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Protozoan and bacterial populations in biological water-treatment processes

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ABSTRACT

Bacterial populations are one of the most important micro-organisms playing a role in the flow of carbon, nitrogen and phosphorous in nature. Bacteria perform an important role in releasing nutrients from dead organic matter and their ability to perform this role appear to be influenced by the presence of protozoa. It was proved that protozoa may play a direct and indirect role in nutrient recycling. The role of the bacterial-feeding protozoa is to increase the bacterial production and to enhance the oxidation of organic compounds in different water treatment processes. Nutrient regeneration, which means the release of soluble organic and inorganic nutrients from dead organic matter in different habitats is necessary for primary production. Simultaneously, much of the phosphorus and nitrogen incorporated into aquatic primary and secondary production may be regenerated by processes other than the bacterial action. Accordingly, it is possible to conclude that the grazing activities of protozoa on bacteria, their small size, their stimulatory effect on the bacterial production rates, their high growth, and other metabolic rates showed the valuable role played by these microorganisms in regenerating the nutrients in nature.

It was found that maximal densities of ciliates develop mainly at the top 5cm of sand and towards the second half of the run whereas, below 5cm, densities were much lower and either constant or increased slowly during the course of the run.

Regarding the bacterial populations, it was found that their numerical densities were low and more or less similar during the first sampling occasions. After that, there was an increase with the duration especially at the top 5 cm sand, while throughout the lower 20 cm the bacterial densities were much less than those of the upper layer.

Key words: Bacteria - Protozoa - water treatment

INTRODUCTION

Water pollution is the disruption of physical, biological, and chemical conditions of the ecosystem. Accumulation of pollutants in the water is a result of waste which has not been destroyed faster than produced. Pollutants contain also micro-organisms like bacteria, protozoa, fungi and viruses. Nutrients available to microorganisms, including: carbon, nitrogen and phosphorous can also be found in sewage. Sewage water treatment plants filter wastewater through several steps known as pre-treatment, primary treatment, secondary treatment, and tertiary (advanced) treatment (Curds and Hawkes, 1975).

Primary Treatment where dissolved organic and inorganic solids are removed by the gravity in a sedimentation tank, which precipitate to the bottom of the tank, forming a mass of solid called sludge. The sludge is then usually removed by mechanical equipment.

Secondary Treatment where, up to 90 percent of the organic matter is removed using biological treatment processes. It comprises two methods: trickling filters or activated sludge and then disinfecting, known as secondary clarification, Although Secondary Treatment destroys most of bacteria and viruses; other pollutants, such as nitrogen, phosphorus, solids, and some organic materials may still be left in the wastewater. Tertiary (advanced) treatment is used to remove these remaining pollutants. Some Nitrogen is removed biologically through two processes known as nitrification, where ammonia is oxidized to nitrate and denitrification, where nitrate is converted to nitrogen gas and is released into the atmosphere.

Phosphorus is another nutrient which makes the growth of algae possible. According to Curds and Hawkes (1975), it was proved that chemicals including ferric chloride, alum, or lime are added to the wastewater, which allow the smaller particles including phosphorus to group into larger masses which then removed through the sedimentation tanks.

Some organic materials from the wastewater which resisted removal by biological treatment are sequestered by carbon adsorption. In doing so, the wastewater passes through a bed of activated carbon granules , removing 98 % of the organic material. There are some microorganisms that continue on to the tertiary treatment to perform removal of other pollutants like nitrogen, but will be disinfected and killed after that.

Slow sand filtration as a biological water treatment is one of the earliest forms of potable water treatment and remains an important process for water purification throughout the world. Simplicity and low capital and operating costs are other principle advantages of SSF compared with the other water treatment methods (Campos *et al*.; 2002). Biological treatment for potable water was more preferable as compared to the chemical one in order to avoid the various harmful side effects of using chemical compounds. According to Gafvert *et al*. (2002), slow sand filtration proved high removal capacity for certain radioactive substances as uranium, thorium, plutonium, and polonium. It plays also important roles in the treatment of potable water by settling down the heavier suspended particles, breaking down the complicated compounds into simpler forms, removing most of the bacterial populations and viruses, and in order to allow algae, in the presence of sunlight, to absorb carbon dioxide, nitrates and phosphates to form their cell material and liberate free oxygen. There were several attempts to apply this technology to obtain high quality of water such as those made by Mbwette and Wegelin;1984, Vigneswaran; 1982 and Lloyd *et al*.; 1988. In Egypt, treated sewage water was used to irrigate certain arid places (Neis *et al*.; 1987).

