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Marine Biofouling Potential on Reverse Osmosis Desalination Membrane and Other Substrata Corresponding to *in situ* Microbial and Physicochemical Characterization of Seawater

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Abstract: *In situ* investigation was designed for following up the biofouling community formed on the different substrata representative of the desalination unit components including Teflon, Glass, Stainless steel and Reverse Osmosis desalination membrane (RO desalination membrane), submerged in the seawater of the Eastern Harbor, Alexandria, Egypt. The area of study was physico-chemically and microbially characterized along 84 days, the time of the experiment. Some physico-chemical parameters such as Temperature, pH, dissolved oxygen, salinity, and nutrients (ammonia, nitrite, nitrate, phosphate, and silicate) were estimated. For *in situ* microbial characterization of seawater, the total heterotrophic bacteria and total marine fungi, in addition to several bacterial groups were counted. Data indicated that the total count of heterotrophic bacteria increased linearly with time until it reached the peak after 42 days of submerging coupons ($4x10^6$ CFU/ml), then it decreased with the time until the end of submerging trail. The count of common pathogenic bacteria *Staphylococcus* sp., *Vibrio* sp. And *Aeromonas* sp. was in same trend but the counts of *Staphylococcus* sp. were more than of the others. On the other side, *Salmonella/Shigella* group was not detected in samples at all. The fecal indicator bacteria exhibited moderate counts, while the count of total marine fungi in the seawater samples showed low counts along the period of submerging coupons. Regarding the marine fouling, a total of 36 coupons were examined along 84 days of submerging in the seawater. Throughout the study 21 species of marine macro-fouling were recorded in addition to the very thin layer of blue-green algae. These belonged to different groups as follows: Macroalgae (2 species), Hydriods (1 species), Polychaets (5 species; 3 sedentarian and 2 errantias), Cirripeds (4 species), amphipods (4 species), Taniads (1 species), bryozoan (2 species), and tunicates (2 species). There are clear dominances by two sessile species. These are the polychaete (tube worm); *Hydroideselegans* and the barnacle; *Balanusamphitrite*. In average, they constituted about 50% and 29% of total fouling abundance, respectively. Across the different substrates, the average total abundance and biomass of the developing fouling community was comparable during different intervals of immersion except for the glass which showed the lowest values. Responsible factors for these variations are discussed.

Keywords: Marine Biofouling, Reverse Osmosis Membrane, *in situ* Characterization

1. Introduction

Huge quantities of raw seawater are being withdrawn from the sea for the unimpeded production of freshwater, greatly needed for sitting desalination in the Middle East region [1]. Seawater desalination, at present, provides approximately 1%

of the world's drinking water supply, and this percentage is increasing by the year [2]. The effect of marine environment has been found to ramify over the plants, principally as biofouling of intake structures, pumps, seawater piping system, heat exchangers, etc. Despite widespread occurrence, not much information is available on the instances of biofouling encountered in desalination and power plants [1]. Technological advances in reverse osmosis (RO) membranes during the past decade have significantly reduced the energy cost of water production via desalination [2]. Membrane fouling still posts as one of the major obstacles in membrane distillation. So, this approach still cannot successfully compete with other conventional seawater desalination methods [3]. However, membrane biofouling is one of the most important practical problems facing RO plant operators and membrane manufacturers. It is the accumulation of marine organisms and their metabolic products (i.e., extracellular polysaccharides [EPS], proteins, and lipids) on the membrane surface [4, 5]. Excessive membrane biofouling results in increased energy demand for salt separation and the deterioration of product water quality [6, 7]. Although research efforts have been devoted to prevent or alleviate biofouling (e.g., disinfection, chemical cleaning, and aeration) [8, 9], these treatments yield temporary results. Advancements in membrane materials and the optimization of operational conditions have contributed to biofouling prevention [8, 10 and 11]; however, these changes cannot eliminate it. The accelerated growth of biofilm on RO membranes likely is due to the physiological response of the bacteria. Systematic and effective strategies for biofouling control have not been established. Investigations of the microbial community that causes RO membrane fouling have not progressed much beyond studies focused on freshwater or wastewater RO treatment systems [12-15]. Earlier studies on the cultivation and isolation of fouling organisms laid the critical foundation for our understanding of biofilm formation on the membrane surface [16, 17], yet it was discovered recently that these results do not necessarily reflect the true composition of the microbial community of membrane biofilm, because a very small fraction of the bacterial community can be cultivated under laboratory conditions [18]. More recently, culture-independent methods have been used to examine the RO membrane biofilm in drinking water and wastewater treatment plants [12, 13 and 15]. On the other side, the bacterial diversity and physiology of seawater are significantly different from freshwater and wastewater. So, the bacteria that cause biofilm formation on seawater RO (SWRO) membranes may be significantly different from those in freshwater RO systems [13].

Therefore, the current study was suggested to understand the nature of biofilm formation *in situ* and its further development on some materials exposed to sea water (Glass, Stainless steel, Teflon and RO desalination membrane), the composition of biofouling communities in space and time, the succession of flora and fauna in a biofouling environment and the principal ecological factors having a bearing on the phenomenon. It is worth mentioning that investigations of the microbial community and the successive flora and fauna that cause RO membrane biofouling have not studied well.

2. Material and Methods

2.1. Substrata

Coupons measuring (7.5x2.5 cm) of four different artificial substrate types were used, including; Glass, Stainless steel, Teflon and RO membrane. The coupons of the four different substrata were fixed to a wooden rack suspended vertically 0.5 m under seawaters level. This experiment was designed to last for 3 months during summer season. In the present study, 9 wooden racks were used to collect fouling organisms during variable intervals ranged from 1st week to 8th week, in addition to a three months interval (12 weeks), during summer 22 June –21 September, 2012.

2.2. Media

The composition of each media used throughout the work was given in gl-1. The pH value of the media was adjusted to 7.2 prior to sterilization for bacteria and 5.6 for fungi. Autoclaving was generally carried out 121^{o}C for 15 min except the media that needed for boiling not for autoclaving. Seawater agar [18] was used for enumerating the total heterotrophic bacteria, while potato dextrose agar was used for counting the total marine fungi. Seven selective media were also used for counting the other bacterial groups, such as; mannitol salt agar [19] for staphylococcus sp., thiosulfate citrate bile salt sucrose agar [20] for *vibrio* sp., m-endo-les agar [21] for streptococcus sp., mFC agar [21] for *E. coli*, m-enterococcus agar [22] for total coliform, salmonella/shigella agar [23] for salmonella and shigella sp., and aeromonas agar [23], for aeromonas sp.

