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# Ecological Studies on the Protozoan Organisms of Three Different Fish Farms in Kafr El-Sheikh Province, Egypt

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## ABSTRACT

The present study was carried out on three different fish farms in Khafr El-Sheikh province. Water samples were collected during three growing seasons: spring, summer and autumn 2018. The seasonal variations of the physicochemical parameters of aquatic samples showed some different magnitudes in these farms. Data on the drainage units of these fish farms are higher than those of the fish culturing ponds apart from those of BOD, Al, Mn and Fe. This could mainly be attributed to the metabolites of the various developing fish stages and/or contaminants in the agricultural drainage. Ammonia and chlorine concentrations exceeded slightly the universal levels, while nitrate and phosphate altitudes were lower than the permissible international standards. It was proved that the protistan organisms (16 genera) of the present study could be divided into four groups depending on the numerical densities; most common, common, frequent and rare protozoan organisms. Certain statistical applications of the present data illustrated that the Protozoa were significantly affected by a combination of one and/or more parameters in both culturing and drainage fish units in Kafr El-Sheikh province. Due to certain biotic and abiotic interactions besides statistical evidence in these fish farms, a case of chemostat ecosystem was established.

## INTRODUCTION

The Egyptian aquaculture business has witnessed a rapid growth over the last three decades and is considered as the third-largest producer of farmed tilapia after China and Indonesia. During 2018, it was the only African country contributing 17.4% of the world production among the top-ten producers of farmed tilapia **(FAO, 2020)**.

The increasing demand on fish production resulted in a higher intensification of aquaculture to face the various challenges and water pollution **(Zahran *et al*., 2021)**.According to intensive studies, there is a risk of human exposure to heavy metals and pesticides from eating farmed tilapia **(Eltholth *et al*., 2018)**. Agriculture drainage canals are a source of pesticide residues, runoff-derived fertilizers and metals that may contaminate farmed fish

**(Authman *et al*., 2013; Zohry & Ouda, 2022)**. **Madoni *et al.* (1996)** has experimentally addressed the toxic effects of certain heavy metals against protozoan community to detect the most hazardous metals and the most sensitive protozoan organisms.

Given that there is no exchange of water or discharge of effluents into the environment, closed systems that use water recirculation can combine intensive production with environmental sustainability **(Martins *et al.,* 2010; Wang *et al*., 2022)**. To achieve this, sophisticated filtering systems are required to ensure the suitability of water quality for aquatic organisms' cultivation **(Zadinelo *et al*., 2018)**.

Protozoa is a complex collection of relating organisms that frequently includes genera and species that are susceptible, resistant or intermediate in their sensitivity to contaminants **(Sa'idi, 2010; AL-Keriawy, 2021)**. Protozoa are responsible for increasing effluent quality and maintaining the density of dispersed bacterial communities through predation **(Madoni, 2011; Yang *et al*., 2021)**.

The present study aimed to address the abundance and diversity of the protozoan organisms in different fish farms and assess the influence of certain parameters on their distribution.

## MATERIALS AND METHODS

Three different fish farms in Khafr El-Sheikh province (EL-Reyad, Motobas and Sidi Salim villages) were subjected to study. Water samples were collected in sterile plastic bottles (2 L volume) at a depth of 30cm from the water surface. Three water stages (water source, culturing and drainage units) were investigated for each farm during three successive seasons (spring, summer and winter according to the fish culturing strategy). These samples were immediately transported in an ice box to the laboratory (Zoology Dept., Faculty of Science, Ain Shams University) to determine the values of various parameters.

The physicochemical parameters (temperature, BOD, COD, NH3, NO3, PO4, etc...) were measured using the Central Laboratory facilities. Simultaneously, a gentle shaking was carried out for some other water samples to avoid the protozoan damage. Microscopical examination was performed using a microscope equipped with an eye piece micrometer and a digital camera to detect, count down and identify the major alive protozoan genera. These organisms were carefully examined using an objective lens of 100X over approximately 100 fields **(El Said, 2012)**. The identification adjusted followed the description of **Donner (1966)** and **Patterson and Hedley (1996)**.