MATERIALS AND METHODS

From the biological point of view, it is very important to collect both water and sediment samples in order to be examined biologically and physico-chemically. Water samples were picked up by means of a transparent perspex sampler of 1.2 liter size, while sediment samples were collected by using sediment corers' system made of P.V.C drain pipes of 10 cm in internal diameter and 31 cm in length. Sediment sampling technique was carried out at different depths of the sediment as mentioned by Galal (1989) and Galal *et al.* (2008). Protozoan organisms were precepitated at 7 ^{o}C according to Goddard (1980), examined and counted by using Carl-Zeiss Jena transmitted-light inverted microscope. Detection and identification of protozoan organisms were carried out according to Bick (1972) and Patterson and Hedely (1992). Bacteria was detected and counted according to the method adopted by jones and Simon (1975).

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RESULTS

Numerical abundance of the common ciliate organisms at various depths within the sampling cores were detected at different sampling intervals of the run. It was found that maximal densities of ciliates develop mainly at the top 5cm of sand and towards the second half of the run whereas, below 5cm, densities were much lower and either constant or increased slowly during the time course of the run as shown in figure (1). During the first two sampling dates of the run, the ciliate densities at 1 cm depth were 2254 and 4914/cm^3 respectively and then declined to 1442/cm^3 by day 28. Another peak densities developed at lower depths after day 14 to day 35 where the maximal densities achieved 5586/cm^3 at 5 cm depth. The initial high protozoan densities on day one were caused by the upward transport of the organisms during back-charging. By day 13, densities increased in the top 5cm of sand (7112/cm^3 at 1cm). It is possible to classify the depth distribution of the ciliated protozoan organisms into three characteristic phases:

1- The first phase: In which the ciliate densities are more or less uniform throughout the whole depth of the sampling core, but with slightly higher densities at the surface or bottom depths. The ciliates in this early stage of the run may come from the water passing downwards through the filter bed and/or from water moving upwards during the back-charging of the bed. They may have already been present in the moist sand used to fill the sampling cores but in certain state of encystment.

2- The second phase: The ciliate populations show a characteristic depth distribution with the development of higher surface densities at the top 5 cms, an exponential decrease with depth till 10 cm downwards and a more or less similar constant low density as going down to 30 cm sand depth. The increase in the ciliate numbers in the surface layers is likely to be associated with the increased availability of food in the form of bacteria , algae and diatoms as well as the presence of plentiful oxygen.

3- The third phase: This is the phase during which the ciliated protozoa seem to avoid the sand surface and peak densities appear at lower depths. This phase usually does not appear mostly before day 14 of the run.. The reasons for this surface avoidance are not exactly well known but may be associated with worsening oxygen condition or various kinds of biological interactions with meiofauna and/or carnivorous ciliates.

Regarding the bacterial populations, it was found that their numerical densities were low and more or less similar throughout different depths of the runs during the first sampling occasions. After that, there was an increase with the duration especially at the top 5 cm sand, while throughout the lower 20 cm the bacterial densities were much less than those of the upper layer (Fig. 2).

It was found that both particulate organic carbon and chlorophyll-a levels were much higher at the uppermost 1 cm during all the run ages, followed by more or less similar lower values within the next four cms and then minimal densities were gained as going downwards till achieving 30 cm depth (Figs. 3 and 4).

The slope of the regression between organic carbon against sand depth quantifies how the carbon declines with depth and so is negative throughout the different sampling occasions. It was found that chlorophyll-a behaved similarly to the sand carbon.