2.3. In situ Biofouling Estimation

For investigating and counting the biofouling community upon the different coupons submerged in seawater of the Eastern Harbor, Alexandria, Egypt, the samples were withdrawn every week along the period of the experiment. In addition, the total heterotrophic bacteria occupied on the different coupons were counted weekly during withdrawing the water sample. As well as, total fungi besides the selective bacterial groups were enumerated. Moreover, the physico-chemical parameters and bacterial count of the seawater were estimated weekly too.

2.4. Study Area

This work was carried out in the Eastern Harbor of Alexandria, Egypt. This Harbor is a relatively semi-closed basin, situated at longitudes 29º 53´ to 29º 54´ 40" E and latitudes 31º 12´ to 31º 13´ N (Figure 1). It is sheltered from the sea by a break-water leaving two openings (Boughaz) through which the exchange of water between the Harbor and the open Mediterranean Sea takes place. The area of the Harbor is about 2.53×10^{6} m², with a maximum depth of 11m.

The average water depth of the bay is about 6.0 m and it receives many kinds of vessels especially fishing boats.

Fig. 1. *Location map of the Eastern Harbor of Alexandria and sampling site.*

2.5. Coupons Preparation

Four test coupons (Glass, Stainless steel, Teflon and RQ membrane, measuring; 7.5 x 2.5 cm) were used to collect the biofilm and marine fouling organisms. Panels were submerged vertically 0.5 m under water level in seawater of the Eastern Harbor, Alexandria, Egypt, where the transparency of water at the Harbor ranged between 120 cm and 190 cm during different periods of panel immersion. The test couponswere fixed to a wooden rack suspended vertically 0.5 m below water surface. The surface of Stainless steel and Teflon that facing seawater was roughened at the beginning of the experiment, using sand paper. FT30 Reverse Osmosis membrane was used which is single-pass seawater desalination. The couponswere suspended beside the jetty of the National Institute of Oceanography and Fisheries, Alexandria.

2.6. Sample Collection

Seawater samples were collected from marine environment during summer 2012 at Eastern Harbor, Alexandria, Egypt. The samples were collected for analysis using the Nansen water sampler according to the method described by Grasshoff (1976) [24]. Samples were collected in sterile bottle with screw cap from a depth of 1.5 meter at the sites under investigation. The water samples were collected in 500 ml sterile screw-caped bottles as described by Austin (1988) [25]. The collected samples must be kept in the dark and transferred into laboratory immediately and maintained at 4ºC in refrigerator for further studies.

2.7. Counting the Marine Microbes

Counting of marine bacteria from the seawater samples was performed by serial dilution and spread plate method. One ml of collected samples was serially diluted in sterilized seawater to get a concentration range from 10^{-1} to 10^{-4}. A volume of 1 ml of each dilution was transferred aseptically to nutrient agar plates and other selective media for other microbial groups. The plates were incubated at appropriate temperature for 24-48 h for bacteria and 5-7 days for fungi [26].

2.8. Physico-chemical Analysis of Seawater

Seawater temperatures were measured by a normal thermometer (0-50). The pH values of the water samples were measured using Beckman digital pH meter model 3560. There were five parameters were measured as the main chemical parameters of seawater; dissolved oxygen [27], dissolved phosphate [24], dissolved nitrate [27], dissolved nitrite [24] and dissolved ammonia [28].

2.9. Estimation of the Biofouling Communities

The settlement of fouling organisms on coupons was estimated either by counting or determining the covering percentage. The total abundance of fouling organisms was estimated only for the countable species per coupon area whereas; the total biomass (wet weight in grams) was estimated for the whole community after removing excess water by blotting paper.

2.10. Identification of the Biofouling Communities

Each coupon was examined carefully for marine fouling and associated foulers using stereo zoom microscope (20X). Identification of marine fouling was almost done to the species level, using standard literature and keys [29-33].

3. Results and Discussion

The location (Eastern Harbor, Alexandria, Egypt) at which the different coupons submerged for studying marine fouling biodiversity was physico-chemically and microbially characterized along 84 days; the period of *in situ* experiment.

3.1. Microbial Characterization of Seawater

In seawater samples, the total heterotrophic bacteria and the total fungi were counted. In addition to several bacterial groups (*Staphylococcus* sp., *Vibrio* sp., *Aeromonas* sp. and *Salmonella*/*Shigella* sp.) were counted. As well as, fecal indicator bacteria (Total coliform, *E. coli* and *S. feacalis*) were detected in such location. However, these results are presented in Fig. 2-5.

Data in Fig. 2 indicated that the total count of heterotrophic bacteria increased linearly with time until it reached the peak after 42 days of submerging panels ($6.5x10^6$ CFU/ml), then it decreased with the time until the end of submerging trail. However, these counts were moderate. Moreover, the count of total marine fungi in the seawater samples (Figure 3) showed low counts along the period of submerging panels. The counts ranged from 16 to 64 CFU/ml after 56 and 21 days, respectively. Figure 4 illustrates that the count of common pathogenic bacteria *Staphylococcus* sp., *Vibrio* sp. and *Aeromonas* sp. was in same trend but the counts of *Staphylococcus* sp. were more than of the others.

Also, the highest count of each was at 42 days; 3700, 220 and 150 CFU/ml for *Staphylococcus* sp., *Vibrio* sp. and *Aeromonas* sp., respectively. On the other side, the count of *Salmonella/Shigella* group was not detected in samples at all. As shown in Fig. 5 the fecal indicator bacteria (Total coliform, *E. coli* and *S. feacalis*) exhibited moderate counts but the highest counts were observed after 56 days as; 6000, 2400 and 2300 CFU/100ml for Total coliform, *E. coli* and *S. feacalis*, respectively.

The existence percentage of total heterotrophic bacteria (%) in seawater of Eastern in situ during the period of exposure was calculated. Data presented in Figure 6 showed that the heterotrophic bacteria were more existant in seawater samples than the other isolated on the selective media. The most existence percentage was detected on 6th and 7th week of exposure time (99.9%), while the lowest was detected after the 12th week (88.7%). This result confirmed the need to the package of confirmatory tests to characterize and identify the bacterial species.

