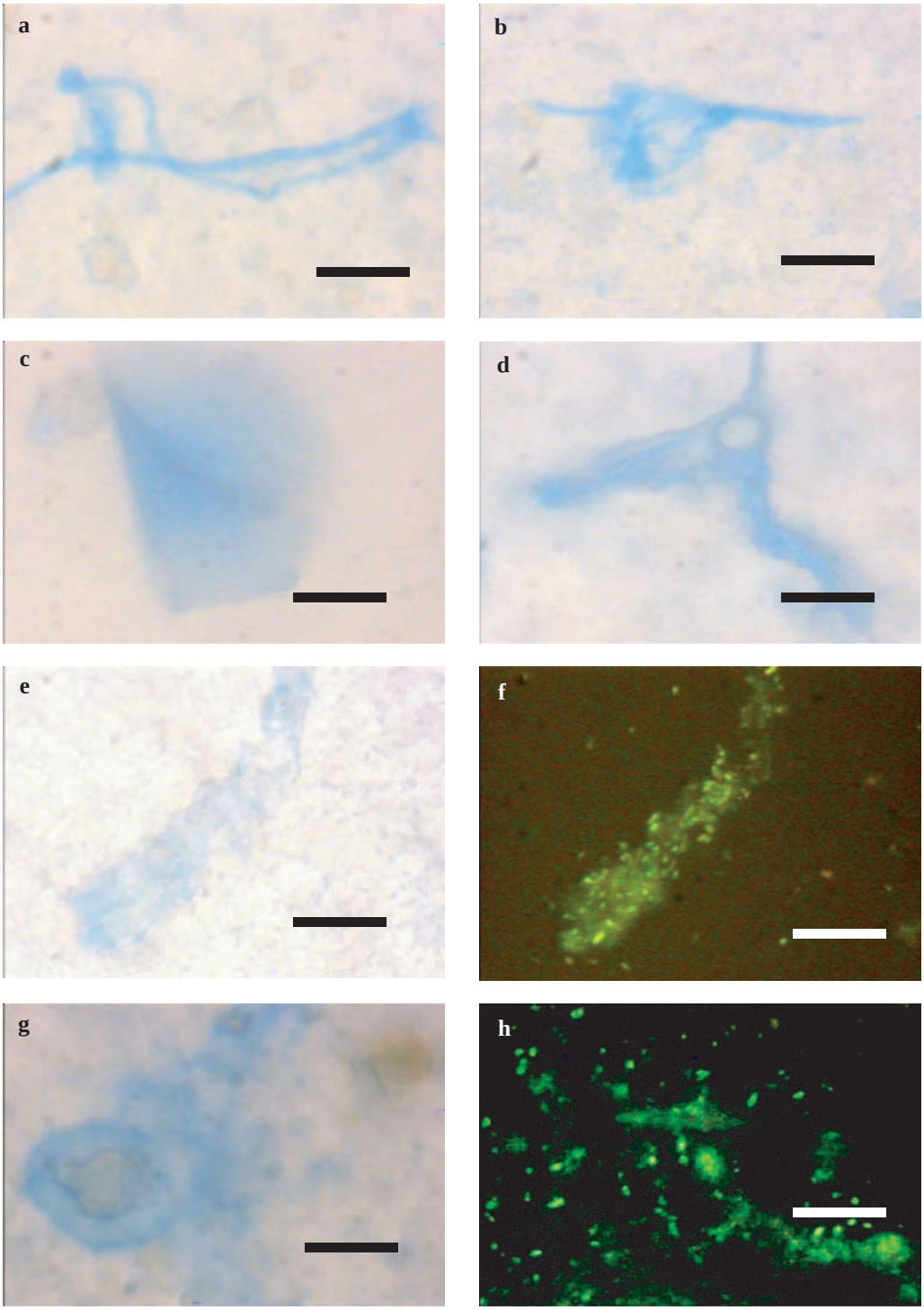

Fig. 2. TEP in Coastal Mediterranean Sea Water (Palmachim Desalination Plant). Legend as in Fig. 1. With the exception of Figs. 2f and 2h, images were viewed under regular illumination. See text for further details. Scale bars = 10 microns.

