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Assessment Performance of Aerated Lagoons for Removing Pathogenic Bacteria at Port Said Wastewater Treatment Plant

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Abstract

Safe disposal of waste water is the most critical environmental challenge facing our government. This study aims to evaluate the performance of aerated lagoon system in Port Said city for removal efficiency of bacteriological and physicochemical contaminants. Variations of bacteriological and physicochemical parameters along the ponds system were observed and evaluated. One year (2018) analysis was done in monthly intervals from January to December. The plant consists of aerated, facultative, and maturation lagoon in series. The study was done by analyzing ten parameters in groups of bacteriological and physicochemical parameters; the microbiological parameters monitored were; heterotrophic plate count (HPC), total coliforms (TC), fecal coliforms (FC), *Enterococcus*, and *Salmonella* spp. the physicochemical parameters monitored were; total suspended solids (T.S.S.), biological oxygen demand (BOD5), chemical oxygen demand (COD), ammonia and phosphorus. We targeted on *Salmonella* spp as a result of *Salmonella* spp causes numerous infections in humans. Removal efficiency of Heterotrophic bacteria ranged from (52.3-86.9%), (54.1- 93.6%) and (99.5- 99.7) for aerated, facultative and maturation lagoon respectively. Removal efficiency of Total Coliform bacteria ranged from (52.2- 83.3%), (51.6- 91%) and (99- 99.3%) for aerated, facultative and maturation lagoon respectively. Removal efficiency of Fecal Coliform bacteria ranged from (49.7- 81.7%), (48.1-94.2%) and (79.1-99.1%) for aerated, facultative and maturation lagoon respectively. Removal efficiency of *Enterococcus* ranged from (53.2- 87.7%), (55.5- 98.5%) and (97.7- 98.8%) for aerated, facultative and maturation lagoon respectively. Removal efficiency of *Salmonella* ranged from (32.5-57.6%), (36.1-66.1 %) and (95.2-99.7%) for aerated, facultative and maturation lagoon respectively. Removal efficiency of T.S.S. ranged from (25.1-43.4%), (37.2- 66.7%) and (19.6- 37.8%) for aerated, facultative and maturation lagoon respectively. Removal efficiency of BOD5 ranged from (28.8- 49.9%), (42.7-76.7%) and (22.5-43.4%) for aerated, facultative and maturation lagoon respectively. Removal efficiency of COD ranged from (34.6-53%), (44.5-77.5%) and (20.6-42%) for aerated, facultative and maturation lagoon respectively. Removal potency of Ammonia ranged from (16.6-37.3%), (25-51.5%) and (13.3- 47.9%) for aerated, facultative and maturation lagoon respectively. Removal efficiency of Phosphorous ranged from (0-12.9%), (8.4- 20%) and (0 - 7.1%) for aerated, facultative and maturation lagoon respectively.

Keywords: Wastewater Treatment; Aerated Lagoon; Pathogenic Removal

Abbreviations: HPC: Heterotrophic Plate Count; TC: Total Coliforms; FC: Fecal Coliforms; TSS: Total Suspended Solids; BOD_5: Biological Oxygen Demand; DO: Dissolved Oxygen; COD: Chemical Oxygen Demand; AL: Aerated Lagoon System; WSPs: Waste Stabilization Ponds; MF: Membrane Filter; MPN: Most Probable Number; KIA: Kligler iron agar.

Introduction

Urban wastewater contains varied unhealthful microorganisms and a high content of organic matter; so it poses variety of potential risks for public health and also the environment. Aerated lagoon system (AL) is taken into account associate in nursing acceptable technique for the treatment and removal of unhealthful microorganisms from effluent in tropical and climatic zone regions of the globe. The employment of eco-technologies for effluent treatment like pool systems is changing into common in developing countries attributable to its affordability and potency of infectious agent removal in heat climates. Aerated lagoon system utilize shallow basins for effluent treatment through natural medical care mechanisms by desegregation the activity of phototrophic, plant life and heterotrophic microorganisms [1]. The infectious agent removal mechanisms like the role of algal biomass, attachment and illuviation of unclean coliforms and also the role of predation by macro invertebrates and protozoans. The first purpose of effluent treatment is that the reduction of unhealthful contamination, suspended solids, BOD_5 and nutrient enrichment [2].