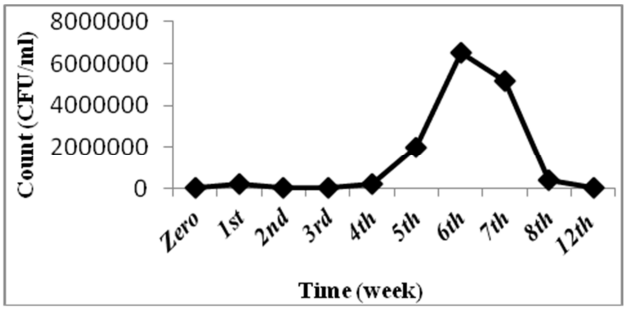

Fig. 2. *Total bacterial counts (CFU/ml) in the seawater of the Eastern Harbor during the period of exposure.*

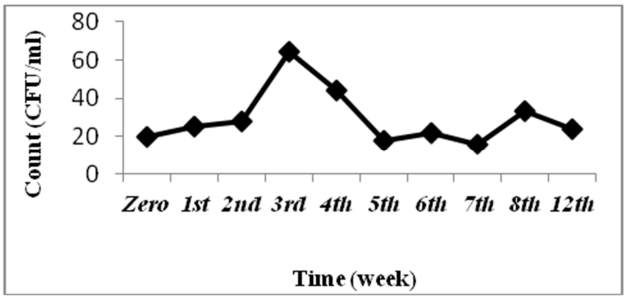

Fig. 3. *Total Fungal bacteria counts (CFU/ml) in the seawater of the Eastern Harbor during the period of exposure.*

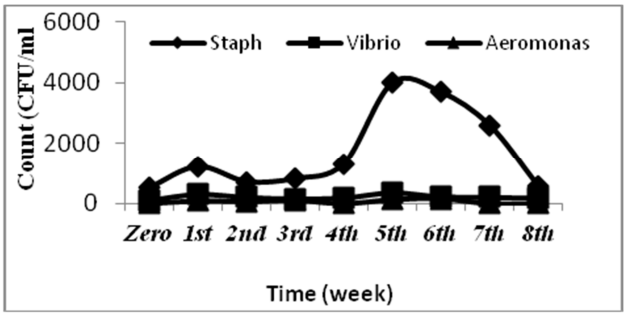

Fig. 4. *Pathogenic bacteria counts (CFU/ml) in seawater of the Eastern Harbor during the period of exposure.*

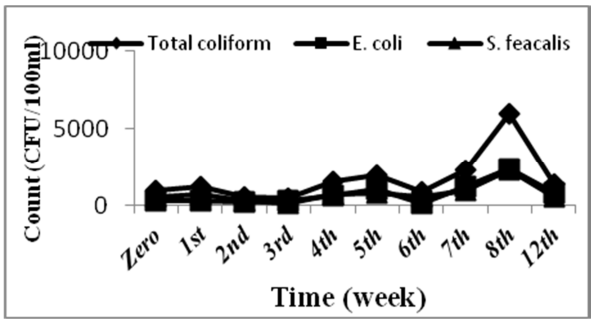

Fig. 5. *Fecal indicator bacteria counts (CFU/100 ml) in seawater of the Eastern Harbor during the period of exposure.*

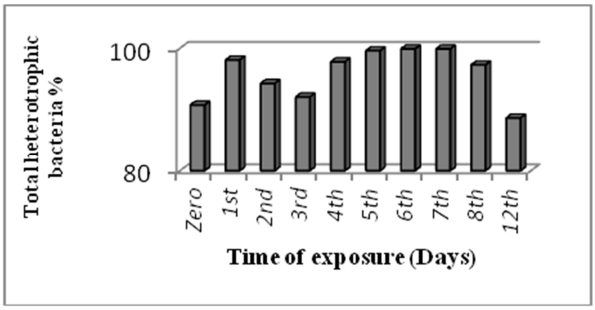

Fig. 6. *The existance percentage of total heterotrophic bacteria (%) in seawater of the Eastern Harbor during the period of exposure.*

These findings may be due to the fluctuation of the environmental conditions of the study area; it is a fishery harbor, besides there are many constructions such as clubs and other human activities throw several types of wastes into seawater. These wastes actually activate many microorganisms and flourish many algal species and microbes associated. We found that the counts of heterotrophes were considerable moderate. The more counts of marine microbes *in situ*, the more chance for the biofilms formation on the artificial surfaces in such location.

Although of these findings, we agreed with Dudley & Christopher, 1999) [34] who stated that the additional microbiological research, such as total cell and heterotrophic plate counts, provides some basic information. However, such experiments do not allow for a reliable evaluation of microbial abundance and diversity of species, because the majority of the microorganisms in ecosystems cannot be cultured[35]. While knowledge of real biofilm microbial composition is essential in identifying the most effective cleaning protocols, only a few molecular-based microbial diversity studies on RO membrane surfaces are reported [36]. In addition, limited data about the formation and development of biofilms over time are available. What little is known comes from laboratory-controlled biofilm monitoring studies using one or a few bacterial strains for biofilm formation on RO membrane surface and other substrata[37, 38]. These studies, therefore, may not provide a true representation of the RO biofilm problem *in situ*.

3.2. Physical Characterization of Seawater

The effect of environmental parameters on the biofouling including; temperature, pH dissolved oxygen and salinity

were determined and the results are shown in Figures 7. The continuous input in the Eastern Harbour basin of seawater, diluted with domestic effluents from the main pipe line situated outside the Harbor, affects markedly its water characteristics creating an eutrophic condition and abnormal flourishing of flora and consequently several kinds of fouling organisms.

Temperature is one of the most important environmental factors affecting markedly the physicochemical characteristics of ecosystem as well as growth, survival and distribution of aquatic organisms [39]. It also affects the decomposition of organic matter of nutrient recycling [40] and liberation or solubility of dissolved gases. Owing to the relative shallowness of the Harbor (<16 m depth) and its semi-enclosed shape, the water temperature is little higher than that of the open water outside the harbor. Surface temperature varies between 27.9 and 30.1ºC with an average of 29.23ºC which is comparable to summer average value recorded by Nessim (2014) [41].