## RESULTS AND DISCUSSION

The microscopical examination of the aquatic protozoan organisms in these fish farms proved the presence of 16 different genera. Their total numerical densities and seasonal variations in culturing and drainage units are illustrated in Tables (1, 2, 3). According to **Patterson and Hedly (1996)**, the protozoan organisms under study are classified into two main groups: Phytomastigophora and Ciliophora. The first group includes mainly one order (Euglenoida), which is represented by *Euglena* and *phacus* spp. While, ciliates comprise four subgroups: Haptorids (*Litonotus* and *Spathidium* spp.) and Prostomes ( *Coleps* and *Urotricha*). Oligohymenophora consists of two suborders: Peritrichs (*Vorticell* sp.) and Peniculina ( *Paramecium* and *Urocentrum* spp.), while Polyhymenophora (Spirotrichs) is represented by three suborders; Hypotrichs (*Euplotes*, *Oxytricha* and *Tachysoma* spp), Heterotrichs (*Metopus* and *Spirostomum* spp.) and Oligotrichs (*Strombilidium* spp.).

In spring, *Euglena*, *Litonotus*, *Paramecium*, *Coleps* and *Euplotes* showed the highest counts, with 3800, 3250, 3050, 2950 and 2900 individual/ L of water, respectively. Whereas during summer, *Paramecium*, *Euplotes*, *Coleps* and *Tachysoma* spp. displayed the highest abundance, with 4450, 3850, 2300 and 2000 organism/ L, respectively. Finally, autumn witnessed the highest numerical densities for *Euplotes*, *Paramecium*, *Coleps* and *Euglena*, with 5300, 3650, 3150 and 2200 protozoan/ L, respectively. Simultaneously, the total protozoan numerical densities were higher in fish culturing ponds, compared to the drainage unit during different seasons. Accordingly, protozoa in these three fish farms could be divided into four groups, based on their numerical densities as follows: Most common ($> 3 \times 10^2$ /L ), Common (between 2 and $3 \times 10^2$ /L), Frequent (between 1 and $2 \times 10^2$ /L ) and Rare ( less than $1 \times 10^2$ /L) protozoan organisms, as seen in Table (3). Data in Table (3) prove that the number of the protozoan orders belonging to each fish farm are similar; however, the individual numerical densities and their types are different.

For the season of spring, the most common protozoa included *Euglena*, *Paramecium* and *Litonotus*. The common protozoa illustrated were *Phacus*, *Peranema*, *Euplotes*, *Coleps* and *Vorticella* spp., while the frequent protozoa were represented by *Metopus*, *Urotricha*, *Tachysoma* and *Oxytricha*.. On the other hand, rare protozoa comprised *Stromilidium* and *Spathidium*.

During summer, the parallel groups are represented as follows; the most common comprise *Paramecium*, *Euplotes*, the common types are *Coleps*, *Tachysoma*, the frequent type consists of *Spathidium*, *Vorticella*, *Phacus*, *Peranema*, *Litonotus*, while rare protozoa is represented by *Euglena*, *Metopus*, *Urotricha*, *Urocentrum*, *Oxytricha*, *Spirostomum* and *Strombilidium* spp..

On the other hand, Autumn samples are shown as follows: The most common includes *Paramecium*, *Euplotes*, *Coleps*, the common genera have *Euglena*, *Peranema* spp., the frequent individuals show *Phacus*, *Litonotus*, *Vorticella*, *Urocentrum*, *Tachysoma*, *Oxytricha*, while rare Protozoa illustrates *Metopus*, *Urotricha*, *Spirostomum*, *Strombilidium*, *Spathidium* spp.

The resemblance between the protozoan organisms in these three fish farms could be referred to their main water source (River Nile agricultural drainage) in that area of Kafr El-Sheikh province. It was proved that the total numerical densities of Protozoa in the fish culturing pond were obtained on spring followed by those of autumn and then those of summer in Motobas and El-Reyad respectively, while Sidi-Salim illustrated the uppermost protozoan numbers during autumn followed by those of spring and then summer (Table 2).