The ratio of integrated bacteria to those of ciliates in the sand column was found to provide a crude estimate of the number of bacteria that were 'available' to each bacterivore, assuming that all the bacteria were grazeable. The ratio bacteria/ bacterivorous ciliate varies with run age and it appears to be more or less constant between 2 and 3 10^6 bacteria per each ciliate during the first 12 days of the run, while that of day 14 was 16.57 10^6 bacteria per ciliate . On the other hand, bacteria/total ciliates ratio was less than one apart from that of day 13 (Table 1).

DISCUSSION

It was found that the aerobic protozoan communities of the water treatment works play an important role in producing a clean water of good quality through activation and stimulation of the aerobic bacterial populations (Curds; 1973, 1975; Curds and Cockburn; 1970 a & b and Goddard; 1980). Bacteria in biological water treatment were found to be efficient in removing various organic compounds (Windle-Taylor; 1974 and Schmidt; 1977).

The availability of food was found to be one of the most vital ecological factors affecting the abundance and the distribution of living organisms like protozoa in nature (Curds, 1975 and Laybourn-Parry, 1984). The highest densities of these organisms were recorded on the surface of sediments (Agamaliev, 1970), in activated sludge and percolating filters (Curds, 1971 and Ramadan, 1998) and in slow sand filter beds (Lloyd, 1974; Goddard, 1980 and Galal and Duncan, $2010_{a,b}$. The role of bacterivorous protozoa is to increase indirectly the bacterial production, oxidation of organic compounds which could be explained as a result of preventing bacteria from reaching self-limiting numbers which consequently maintains bacteria in a prolonged state of physiological youth (Johannes; 1965) and secreting growth-promoting substances; Nikoljuk (1969). The physiological characteristics of protozoa such as high growth rates, division and assimilation efficiencies give them an important role in the cycling of carbon (Stout; 1980) via their high carbon assimilation rates and loss through the respiration in the form of carbon dioxide.

The number of protozoa within the different water treatment processes including slow sand filtration and activated sludge including mixed liquor varies greatly by processing and operational conditions, especially hydraulic and organic loadings. The relative numerical density of protozoa may be varied between 100 and100,000/ ml. Protozoa, especially ciliated protozoa, perform several beneficial roles in water treatment. These roles include cropping action, coating action, and recycling of mineral nutrients.

Bacteria are the primary food source for protozoa, and the consumption of suspended or dispersed bacteria by protozoa is referred to as "cropping" action which removes many dispersed bacteria from the bulk solution. Dispersed growth as well as colloids and particulate materials, collectively known as "fine" solids are also removed from the bulk solution by the "coating" action of ciliated protozoa. This group releases sticky secretions that cover the surface of fine solids. Through coating action, the surface charge of fine solids is made compactable for adsorption to floc particles in the activated sludge process. The adsorption reduces the quantity of fine solids in the final effluent.

Protozoa also release excretions to the bulk solution. These excretions contain many mineral nutrients, including nitrogen and phosphorus, and help to recycle mineral nutrients in the biological water treatment process. These nutrients then are available for bacterial activity in degrading different pollutants. Protozoa in this biological process are commonly include five groups: amoebae, flagellates, free-swimming ciliates, crawling ciliates and stalked ciliates. Amoebae and flagellates are considered "lower" life forms, while crawling ciliates and stalk ciliates are considered "higher" life forms. Free-swimming ciliates are considered "intermediate" life forms. Some treatment plant operators often perform routine microscopic examinations of the protozoa to determine the health of the water treatment process. Accordingly, when lower life forms are dominant, the biological treatment is considered unhealthy and unacceptable where the effluent BOD is great. If the higher life forms are dominant, the treatment process is considered healthy and acceptable with lower BOD values (Mansour *et al.*, 2008).

It was obvious that the more or less remarkable stability in the ratios between bacteria and bacterivorous ciliates seem to indicate a state of balance between the ciliated protozoans and the apparent ‘ availability’ of bacterial food, particularly during the early part of the runs of the bed (Galal and Duncan, 2011).