Hydrogen ion concentration plays an important role in many of the life processes. Living organisms are very dependent on, and sensitive to the pH. Not only is the hydrogen ion a potential pollution in itself, but it is also related intimately to the concentration of many other substances, particularly the weakly dissociated acids and bases. The values of pH ranged from 7.2 to 7.4. The values of pH measured in Eastern Harbor are still lying on the alkaline side. The present data is in agreement with that reported by Abdel-Halim & Khairy (2007) [42]; and Nessim (2014) [41] they showed that the changes of pH in the area are mainly related to photosynthetic activities of phytoplankton, aquatic plants, respiration and variation in water temperature and may be due to the effect of waste water discharged to the harbor.

Dissolved oxygen is a fundamental requirement for life of fauna and flora population in natural water and its concentration is generally changeable and represents a momentum balance between the rate of supply and consumption. The survival of marine biota depends on the ability of the water to maintain certain minimal levels of this vital gas. Oxygen content of seawater depends on a number of physical, chemical, biological and microbiological processes. The deficiency in DO is an important indicator of pollution in a natural water body, describing its biological state, the predominant processes occurring in it, the destruction of organic substances and the intensity of self purification, Dyrssen & Wedborg (1980) [43] and Grasshoff (1975) [44]. The amount of DO varied between 3.3 and 7.2-mg/l with an average of 5.3mg/l. Generally, the deficiency of DO values in summer are related mainly to the increase in the rate of oxygen consumption through decomposition of organic matter supplemented by the rise of water temperature [41].

Salinity is defined roughly as the amount of salts dissolved in one kilogram of sea water and reflects the water criteria of such water body. It is a very important environmental factor that affects the distribution of fauna and flora in such water body. Salinity of Eastern Harbor is mainly controlled by the amounts of sewage waters discharged into this basin, as well as the rate of exchange of the Harbor water with the adjoining open seawater through its two openings (El-Boughaz and El-Silsila). It showed variations (35.3-31.8‰) with an average of 33.75‰, which is lower than that of the open water (38.8-39.4‰) being diluted by sewage effluents discharged into the coastal area through KayetBey Pump station, Aboul-Kassim (1987) [45]. It is in a good agreement with Nessim (2014) [41] at the same area during summer (34.7‰). According to Ahmad & Abdullah (1979) [46] coastal seawater enters the Harbor during winter and summer seasons via El-Boughaz, consequently additional amounts of polluted water from the main sewage outfall of KayetBey, reached the Harbor and thus reduced the salinity during these seasons.

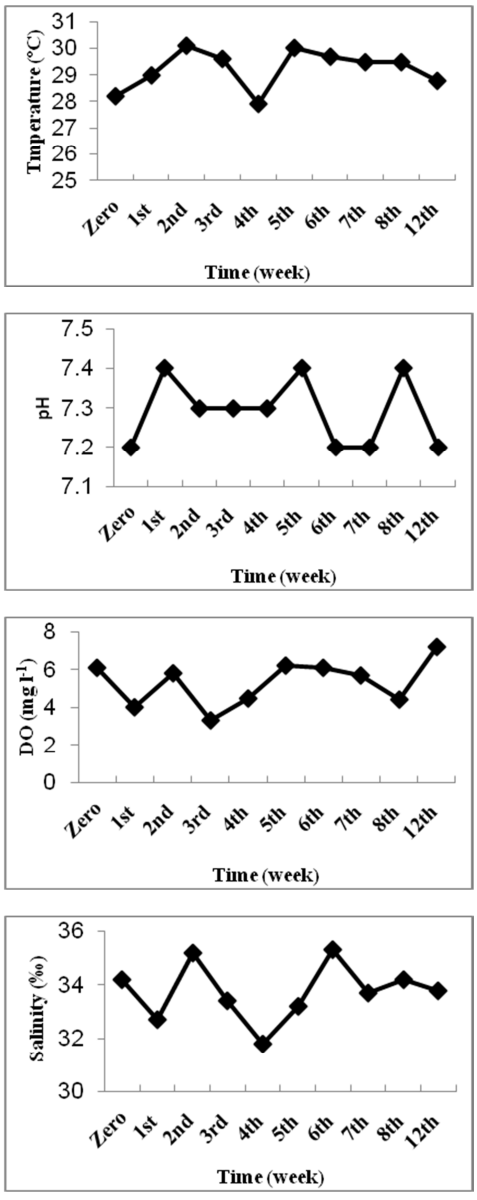

Fig. 7. *Temperature (ºC), pH, dissolved oxygen (mg/l), and salinity (‰) of seawater in submerging site of different coupons, in the Eastern Harbor, Alexandria along 12 weeks of exposure.*

3.3. Chemical Characterization of Seawater (Nutrients)

In marine chemistry the term "Nutrient" has been applied to phosphorus, inorganic nitrogen and silicon, together with a large number of essential trace metals. In this study dissolved inorganic nitrogen fractions (NH_3/N, NO_2/N and NO_3/N), inorganic phosphate and reactive silicate were discussed. The concentration of nutrient salts in seawater is mainly controlled by physical, chemical and biological factors; sinking and degradation of dead organisms are also considered. However, the results of chemical factors affecting the biofouling community are illustrated in Fig. 8.

Ammonia is the major nitrogenous product of the bacterial decomposition of organic matter containing nitrogen, and is an important excretory product of invertebrates and vertebrates. As for the utilization of nitrogenous materials, ammonia is the preferred inorganic source because of its ease uptake and incorporation into amino acids (N-assimilation), Faragallah (2009) [47]. In present study, concentration of ammonia is ranged between 2.93 and 5.21 with average 4.31 µM. Faragallah (1995) [48] pointed out that summer was characterized by the highest level of NH_4 (4.07 µM). Madkour et al. (2007) [49] respect this to the stratification and the effect of the rise in water temperature which may induce the mineralization from the sediment, decomposition rate of sewage and other organic wastes.

Nitrite concentration ranged from 0.63 to 2.43 µM with an average value 1.33 µM. Nitrite concentration average is slightly increasing comparing with Nessim (2014) [41] at the same area during summer (0.47 µM). Nitrate form is generally considered as the most stable and predominant inorganic nitrogen compound in oxygenated sea water. Nitrate concentration ranged between 0.77 and 5.23 with average 3.15 µM which is comparable with Nessim (2014) [41] at summer season. Low averages of nutrients salts during spring-summer period coincided with the onset of phytoplankton bloom. Zaghloul (1976) [50] stated that the increased quantity of phytoplankton during summer months increases the rate of nutrients uptake. According to Meybeck et al. (1988) [51] primary production is responsible for the remarkable spring and summer depletion of nutrients. Strickland and Parson (1972) [27] attributed the increase in plant nutrients utilization to heating of water surface which stimulate plankton growth.