The same behaviour was exhibited in the drainage units of these examined fish farms which could be interpreted as a case of a chemostat ecosystem. It is necessary to mention that the water samples of both units of the three examined fish farms have no protozoan organisms such as *Epistylis*, *Campanell*, *Metobus*, *Carchesium*, *Vaginicola*, *Platycola*, *Thuricola* spp. or any other colonial forms of Peritrichida which are used as a biological indicator for sewage pollution. This is equivalent to the universal standards of both Health and Agricultural World Organizations for international hygienic situation. In addition, Sarcodina exhibited a very rare existence which confirmed an appropriate situation for water in these farms which is concomitant with data obtained by **Curds and Hawke (1975).**

The seasonal variations of the physicochemical parameters of water illustrated slightly different magnitudes between the examined three fish farms. Having a glance at the data belonging to fish culturing units (Table 1), it was clear that water temperature ranged between 27-28 ºC, 27-29 ºC and 23-24 ºC during spring, summer and autumn, respectively. Moreover, the pH values recorded 7.3-7.7, 8.2-8.5 and 7.5-7.9, respectively. Electrical conductivity (EC) values ranged between 3.2- 4.2, 4.6-5.7 and 3.7- 4.1 ms/cm, during spring, summer and autumn, respectively. Meanwhile, total dissolved solid (TDS) values were 2.4-2.9, 3.2- 4.0 and 2.7-2.9 g/l, respectively. On the other hand, the Chemical oxygen demand (COD) and the biological oxygen demand (BOD) recorded 89-98 and 50.4-55.6 mg/l, respectively in spring, while in summer were 78-89 and 43.7-50.1 mg/l. Moreover, during autumn their corresponding values represented 139-155 and 77.1- 85.8 mg/l, respectively.

Concerning the environmental parameters, it was found that ammonia varied during seasons with high concentration during summer, which ranged from 0.24-0.55, 0.75-0.95 and 0.11-0.95 mg/l during spring, summer and autumn, respectively. But it is worth keeping in mind that concentration levels below 0.05 mg/l are considered safe for most aquatic organisms including fishes. Nitrate concentrations are 3.5- 7.4, 1.67 – 2.55 and 1.18- 2.92 mg/l during spring, summer and autumn, respectively. However, the accepted level of nitrate was up to 50 mg/l for freshwater fishes. Moreover, phosphate values ranged between 1.7-3.2, 1.5-2.6 and 1.6- 2.5 mg/l, respectively. Quality standards on phosphorous levels are between 0.02 and 0.20 mg/l for freshwater aquacultures. Sulphate values were 0.15-0.23, 0.29-0.42 and 0.34-0.72 mg/l during spring, summer and autumn, respectively. The current results are less than the safe concentration (100 mg/l) for aquatic life. On the contrary, chlorine measurements proved very high concentrations especially during spring and autumn ( 0.67-1.11 mg/l and 0.84-1.89 mg/l respectively). It is necessary to remember that chlorine concentration must be below 0.05 mg/l as a safe level for culturing organisms, particularly fish.

The data of the drainage units of these fish farms are more or less high to different levels than those of the fish culturing ponds apart from those of BOD, Al, Mn and Fe which illustrated lower values. This could be interpreted mostly due to the metabolites of the developing various fish stages during their growth as shown in table (2) beside pollutants belonging to the agricultural drainage ( fertilizers, pesticides and herbicides).

Regarding the heavy metals pollution, it was proved that the order of toxicity against protozoa is as follows: Cd > Cu > Pb > Zn > Cr. Most of the protozoan organisms are very sensitive to these metals. This was parallel to most of the data belonging to **Madoni, *et al.* (1996).** All the previous data were examined and compared together collectively to estimate the actual influences of certain factors on the diversity and abundance of the free-living protozoa. Simultaneously, these data were examined statistically using the regression and time series analyses. Applying the stepwise regression analysis proved that the protozoan organisms were significantly affected by one or more parameters in both culturing and drainage fish farm units in Kafr El-Sheikh province as could be seen in Table (4). Applying the time-series analyses for the various combinations of the examined parameters against the protozoan organisms were summarized and illustrated in Figures (1 and 2).