Aerated lagoon system area unit an inexpensive and effective shallow basins to treat effluent in things wherever the price of land isn't an element. The best methodologies of municipal effluent treatment are aerated lagoon system. Lagoons area unit straight forward stuff basins within which effluent is treated by the removal of stuff and also the biological degradation of settled solids. Aerated lagoon system considers drawn-out detention times and environmental factors (wind and solar radiation) for treatment efficiency [3].

Using oxidization pond technique is worldwide used for sewage treatment. Ponds became extremely popular with tiny communities as a result of their low construction and operative prices supply a big monetary advantage over different recognized treatment ways. One in every of the foremost important blessings is that the simplicity in construction and operation. These ponds area unit sometimes classified in line with the character of biological activity that's going down such as: aerobic, aerobic–anaerobic and anaerobic. However, the disadvantage is that the biomass concentration is comparatively low (25–50 mg/l) compared with activated sludge method (3000–5000 mg/l). Additionally, the particular volume reaction rate is low; so larger area units are necessary for enough effluent quality [4].

Temperature, starvation, the interactions of daylight with pH scale and O_2, algal toxins, algal biomass, predation and sedimentation of hooked up coliforms as key factors moving the removal of pathogenic bacteria from maturation ponds, that also are called tertiary lagoons [5].

Aerated lagoons treatment system was developed from the normal waste stabilization ponds (WSPs), wherever mechanical aeration was put in to extend the dissolved oxygen offer within the ponds system. It had been suggested that WSPs be placed next the aerated lagoon to allow the microorganism solids to settle and be stable. The inorganic parts remaining can stimulate the algae to grow within the WSPs. The aerated lagoons have additional blessings than the normal WSPs, in terms of eliminating odor nuisance and fewer land demand [6].

The aim of this study is to evaluate the performance of aerated lagoon system in Port Said city for removal efficiency of bacteriological and physicochemical contaminants. With the spotlight on *salmonella* bacteria because of their great danger, as it may cause health problems for humans and animals alike, as it is one of the most important waterborne diseases.

Plant Location and Design Description

The plant placed 5 km linear unit to the west of Port Said town. It's operative since 1996 to serve 450,000 populations. The capability of the plant is to treat one 190,000 m^3/day sewer water. It's designed as a secondary treatment plant on a part of 350 feddan (about 152 hectare) as aerated ponds. The plant is supplied with four mechanical screens to retain solids of quite 2.2 cm, then four sand grit removal lines in parallel series. Plant consists of aerated lake, facultative lake, and a maturation lake as shown in Table 1.

Lagoons	Retention time (day)	Volume(m^3)	Dimensions (m)	No. of aerators
Aerated	1	190000	365 × 150 × 4.5	92
Facultative	5	950000	1865 × 150 × 4.5	101
Polishing	5	950000	1865 × 150 × 3.5	-

Table 1: Design description of the aerated lagoons in Port Said city.

The aerated lake was designed as a whole mix reactor, wherever all contents of the lake were control in suspension. Operative aerators with homogenous distribution through the lake were enough to succeed in the conditions of complete mix and full aeration. Dissolved oxygen (DO) contents were retained at range from 3–5 mg/l. The facultative lake was designed as associate degree incomplete mix reactor. Solely the spread microbe's area unit maintained in suspension with the soluble organics. The organic matter that settles to bottom of the facultative lake undergoes anaerobic digestion and slowly releases organics into answer. Operative of aerators was enough to succeed in the conditions of incomplete mix. The DO was retained at range from 1–3 mg/l. The maturation lake was designed with hydraulic retention time Retention time of five days; its main function is to remove pathogens. The algae and surface air area unit liable for increasing DO content within the lake. The ultimate effluent is discharged by pump station to El-Manzala Lake.

Materials and Methods

Wastewater Samples

Samples were obtained from the Port Said, municipal sewer water treatment plant. The samples were analyzed over one year (January 2018–December 2018). They were collected at distinct points throughout the treatment process—from raw sewage taken at entry to the treatment plant, from effluent of Aerated lake, effluent of Facultative lake and from treated sewer water once Maturation lake.