Phosphorus plays a major role in biological metabolism; it is an essential nutrient element in photosynthesis and other processes in plants. Reactive phosphate fluctuated between 6.58 to 7.39 with an average of 6.95 µM. It was in a good agreement with Faragallah (2009) [47] data. Faragallah (1995) [48] pointed out that summer had the maximum values of PO_4-P and referred that to the accumulation of the sewage effluent in the harbor during summer season.

Silicate is one of the major constituents in the sea water. It is a good indicator of fresh water dispersion and of the potential for diatom [52]. The concentration of SiO_4 in the present study varied from 28.85 to74.16 µM, its average 45.58 µM. The present level of silicate data was markedly higher than those recorded by Nessim (2014) [41] (summer average 4.54 µM) and Faragallah (2009)[47] (summer range < 3.0 µM). Probably, during summer and spring, the dissolution of diatom skeletons by increasing temperature is responsible for the high level of silicate content.

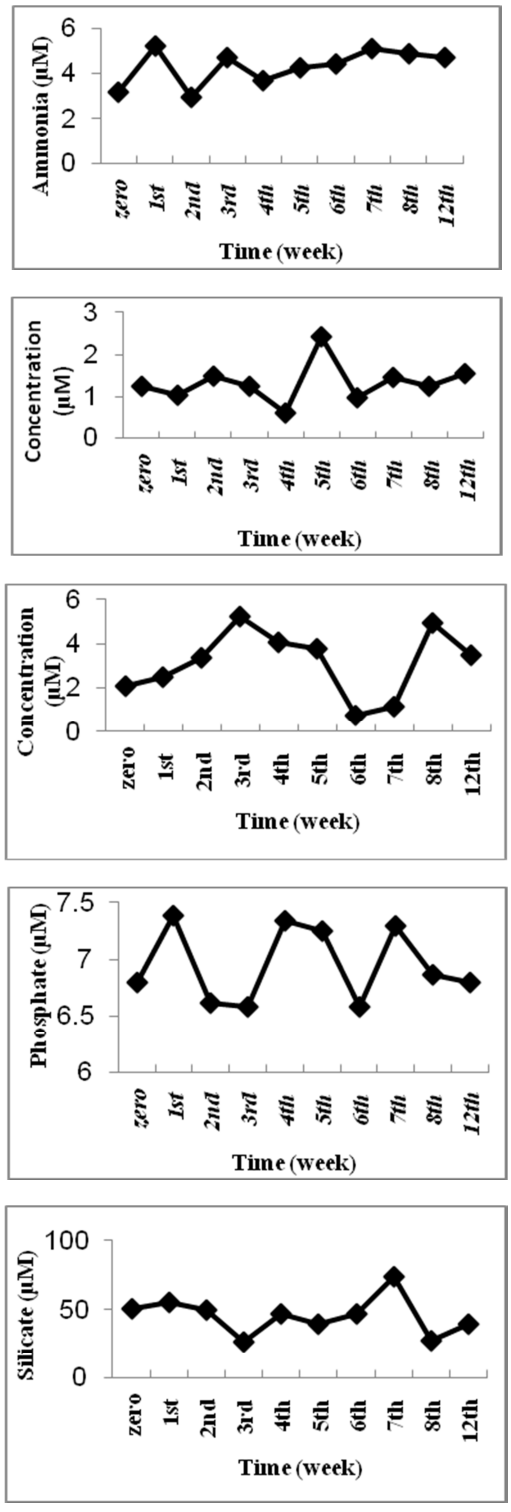

Fig. 8. *Ammonia (µM), nitrite (µM), nitrate (µM), phosphate (µM), and silicate (µM) in seawater in submerging site of different coupons, in the Eastern Harbor, Alexandria along 12 weeks of exposure.*

Dissolved inorganic nitrogen (DIN) and PO_4-P are the

main forms of N and P that are readily bioavailability for the growth of phytoplankton. According to Chiaudani & Vighi (1978) [53] when N/P ratio is higher than 6, the marine algae are considered to be P-limited and when it is lower than 4.5, they considered to be N – limited. In the present work, DIN/P ratio is lower than 6, this consideration shows that nitrogen was limiting factor in the harbor. This is in a good agreement with Faragallah (2009) [47].

3.4. Diversity of Marine Fouling on Different Coupons

A total of 36 coupons were examined in the present investigation along 84 days of submerging under the seawater surface. As mentioned before, the location at which the different panels submerged in marine environment was physico-chemically and microbially characterized. In general, the following photographs (Fig. 9) show the development of biofilm with the biofouling formation after the 1st week and passing to 2nd, 3rd, 4th weeks and finished after 12th week. Throughout the study a total of twenty-one species of marine macro-fouling were recorded in addition to the very thin layer of blue-green algae. These belonged to different groups as follows: Macro-algae (2 species), Hydriods (1 species), Polychaets (5 species; 3 sedentarian and 2 errantias), Cirripeds (4 species), amphipods (4 species), Taniads (1 species), bryozoan (2 species), and tunicates (2 species) (Table1).

In the present study, there are clear dominances by two sessile species. These are the polychaete (tube worm); *Hydroideselegans* and the barnacle; *Balanusamphitrite*. In average, they constituted about 50% and 29% of total fouling abundance, respectively.

There is a general increase in total abundance and biomass of fouling with longer time of exposure (Fig. 10 and 11). Across the different substrates, the average total abundance of the developing fouling community was comparable during different intervals of immersion except for the glass which showed lower abundance (Fig. 12). As well as, the glass demonstrates less biomass value while stainless steel showed the highest value (Fig. 13).

Regarding the species richness, there is a gradual increase in the species richness with increasing the time of immersion until 6 weeks followed by a gradual decrease (Fig. 14); however, the highest species richness was recorded during the twelve weeks interval. The glass showed the lowest average species richness during different intervals of immersion (Fig. 15). Moreover, the rank of species richness follows the order; Teflon> RO membrane > Stainless steel > Glass coupon.