**Table (1) Total protozoan numerical densities (y x $10^2$/L), physicochemical, environmental and heavy metal parameters in culturing fish unit at different seasons and stations.**

| Parameters | Motobas | | | El-Reyad | | | Sidi Salem | | |
|---|---|---|---|---|---|---|---|---|---|
| Seasons | Spr. | Sum. | Aut. | Spr. | Sum. | Aut. | Spr. | Sum. | Aut. |
| T.protz/L | 51.0 | 37.50 | 42.00 | 5850 | 35.00 | 43.00 | 37.0 | 37.50 | 40.50 |
| Temp. $^oC$ | 28 | 27 | 24 | 28 | 29 | 23 | 27 | 28 | 24 |
| pH | 7.3 | 8.2 | 7.5 | 7.5 | 8.5 | 7.7 | 7.7 | 8.3 | 7.9 |
| EC ms/cm | 3.2 | 4.6 | 3.7 | 4.2 | 5.7 | 4.1 | 3.5 | 4.9 | 3.8 |
| TDS ppt | 2.4 | 3.2 | 2.7 | 2.9 | 4.0 | 2.9 | 2.4 | 3.4 | 2.7 |
| COD mg/L | 94 | 78 | 139 | 89 | 89 | 146 | 98 | 88 | 155 |
| BOD mg/L | 52.1 | 43.7 | 77.1 | 50.4 | 49.9 | 81.9 | 55.6 | 50.1 | 85.8 |
| NH3 mg/L | 0.32 | 0.81 | 0.11 | 0.24 | 0.75 | 0.86 | 0.55 | 0.95 | 0.95 |
| NO3 mg/L | 7.4 | 1.67 | 1.18 | 3.5 | 2.07 | 2.12 | 4.6 | 2.55 | 2.92 |
| PO4 mg/L | 2.6 | 2.6 | 1.6 | 1.7 | 1.5 | 1.9 | 3.2 | 2.2 | 2.5 |
| SO4 g/L | 0.23 | 0.37 | 0.72 | 0.15 | 0.42 | 0.34 | 0.20 | 0.29 | 0.61 |
| Al mg/L | 0.47 | 15.48 | 1.74 | 0.43 | 35.17 | 0.62 | 0.51 | 31.80 | 0.69 |
| Cu mg/L | 0.02 | 0.83 | 0.05 | 0.01 | 1.56 | 0.02 | 0.02 | 0.78 | 0.03 |
| Pb mg/L | 0.01 | 0.02 | 0.05 | 0.01 | 0.13 | 0.02 | 0.02 | 0.03 | 0.04 |
| Cd mg/L | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| Ni mg/L | 0.01 | 0.02 | 0.01 | 0.01 | 0.12 | 0.01 | 0.01 | 0.08 | 0.01 |
| Mn mg/L | 0.04 | 1.22 | 0.18 | 0.03 | 4.94 | 0.07 | 0.08 | 1.19 | 0.12 |
| Fe mg/L | 0.56 | 29.81 | 1.45 | 0.53 | 76.53 | 0.46 | 0.75 | 74.55 | 0.52 |

**Table (2) Total protozoan numerical densities (y x $10^2$/L), physicochemical, environmental and heavy metal parameters in the drainage unit at different seasons and stations.**