Bacterial Analysis

Total bacterial count was determined by using the spread plate method. Several well-described classical biological indicators of contamination were chosen for inclusion during this study. Total Coli form bacteria, Fecal Coli form bacteria, and *Enterococcus* was all enumerated using the membrane filter (MF) technique [7].

Total microorganism count was determined by spread plate methodology. Samples were screened for Total bacterial count by spread of 50 ul of diluted sample on plate count agar medium and incubated at 35ºC for 24 hours. Total coliform count was determined by using the membrane filter (MF) technique. Samples were screened for total coliform bacteria by filtration of 100 ml diluted sample through 0.45 μm-pore-size membranes. Enumerations were accomplished by placing the membranes on m-Endo medium and incubated at 35ºC for 24 hours. Thermo tolerant fecal coliform count was determined by using the membrane filter (MF) technique. Samples were screened for fecal coliform bacteria by filtration of 100 ml diluted sample through 0.45 μm-pore-size membranes. Enumerations were accomplished by placing the membranes on m-FC medium and incubated at 44.5ºC for 24 hours. *Enterococcus* bacteria count was determined by using the membrane filter (MF) technique. Samples were screened for *Enterococcus* bacteria by filtration of 100 ml diluted sample through 0.45 μm-pore-size membranes. Enumerations were accomplished by placing the membranes on m-*Enterococcus* medium and incubated at 35ºC for 48 hours [7].

The enumeration of *Salmonella* spp. was performed mistreatment the Most probable number (MPN) procedure 5 samples of 3 volumes (10, 1, and 0.1 mL) were extra to tubes of selective Rappaport–Vassiliadis medium and incubated for 24 h at 43°C. These tubes were afterwards spread-plated onto *Salmonella*–*Shigella* agar and incubated at 37°C for 48 hours [8].

The middle of the *Salmonella* colony was lightly touched and was transferred to Kligler iron agar (KIA) slants, slant inoculated by stabbing the butt and streaking the slants. These were then incubated at 37ºC for 24 hours. Urea broth media and Simmons Citrate agar were inoculated and incubated at 37ºC for 24 hours [9].

Salmonella Identification

A single colony of a pure nutrient agar culture was grown overnight at 37°C in nutrient broth. DNA was extracted using Gene JET Genomic DNA purification kits (Thermo scientific-Germany).

A 30-bp forward primer (5'-CGGAACGTTATTTGCGCCATGCTGAGGTAG-3') and a 27-bp reverse primer (5'-GCATGGATCCCCGCCGGCGAGATTGTG-3'), targeting the invA gene of *Salmonella sp*., were utilized in PCR to get a 784-bp product. Amplification was carried out in a total volume of PCR reaction mixture (25 μL) contained 10 μL template DNA, 12.5 μL PCR Master Mix (PCR Buffer, 4 mM $MgCl_2$, 0.4 mM of each dNTP, 1.25 U Taq polymerase; Fermentas), 1 μL of each primer and ddH_2O to the total volume of 25 μL. PCR was dispensed in a programmable thermal controller (Bio metra).

An initial denaturation at 94 ºC for 5 min was followed by 36 cycles of denaturation at 94 ºC for 30 sec, annealing at 58 ºC for 30 sec and extension at 72 ºC for 1 min. Finally, an additional extension was achieved for 10 min at 72 ºC. A 10 μL aliquot of each PCR product was electrophoresed on a 2% agarose gel for 1.5 h at 100 V, stained for 10 min in ethidium bromide (0.5 μg ml^{-1}) and visualized and photographed under UV illumination. The molecular mass marker used was 4 kb plus DNA ladder marker (Invitrogen), a product of 784 bp was considered the invA gene [10].

Physicochemical Analysis

All determinations routinely performed at wastewater treatment plants are physiochemical determinations. Those included in our study for comparison with the variables described above were: total suspended solids (T.S.S.), chemical oxygen demand (COD) closed reflux colorimetric method, biological oxygen demand BOD_5, ammonia and phosphates. Analysis for all parameters was done according to the Standard Methods for the Examination of Water and Wastewater [7].

Statistical Analysis

Statistical analyses were performed using SPSS version (19). One way analysis of variance (ANOVA) was performed.