According to Laybourn-Parry (1984) water effluent of good quality, low BOD and low suspended solids was obtained in the presence of a wide variety of ciliated protozoan organisms due to high grazing densities of bacteria and their ability to flocculate suspended particulate matter and bacteria.

Duncan (1988) proved that POC, bacterial, algal and protozoan biomasses of the sand in the SSF exhibited initial faster increase from day 1-12 (phase 1 and 2) and a subsequent slower increase from days 12-22 (phase 3). She also mentioned that these biological changes parallel the periods of slow and fast variations in the head loss and the periods of high and low vertical flow rates.

The integrated area below the depth distribution figures of the various examined parameters during the filter run proved higher values at the top 10 cms as compared to the lower 20 cms (Galal and Duncan, 2010, a). Simultaneously, the daily instantaneous rates were found to be either parallel to the integration values or equal within these two layers. This could be attributed mainly to the more accumulation of POC and chlorophyll-a in the former layer with particular reference to the most superficial outer covering layer (Schmutzdecke) than those of the lower layer.

Table (1) Bacterial and ciliate densities during various time intervals at slow sand filter bed.

Run age (days)	Water bacteria (10^6/cm^3) Top	Effluent	Sand bacteria (10^9/cm^2)	Total sand ciliates (10^3/cm^2)	Sand bacteriv. ciliates (10^3/cm^2)	Water temp (°C)	R 1 (10^6/cm^2)	R2 (10^6/cm^2)
1	12.73	5.87	40.62	63.64	14.63	14	0.638	2.78
5	10.06	4.91	38.86	58.79	13.32	15	0.661	2.92
7	15.17	7.73	11.48	20.29	3.15	11	0.566	3.64
12	19.74	6.94	45.66	62.87	14.60	16.5	0.726	3.13
13	20.28	9.77	144.06	70.20	24.49	17	**2.052**	5.88
14	5.66	4.44	28.00	75.16	1.69	18	0.373	**16.57**
20	14.12	6.79	41.37	73.85	13.12	16	0.560	3.15
21	20.80	9.89	6.76	47.78	9.54	15	0.142	0.71
22	17.02	6.96	41.60	59.90	7.64	14	0.695	5.45
35	10.68	4.87	12.05	47.26	17.07	15	0.255	0.71

R1 = Total sand bacteria / Total sand ciliates .

R2 = Total sand bacteria /Bacterivorous ciliates .

Figure (1) Ciliate densities at different depths at various sampling intervals.

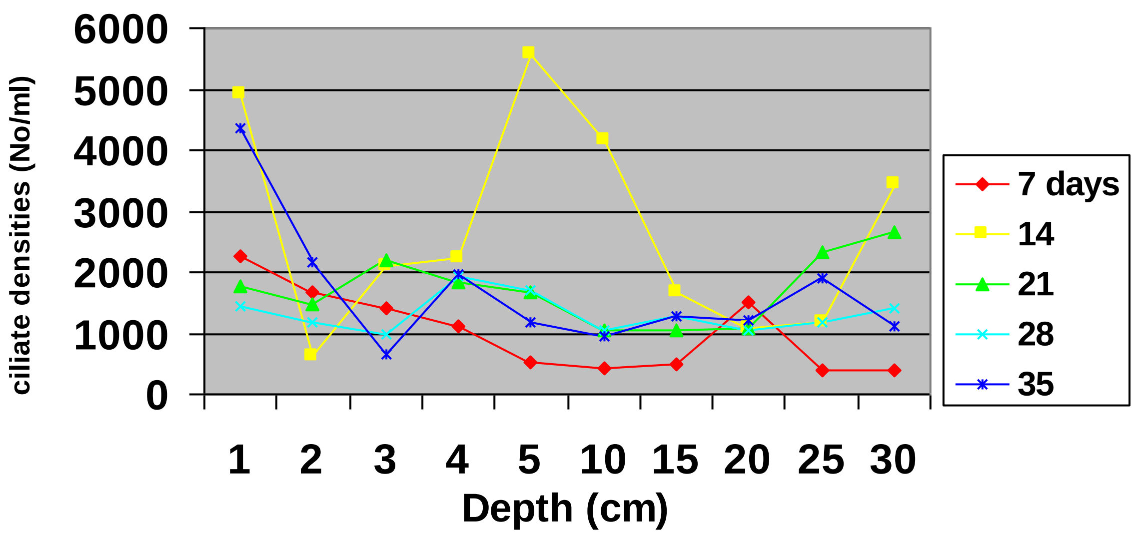


Figure (2) Bacterial densities at different sand depths at various sampling intervales.