Regarding the most dominant species, the maximum average abundance of the tubeworm *H. elegans* was recorded on Glass substratum meanwhile; the minimum was recorded on Teflon coupon. On the other hand, the highest average abundance of the barnacle *B. amphitrite* was recorded on Stainless steel and/or Teflon meanwhile the lowest average was recorded on RO membrane (Fig. 16).

In colonizing a surface, invertebrates rely on a swimming larval stage to make contact with and attach to the substratum. The larvae then metamorphose to the sedentary stage. Critical links in the biofouling process include detection of appropriate substrata and secure adhesion by larvae (Zardus et al., 2008) [54].

The physical nature of surfaces - in terms of roughness, thermal capacity, color, charge, elemental and organic composition - has been shown to affect the settlement of a variety of macro- and microscopic marine organisms during biofouling community development [55-57].

Brown (2005) [58] indicated a reduction of the pronounced effect of substrate on species composition with increasing the time of exposure. He mentioned that the pronounced effect is within the first three months. This was the case of the present experiment.

The present study demonstrated that, there is a general increase in total abundance, biomass and species richness of fouling with longer time of exposure. This finding is expected and supported by other works at different Egyptian waters [59-61]. The mechanisms for succession discussed by Connell &Slatyer (1977) [62] may be an important determinant in how assemblages develop over longer periods of time.

Hydroideselegans and *Balanusamphitrite* were the most dominant fouling species in the area of study. *Hydroideselegans*(Haswell, 1883) [63] is a polychaete tube worm that is found worldwide in tropical and subtropical bays and harbors (ten Hove, 1974) [64]. The barnacle *Balanusamphitrite* Darwin, 1854, occurs circum-tropically and is commonly found on a wide variety of hard surfaces [65]. These two fouler produce exclusively calcium carbonate skeletons and control biomineralization like many other marine calcifiers [66]. It is well known that marine benthic invertebrates protect their soft tissues from predators, pathogens, and abrasions with a tube or shell made of calcium carbonate ($CaCO_3$) built using a sophisticated biomineralization process [67, 68]. Chan et al. (2013) [69] studied the effect of multiple stressors on the tube formation of *H. elegans* at Hong Kong, and indicated that at elevated temperature, the tubes were harder and more elastic, regardless of salinity and pH, indicating a recovery from the negative effects caused by decreased pH. In the present study, pH values lies in the alkaline side (7.2-7.4) which support more settlement of *Hydroideselegans* and *Balanusamphitrite*. Moreover, higher water temperatures were measured during the sampling season (summer season). Nevertheless, the positive effect of elevated temperature on tube hardness might be indirectly related to alterations of seawater carbonate chemistry by changes in temperature, i.e. high temperature increases saturation states [69].

Many authors have investigated the effects of substrate on fouling assemblages. Some species have been shown to colonize materials with a porous nature in greater abundances than smooth hard. Rougher surfaces also have a larger surface area and this provides greater microhabitat diversity [70], a better surface for attachment and better protection from disturbance [71] resulting in a significant effect on the number and abundance of species present in a fouling

assemblage [72].In the present study, this is clearly observed with the Glass (smooth substrate) which showed the least averages of total fouling abundance, biomass, and species richness. However, the roughened surface of RO membrane may support higher total fouling abundance and species richness.

On the other hand, bacteria are known to attach more rapidly to hydrophobic non-polar surfaces such as Teflon and other plastics than hydrophilic materials such as glass or metals [73].This may explains the higher species richness of marine fouling on Teflon than the glass substratum, taking into consideration that biofilms can enhance larval settlement of marine invertebrates and attachment of algal spores [74]. Moreover, we observed that the more counts of marine microbes *in situ*, the more chance for the biofilms formation on different substrata in such location.

Table 1. *List of recorded species in marine fouling upon the different coupons submerged in Eastern Harbor, Alexandria during three months of experiment.*

Fouling category	Species recorded
Macroalgae	*Enteromorpha*sp. and *Cladophora*sp.
Hydrozoa	*Obelia geniculate*
Polychaeta	*Hydroideselegans, Spirobranchustetraceros, Spirorbis* sp., *Syllis hyaline* and *Nereis*sp.
Cirripedia	*Balanusamphitrite, Balanuseburneus,Balanusperforatus* and *Balanustrigonus*
Tanaidacea	*Taniasdulongii*
Amphipoda	*Podocerus variegates, Corophiumacutum, Elasmopus*sp. and *Stenothoegallensis*
Bryozoa	*Schizoporella errata* and *Bugulaneritina*
Tunicata	*Styelaplicata*and *Symplegmaviride*

4. Conclusion

The present study was proposed to investigate the formation of biofouling *in situ* on different substrata including; Glass, Stainless steel, Teflon and RO membrane. Finding more effective ways to deal with biofouling problems in the current RO systems still need more fundamental investigations especially; all aspects of biofilm formation. However, bacteria and environmental factors play an important role in biofilm formation on RO membrane and other surfaces. From the current data, the experimental studies have to be assessed to give a more fundamental insight into the mechanism of the biofouling process, how to quantify and identify it.

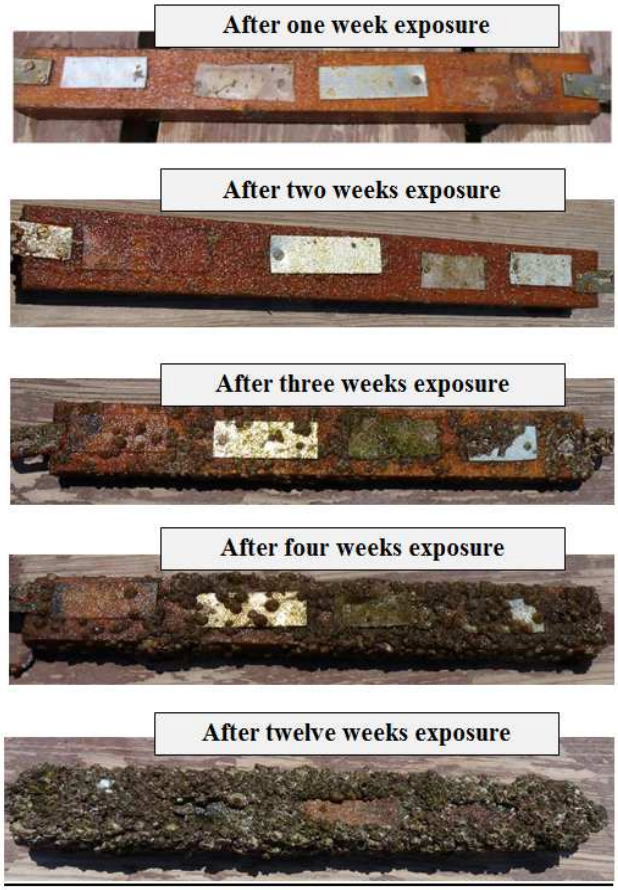

Fig. 9. *Photographs demonstrate the development of biofilm and marine fouling growth on different test coupons submerged in theEastern Harbor, Alexandria during different periods of exposure.*