Results and Discussion

Total Heterotrophic Bacteria Removal Efficiency

Figure 1 illustrated removal efficiency of Heterotrophic Bacteria by using aerated lagoon ranged from 52.31% (least value) which recorded in January and 86.99% in August (highest value) while removal efficiency of this parameter by using facultative lagoon ranged from 54.19% (least value) which recorded in January and 93.62% in July (highest value) but it was founded that when using the Polishing lagoon removal efficiency ranged from 99.54% (least value) which recorded in January and 99.70% in October (highest value). El-Deeb Ghazy, et al. [11] recorded that removal efficiency in facultative and maturation ponds were more than 90% for total bacterial counts (90%).

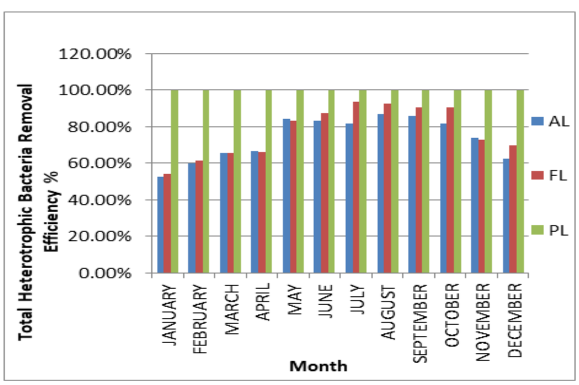

Figure 1: Total Heterotrophic Bacteria Removal Efficiency.
AL: Aerated lagoon
FL: Facultative lagoon
PL: Polishing (Maturation) lagoon

Total Coliform Bacteria Removal Efficiency

Figure 2 illustrated removal efficiency of Total Coliform Bacteria by using aerated lagoon ranged from 52.28% (least value) which recorded in January and 83.37% in August (highest value) while removal efficiency of this parameter by using facultative lagoon ranged from 51.69% (least value) which recorded in January and 91.05% in August (highest value) but it was founded that when using the Polishing lagoon removal efficiency ranged from 99.04% (least value) which recorded in December and 99.36% in October (highest value). Butler, et al. [12] reported percentage removal for total coli form bacteria was TC 99.96% in the summer season. Dar & Phutela [13] reported that over all removal efficiency of maturation ponds were 99%.

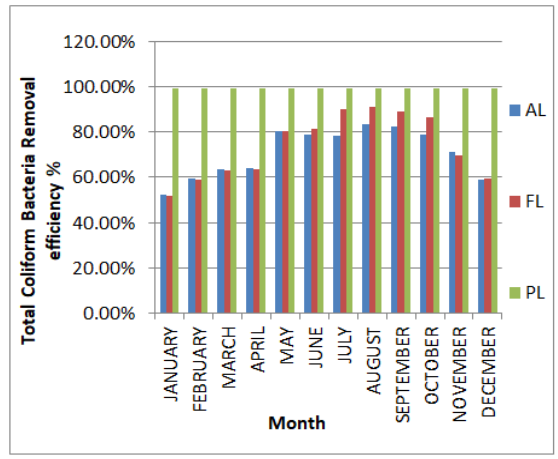

Figure 2: Total Coliform Bacteria Removal efficiency.

Fecal Coliform Bacteria Removal Efficiency

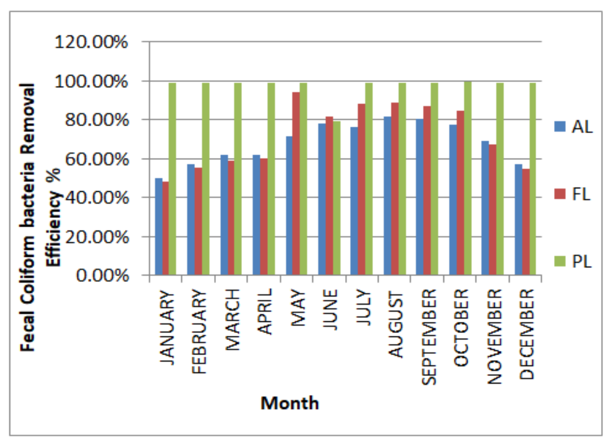

Figure 3: Fecal Coliform bacteria Removal Efficiency.