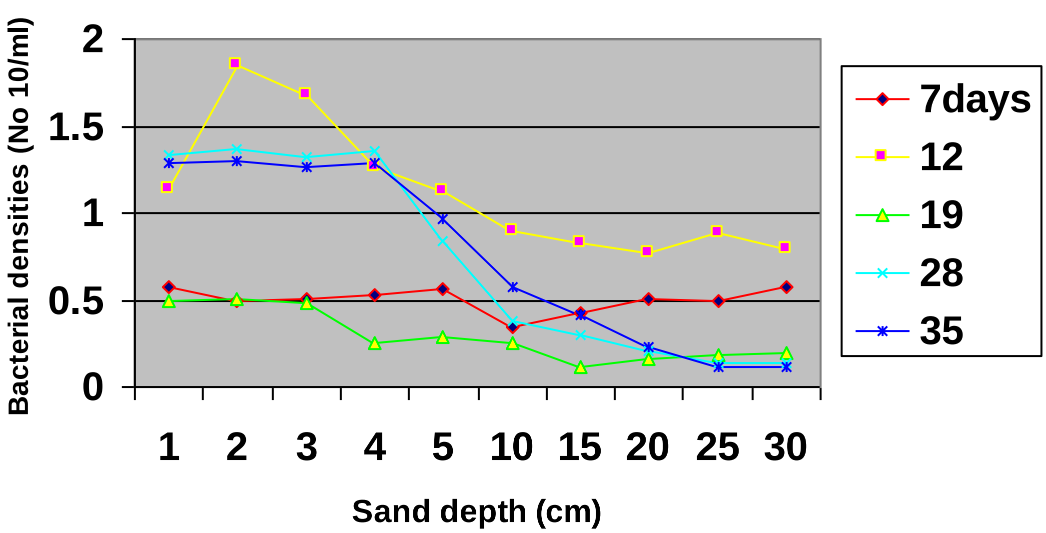


Figure (3) Depth distribution of POC (mg/ml)