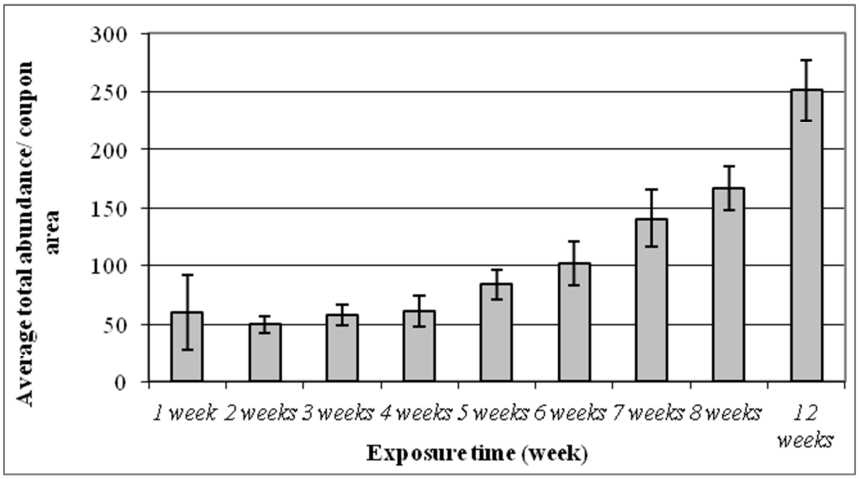

Fig. 10. *Average of total fouling abundance settled on various substrate during different exposure times.*

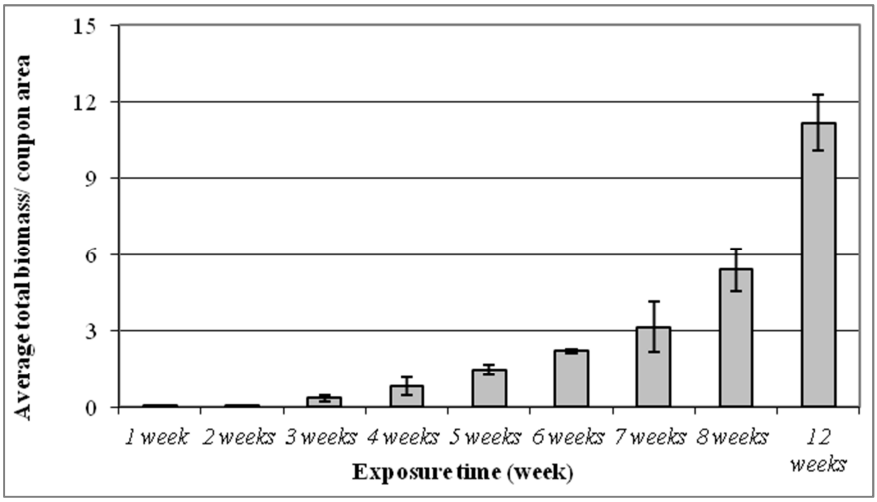

Fig. 11. *Average of total fouling biomass settled on various substrate during different exposure times.*

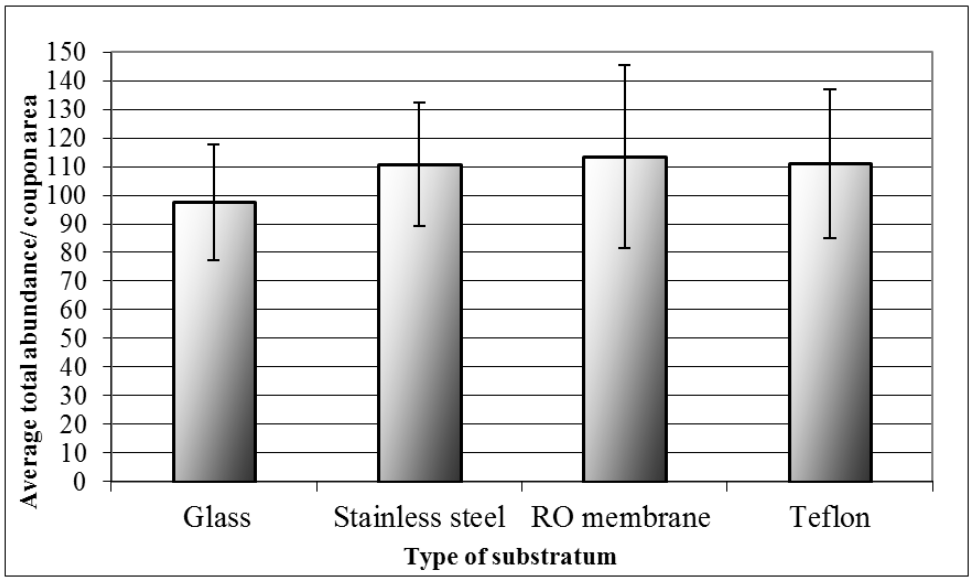

Fig. 12. *Average of total fouling abundance during different time intervals settled on different substrata.*