Figure 3 illustrated removal efficiency of Fecal Coliform bacteria by using aerated lagoon ranged from 49.70% (least value) which recorded in January and 81.79% in August (highest value) while removal efficiency of this parameter by using facultative lagoon ranged from 48.12% (least value) which recorded in January and 94.21% in May (highest value) but it was founded that when using the Polishing lagoon removal efficiency ranged from 79.13% (least value) which recorded in June and 99.14% in October (highest value). Kantachote [14] observed that reduction of fecal coliforms was 98 percent, which displayed a peak during the summer season. El-Deeb Ghazy, et al. [11] reported the removal efficiencies in facultative and maturation ponds were more than 95% for classical bacterial indicators.

Enterococcus Removal Efficiency

Figure 4 illustrated removal efficiency of *Enterococcus* by using aerated lagoon ranged from 53.23% (least value) which recorded in January and 87.76% in August (highest value) while removal efficiency of this parameter by using facultative lagoon ranged from 55.52% (least value) which recorded in January and 98.57% in May (highest value) but it was founded that when using the Polishing lagoon removal efficiency ranged from 97.75% (least value) which recorded in November and 98.89% in June (highest value). Reinoso, et al. [15] observed that reduction of *Enterococcus* was 89%.

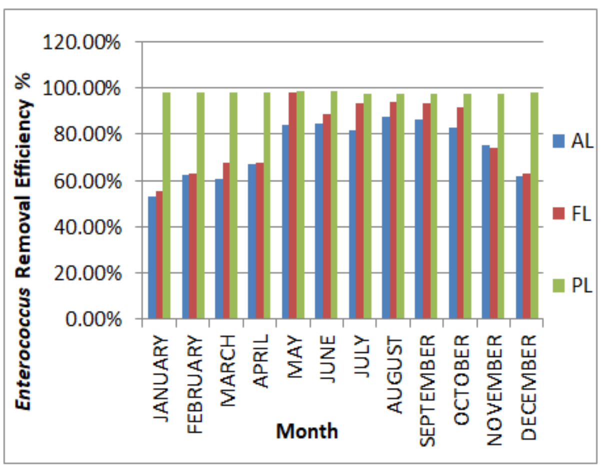

Figure 4: Enterococcus Removal Efficiency.

Salmonella Removal Efficiency

Figure 5 illustrated removal efficiency of *Salmonella* by using aerated lagoon ranged from 32.52% (least value) which recorded in January and 57.65 % in August (highest value) while removal efficiency of this parameter by using facultative lagoon ranged from 36.12% (least value) which recorded in January and 66.10 % in August (highest value) but it was founded that when using the Polishing lagoon removal efficiency ranged from 95.25% (least value) which recorded in December and 99.73% in July (highest value). Reinoso, et al. [15] reported Sunlight, along with other factors may remove up to 99.99% of microorganisms of Public-health importance.

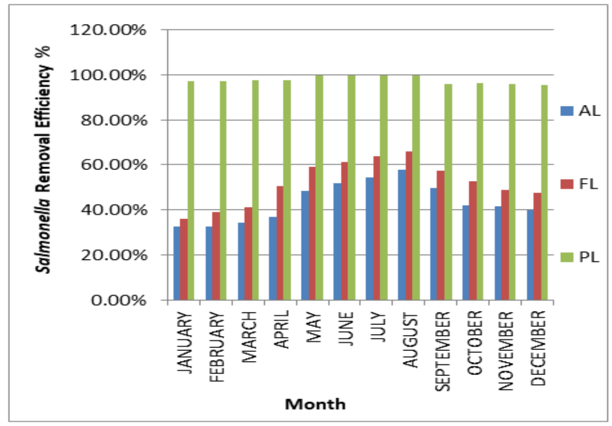

Figure 5: Salmonella Removal Efficiency.