3.2.4. Ketsiot Desalination Plant intake: Saline, sulphide and iron-rich, deep well water

As noted, these saline groundwaters pumped from a 600 m deep well had measurable concentrations of TEP (Table 1). Here the AB staining particles were quite different and more densely packed than TEP observed in other waters. Although there were some cloud-like TEP (Fig. 4a), most TEP were associated

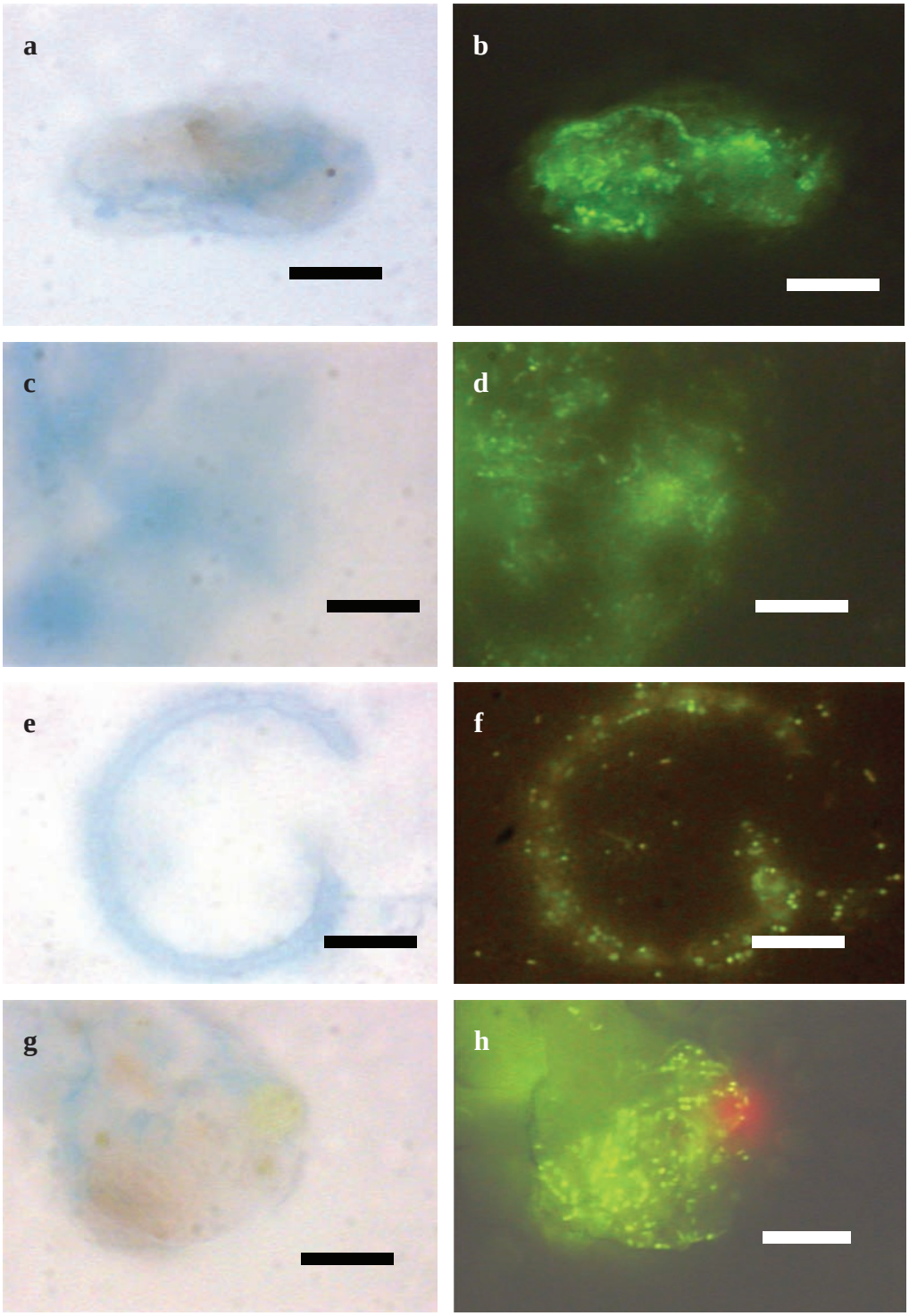

Fig. 3. Recycled secondary treated wastewater (Shafdan Water Treatment Plant). Legend as in Fig. 1. See text for further details. Scale bars = 10 microns.

with large (>10 μm) unidentified biological cells with very few bacteria on their surface (Fig. 4e,f,g,h). Large, probably inorganic, perhaps iron or sulfur containing particles were often attached to TEP in these samples (Fig. 4c,e). No filamentous TEP forms were observed. Rather strangely, although clumps of bacteria were observed, these often appeared on areas not stained by AB (Fig. 4b,d).