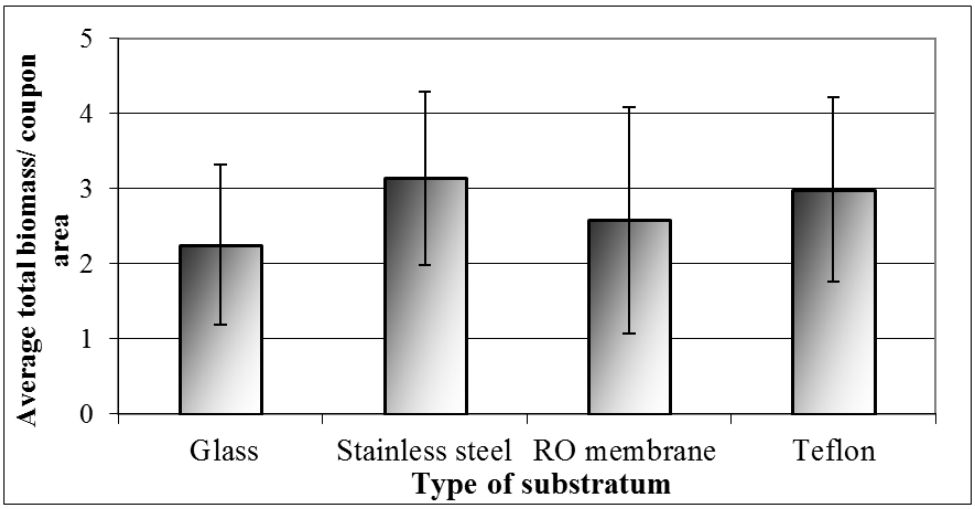

Fig. 13. *Average of total fouling biomass during different time intervals settled on different substrata.*

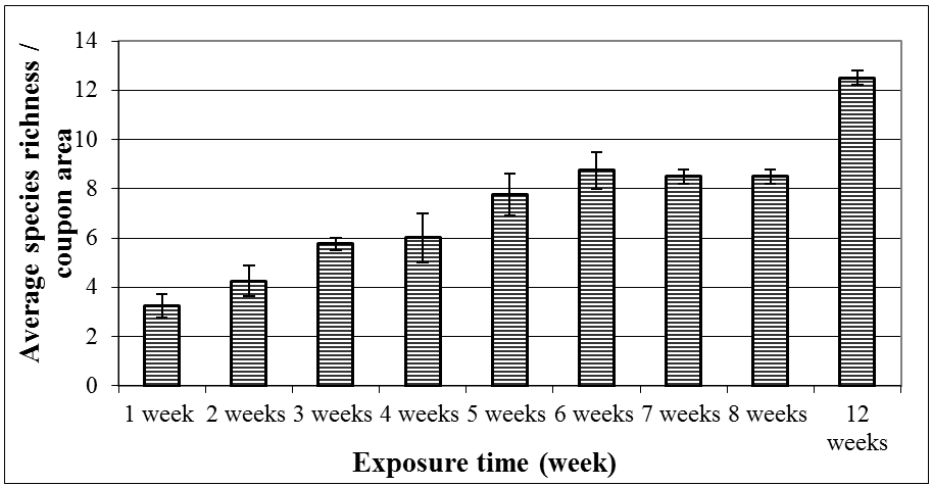

Fig. 14. *Average of fouling species richness settled on various substrata during different exposure times.*

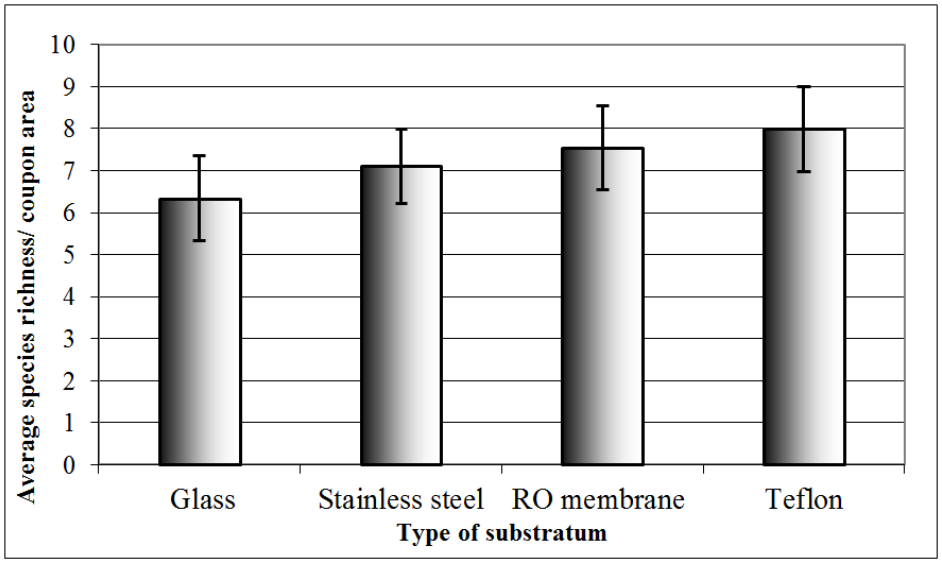

Fig. 15. *Average of fouling species richness during different time intervals settled on different substrata.*

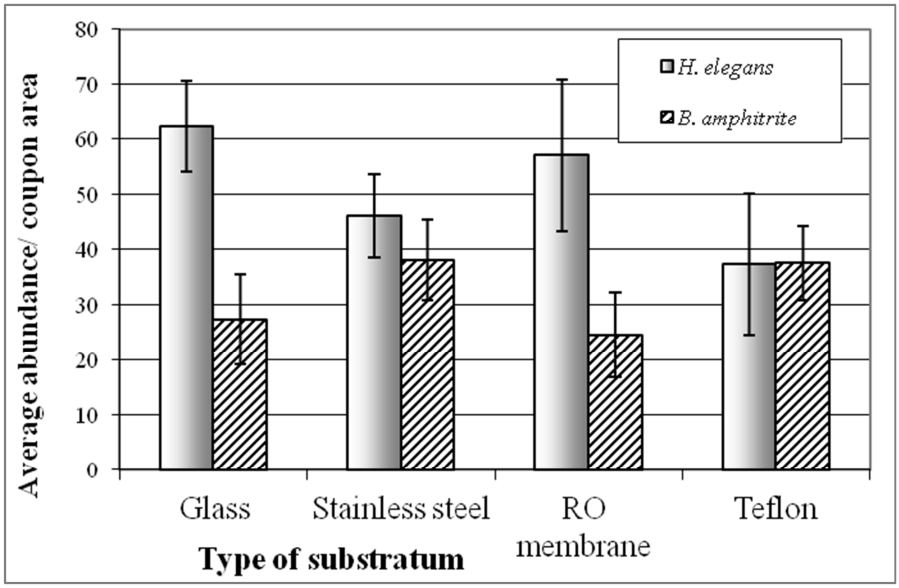

Fig. 16. *Average of H. elegans and B. amphitrite abundance during different time intervals settled on different substrata.*

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