TSS Removal Efficiency

Figure 6 illustrated removal efficiency of Total Suspended Solids by exploitation aerated lagoon ranged from 25.11% (least value) which recorded in January and 43.40% in August (highest value) whereas removal efficiency of this parameter by exploitation facultative lagoon ranged from 37.20% (least value) that recorded in December and 66.78% in June (highest value) however it had been supported that once exploitation Polishing lagoon removal efficiency ranged from 19.66% (least value) which recorded in January and 37.82% in August (highest value). Abdel-Shafy [16] showed that average removal efficiency of WSPs for TSS was 78%. Fernando & Quiroga [17] recorded that ponds in Sadat town was evaluated for wastewater treatment was able to remove 66% for TSS. El-Deeb Ghazy, et al. [11] showed that the overall reductions were 44.3% (TSS).

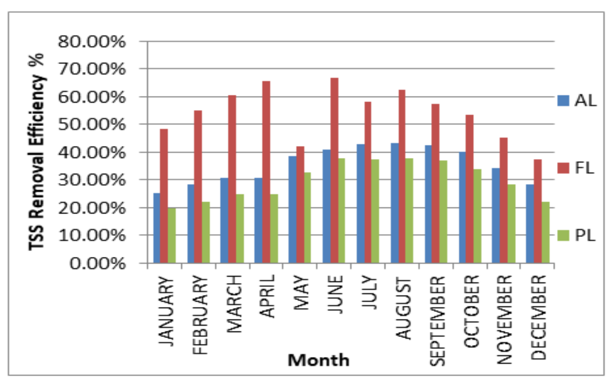

Figure 6: TSS Removal Efficiency.

BOD_5 Removal Efficiency

Figure 7 illustrated removal efficiency of BOD5 by using aerated lagoon ranged from 28.83% (least value) that recorded in January and 49.91% in August (highest value) whereas removal efficiency of this parameter by using facultative lagoon ranged from 42.75% (least value) that recorded in December and 76.71% in June (highest value) but it was founded that when using the Polishing lagoon removal efficiency ranged from 22.54% (least value) which recorded in January and 43.46% in August (highest value). Hamaidi, et al. [18] reported that lagoon system removed 57% of the influent BOD_5. Abdel-Shafy [16] showed that average removal efficiency of WSPs for BOD_5 were 81%. Hayati, et al. [19] showed that the overall BOD_5 reductions were 50.6%.

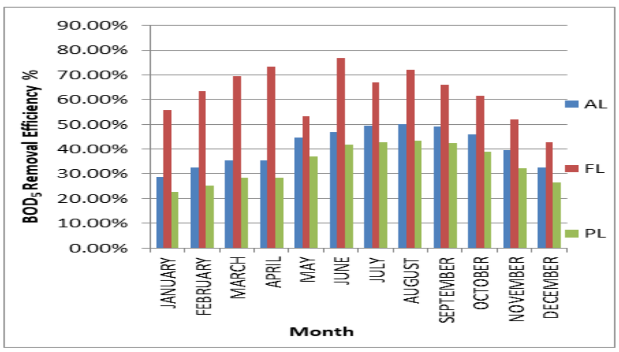

Figure 7: BOD5 Removal Efficiency.

COD Removal Efficiency

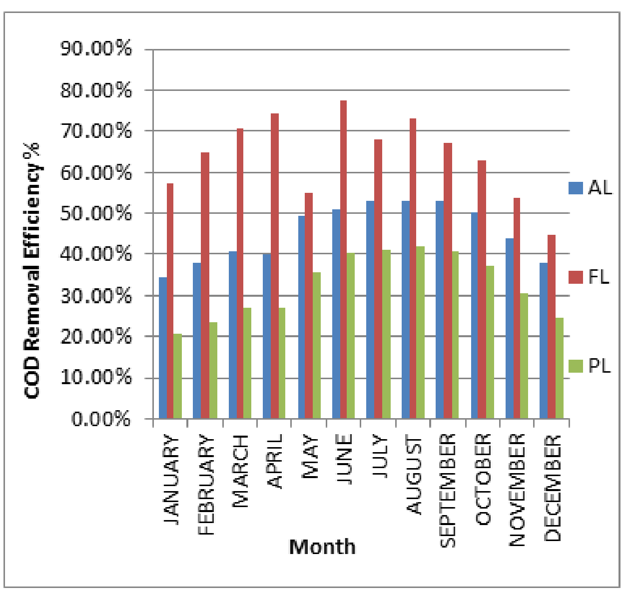

Figure 8: COD Removal Efficiency.