| Parameters | Motobas | | | El-Reyad | | | Sidi Salem | | |
|---|---|---|---|---|---|---|---|---|---|
| Seasons | Spr. | Sum. | Aut. | Spr. | Sum. | Aut. | Spr. | Sum. | Aut. |
| T.protz/L | 54.8 | 42 | 49.5 | 59.8 | 41.5 | 42.8 | 42.25 | 38.96 | 50.85 |
| Temp. oC | 26 | 30 | 23 | 25 | 31 | 24 | 27 | 30 | 25 |
| pH | 8.1 | 8.8 | 8.1 | 7.8 | 8.7 | 8.3 | 8.3 | 8.4 | 8.0 |
| EC ms/cm | 4.0 | 5.0 | 5.7 | 4.5 | 6.1 | 6.3 | 3.9 | 5.5 | 4.9 |
| TDS ppt | 2.6 | 3.5 | 4.0 | 3.2 | 4.3 | 4.5 | 2.7 | 3.8 | 3.4 |
| COD mg/L | 56 | 62 | 138 | 67 | 68 | 129 | 74 | 71 | 142 |
| BOD mg/L | 31.2 | 35.4 | 76.9 | 37.9 | 39.8 | 73.5 | 41.8 | 40.3 | 80.5 |
| NH3 mg/L | 0.48 | 1.88 | 0.2 | 0.52 | 1.72 | 0.16 | 0.45 | 1.96 | 0.24 |
| NO3 mg/L | 11.4 | 2.19 | 1.98 | 7.2 | 3.16 | 3.66 | 9.4 | 3.48 | 3.74 |
| PO4 mg/L | 3.0 | 3.9 | 2.4 | 2.7 | 3.2 | 2.8 | 5.2 | 4.6 | 3.6 |
| SO4 g/L | 0.23 | 0.3 | 0.35 | 0.17 | 0.29 | 0.43 | 0.25 | 0.21 | 0.52 |
| Al mg/L | 0.21 | 11.19 | 1.35 | 0.16 | 29.42 | 0.51 | 0.31 | 25.76 | 0.41 |
| Cu mg/L | 0.01 | 0.59 | 0.03 | 0.01 | 1.21 | 0.02 | 0.02 | 0.64 | 0.02 |
| Pb mg/L | 0.01 | 0.02 | 0.01 | 0.01 | 0.12 | 0.01 | 0.01 | 0.02 | 0.02 |
| Cd mg/L | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| Ni mg/L | 0.01 | 0.01 | 0.01 | 0.01 | 0.11 | 0.01 | 0.01 | 0.06 | 0.01 |
| Mn mg/L | 0.02 | 3.75 | 0.06 | 0.01 | 4.65 | 0.03 | 0.03 | 1.08 | 0.04 |
| Fe mg/L | 0.38 | 23.53 | 0.73 | 0.24 | 64.74 | 0.38 | 0.61 | 61.27 | 0.37 |

**Table (3) Seasonal variations of the protozoan genera in three fish farms in Kafr El-Sheikh Province, Egypt.**

| Season | Most common | Common | Frequent | Rare |
|---|---|---|---|---|
| **Spring**<br><br>**14 gen.** | *Euglena, Paramecium, Litonotus* | *Phacus, Peranema, Euplotes, Coleps, Vorticella.* | *Metopus, Urotricha, Tachysoma, Oxytricha.* | *Stromilidium, Spathidium* |
| **Summer**<br><br>**15 gen.** | *Paramecium, Euplotes* | *Coleps, Tachysoma* | *Spathidium, Vorticella, Phacus, Peranema, Litonotus.* | *Euglena, Metopus, Urotricha, Urocentrum, Oxytricha, Spirostomum, Strombilidium.* |
| **Autumn**<br><br>**15 gen.** | *Paramecium, Euplotes, Coleps* | *Euglena, Peranema,* | *Phacus, Litonotus, Vorticella, Urocentrum, Tachysoma, Oxytricha* | *Metopus, Urotricha, Spirostomum, Strombilidium, Spathidium* |

**Table (4) Summary of the significant relationships between protozoa and certain abiotic parameters at fish culturing and fish drainage units.**