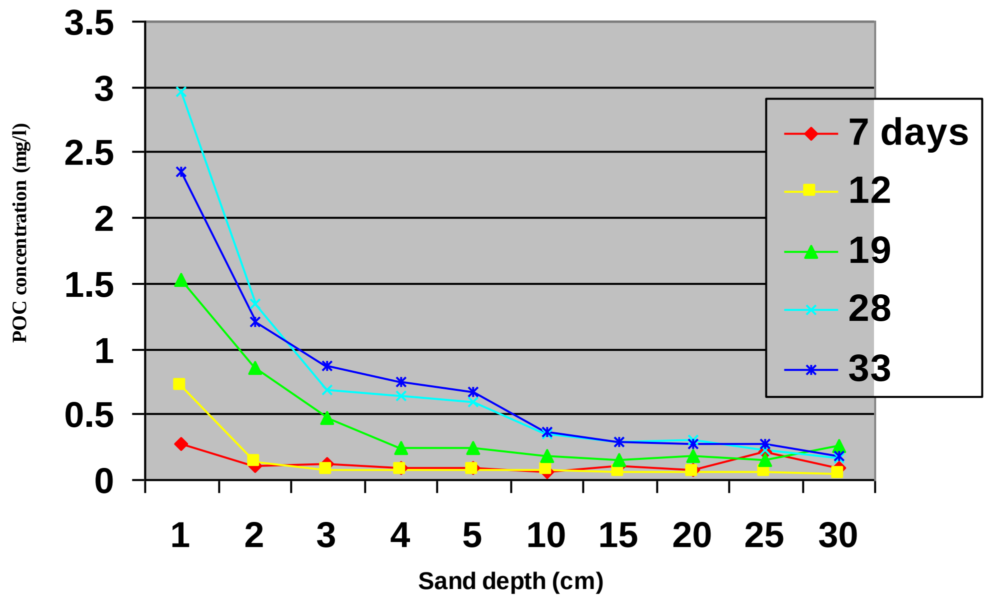
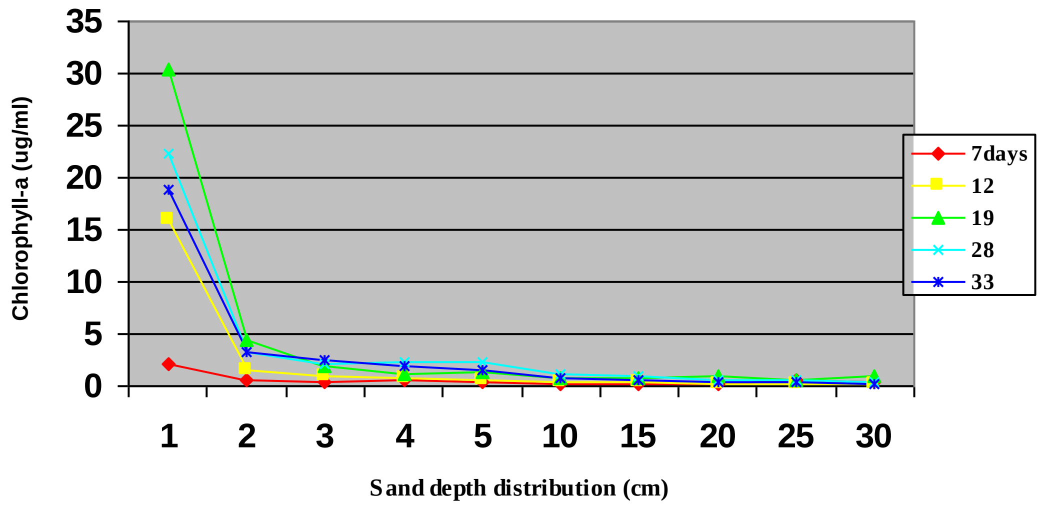


Figure (4) Depth distribution of chlorophyll-a (ug/ml)



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العشائر البكتيرية والأولية فى عمليات معالجة المياه الحيوية

منصور جلال
قسم علم الحيوان كلية العلوم جامعة المنوفية

تعتبر التجمعات البكتيرية من أهم الكائنات الدقيقة التى تلعب دوراً هاماً فى تحرير واطلاق الكربون والنيتروجين والفوسفور من المادة العضوية غير الحية وذلك بمساعدة الكائنات الآولية. ولقد ثبت أن الأوليات آكلة البكتريا تعمل على زيادة تكاثر البكتريا وتحسين أكسدة المركبات العضوية فى عمليات معالجة الماء المختلفة. ولقد لوحظ أن نواتج هذه الأكسدة تكون ضرورية لعمليات البناء الضوئى والعمليات التالية لها. ولقد وجد أن الكثافة العددية القصوى للأوليات الهدبية تتواجد أساساً فى أعلى خمس سنتيمترات سطحية من الرمل وكلما اتجهنا نحو النصف الثانى من دورة تنقية المياه ، وتقل الكثافة العددية كلما ازداد عمق الرمل تحت الخمس سنتيمترات العليا. من الناحية الأخرى ثبت أن التجمعات البكتيرية تكون قليلة ومتشابهه فى أعماق الرمل المختلفة وذلك فى فترات التجميع الأولى ، ولكن تزداد البكتريا بعد ذلك كلما ازدادت فترة تشغيل حوض المعالجة خاصة فى الطبقة العليا من رمل الحوض. ويلاحظ أن تلك الكثافة البكتيرية تقل كثيراً فى العشرين سنتيمتر السفلى الموجودة بجهاز تجميع الرمل.