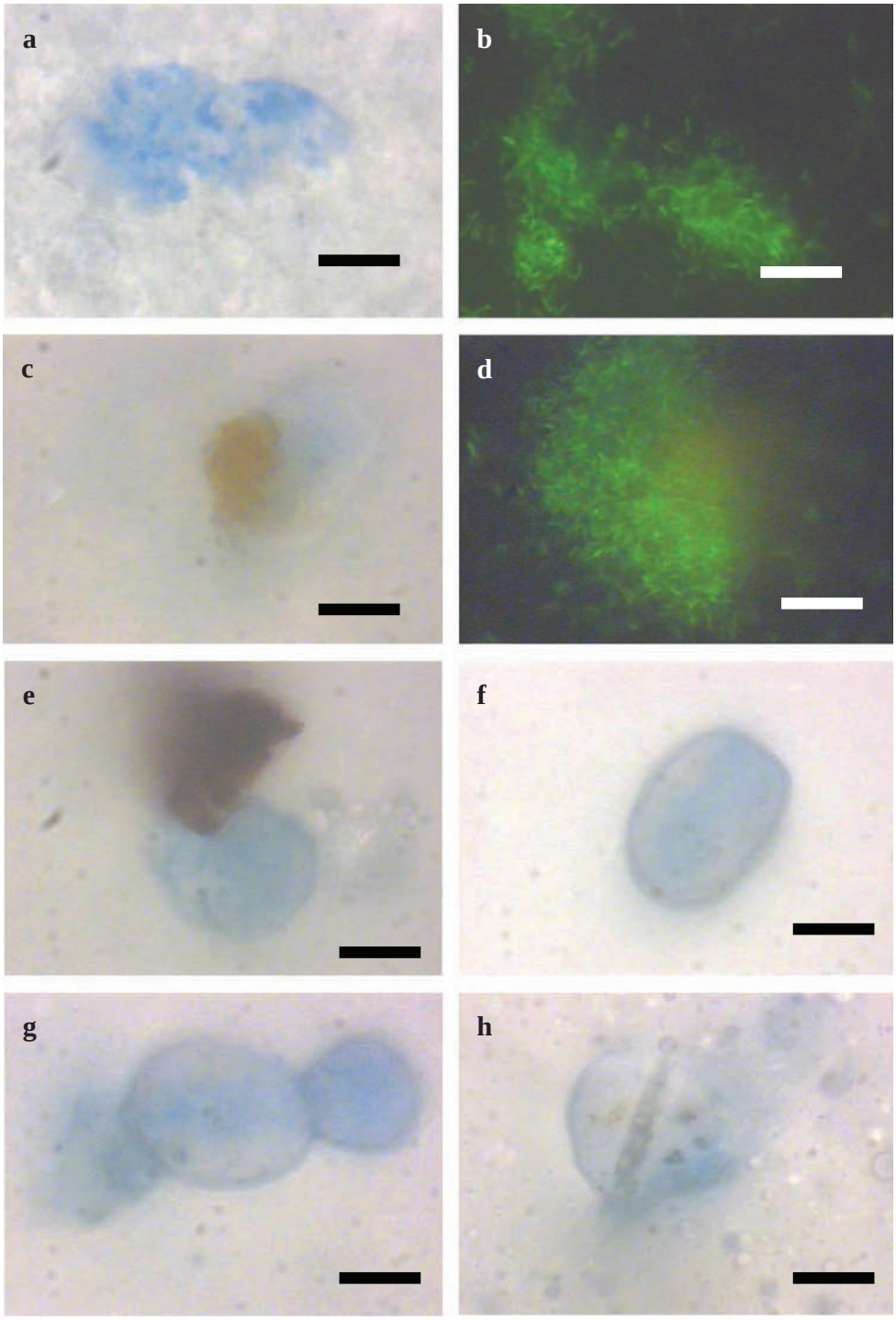

Fig. 4. Saline, sulfide and iron rich groundwater (Ketsiot Desalination Plant intake). Legend as in Fig. 1. See text for further details. Scale bars = 10 microns.

4. Conclusions

Abundant TEP were present in every kind of water source examined. We have been able to show only a few of the many diverse morphological forms of TEP that were observed in these samples. As detailed above, there were some obvious differences in the characteristics of TEP from different source waters; for example, the obvious preponderance of filamentous forms of TEP in marine coastal waters, or the dense associations of bacteria with TEP in treated

wastewater. The visual evidence for the presence and diversity of form of these particles in all kinds of source waters should serve to emphasize the potential impact of TEP as active agents in the process of biofouling. Not only are these highly sticky [8,10] planktonic particles able to adhere to surfaces and become part of biofilm EPS [4] but as others [11] have observed and we have illustrated in this study, many of these TEP are carrying swarms of bacteria. It is as yet unknown whether these bacteria are involved in further development of biofilm. Note that we have shown only TEP that were retained by 0.4 μm filters, our imaging techniques would not show any trace of the 0.05–0.4 μm ''colloidal'' TEP fraction reported by Villacorte et al. [14].

Increasing evidence indicates that lowering the concentrations of TEP by pretreatment of source waters in desalination and water treatment plants could alleviate problems of biofouling on membranes [4,7,16]. However, effective filtration or other means of TEP reduction in feed water reaching sensitive surfaces such as RO membranes will not be easy to achieve [16]. Although experimental evidence is meager, it would seem likely that TEP from different source waters will have very different chemical and physical characteristics. Visualization, combined with physicochemical characterization and quantification of TEP may be helpful in evaluating the efficacy of pretreatment technologies as well as assessing the fouling potential of different source waters.

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