| Parameters | Fish pond unit | | | Fish drainage unit | | |
|---|---|---|---|---|---|---|
| | DF | F | P | DF | F | P |
| Protozoa Vs pH | 1,8 | 8.00 | 0.025 | 1,8 | 8.00 | 0.025 |
| Protozoa Vs Temp. & pH | 2,7 | 5.34 | 0.047 | 2,7 | 5.93 | 0.038 |
| Protozoa Vs temp., pH, EC | 3,6 | 6.40 | 0.036 | --- | --- | ------- |
| Protozoa Vs pH & TDS | 2,7 | 7.59 | 0.023 | 2,7 | 6.6 | 0.03 |
| Protozoa Vs pH & PO4 | 2,7 | 5.03 | 0.05 | 2,7 | 9.4 | 0.014 |
| Protozoa Vs pH & SO4 | 2,7 | 4.97 | 0.05 | 2,7 | 6.26 | 0.034 |
| Protoz Vs temp. &EC | ---- | ---- | ----- | 2,7 | 6.40 | 0.036 |
| Protoz Vs NO3, PO4 | ---- | ---- | ----- | 2,7 | 2.83 | 0.136 |

**Fish Drainage pond**

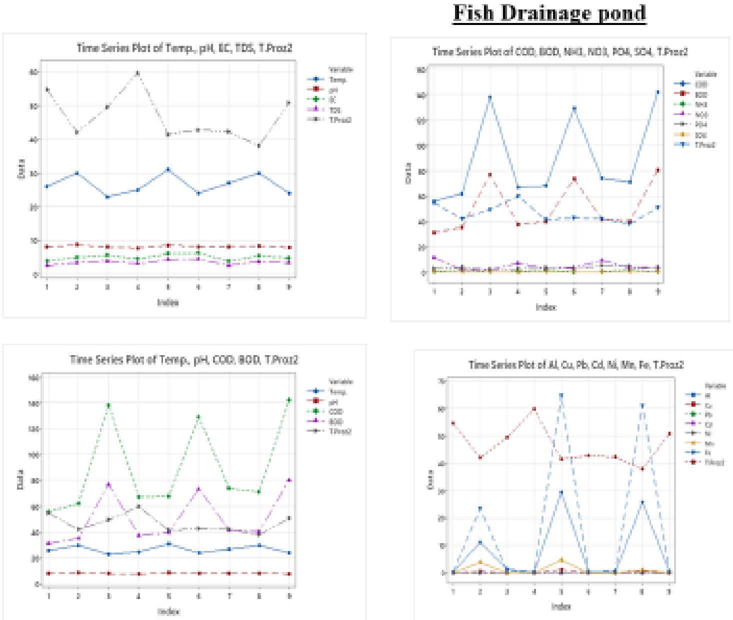

**Figure (1) Summary of the time series analysis for protozoan organisms against various abiotic parameters at the fish drainage unit.**

**Fish culturing pond**

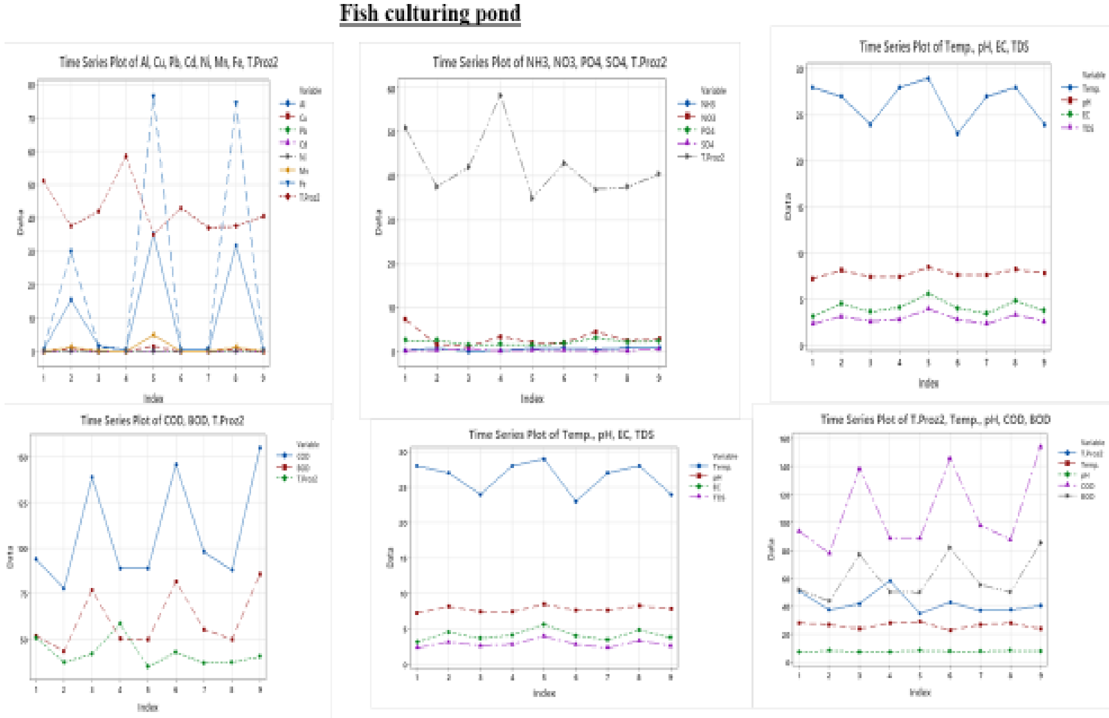


**Figure (2) Summary of the time series analysis for protozoan organisms against various abiotic parameters at the fish culturing unit.**

## CONCLUSION

Three separate fish farms in Khafr El-Sheikh province were used for the investigation. At these farms, the physicochemical properties of aquatic samples exhibited seasonal changes of varying magnitudes. It was proved that the highest total numerical densities of Protozoa in the fish culturing pond were obtained in spring. This may be understood primarily considering the metabolites produced by the various fish developmental stages and/or pollutants produced by agricultural drainage. In the province of Kafr El-Sheikh, it was proved that the protozoan organisms were considerably impacted by one or more factors in both the culturing and drainage fish farm units. It was recommended that the existence of a chemostat ecosystem in these fish farms was demonstrated because of specific biotic and abiotic interactions in addition to statistical data.

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## الملخص العربى

# دراسات بيئية على الكائنات الأولية فى ثلاث مزارع سمكية بمحافظة كفر الشيخ بمصر

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2- قسم بحوث البيئة – معهد تيودور بلهارس للأبحاث – مصر.
3- قسم الإستخدامات البترولية – معهد بحوث البترول – مصر.

**أجريت هذه الدراسة فى ثلاث مزارع سمكية بمحافظة كفر الشيخ وذلك خلال فصول الاستزراع السمكى الثلاثة (الربيع والصيف والخريف) لسنة 2018 فى كل من حوضى التربية والصرف لتلك المزارع حيث أن مصدر المياه لتلك المزارع مشترك وهو الصرف الزراعى لتلك المحافظة. ولقد ثبت أن قيم العوامل الفيزيائية والكيميائية لأحواض الصرف أعلى من مثيلاتها فى أحواض التربية عدا قيم الأكسجين المستهلك بيولوجيا BOD وكل من الحديد والمنجنيز والألومنيوم والذى يمكن ارجاعه الى مخلفات زريعة الأسماك وبقايا صرف الأسمدة والمبيدات الحشرية التى تستخدم فى الزراعية. هذا وقد سجلت قيم أعلى من الموصى بها دولياّ لكل من الأمونيا والكلور، أما النترات والفوسفات فكانت قيمها فى نطاق المسموح به من منظمة الصحة العالمية. كما أثبتت هذه الدراسة أن الكائنات الأولية بتلك المزارع الثلاث تتشابه لدرجة كبيرة من حيث وضع كائناتها الأولية التصنيفى وتختلف من حيث الأنواع والأعداد، كما أنها تتأثر بمعظم العوامل الكيميائية والفيزيائية والبيئية بطريقة متوازية مع بعض الاختلافات فيما بينها. هذا بالإضافة الى غياب معظم انواع الأوليات المميزة لوجود تلوث بمياه الصرف الصحى، ونستنتج مما سبق أن الكائنات الأولية المائية المتواجدة بتلك المزارع تحيا فى تناسق واتزان ( كيموستات ) مع العوامل غير الحية المتواجدة معها مما يؤدى الى تحسن وازدهار النظام البيئى ولذلك يزدهر الاستزراع السمكى بتلك المحافظة.**