Figure 8 illustrated removal efficiency of COD by using aerated lagoon ranged from 34.60% (least value) which recorded in January and 53.07% in September (highest value) while removal efficiency of this parameter by using facultative lagoon ranged from 44.56% (least value) which recorded in December and 77.56% in June (highest value) but it was founded that when using the Polishing lagoon removal efficiency ranged from 20.65% (least value) which recorded in January and 42.08% in August (highest value). Abdel-Shafy [16] showed that average removal efficiency of WSPs for COD were 83%. El-Deeb Ghazy, et al. [11] showed that the overall reductions were 48.9% (COD).

Ammonia Removal Efficiency

Figure 9 illustrated removal potency of Ammonia by victimization aerated lagoon ranged from 16.66% (least value) that recorded in February and 37.34% in October (highest value) whereas removal efficiency of this parameter by victimization facultative lagoon ranged from 25% (least value) that recorded in February and 51.55% in July (highest value) however it had been based that once victimization the Polishing lagoon removal efficiency ranged from 13.33% (least value) which recorded in February and 47.91% in June (highest value). Hodgson [20] reported that WSPs removal efficiencies of ammonia were 92%. Kenneth [21] reported that there's very little proof for nitrification and denitrification (unless the wastewater has high concentrations of nitrate); It's believed that the nitrifying populations are very low in WSP, may be due to the absence of physical attachments.

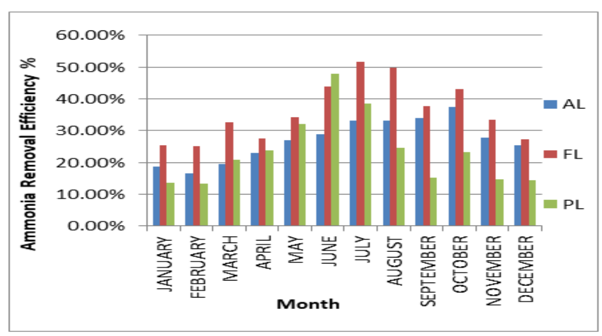

Figure 9: Ammonia Removal Efficiency.

Phosphorous Removal Efficiency

Figure 10 illustrated removal efficiency of phosphorous by victimization aerated lagoon ranged from 0% (least value) that recorded in February and 12.96% in December (highest value) whereas removal efficiency of this parameter by victimization facultative lagoon ranged from 8.47% (least value) that recorded in August and 20% in February (highest value) however it had been supported that once victimization the Polishing lagoon removal efficiency ranged from 0 % (least value) that recorded in February and 7.14% in April and December (highest value). Mara [22] recorded that phosphorus removal has been recorded up to 26% in arid climate in Marrakech, Morocco while in Catalonia; Spain removal of up to 48% has been recorded. A first order plug flow predicts a 45% P removal but this is mainly attributed to sediment adsorption. Alcocer, et al. [23] reported the maximum PO_4-P removal efficiency of WSP was 70%, obtained in July.

This statistical analysis showed that there was a weak (–ve) correlation between temperature and removal efficiency of phosphate (p>0.05), while it showed that there was a strong (+ve) correlation between temperature and removal efficiency of TSS, BOD_5, COD, Ammonia, Total heterotrophic bacteria count, Total coli form bacteria, Fecal coli form bacteria and *Salmonella* spp. (p<0.05).

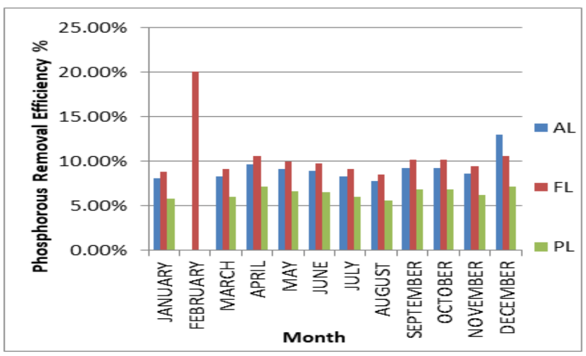

Figure 10: Phosphorous Removal Efficiency.

Salmonella Identification

Molecular identification of *Salmonella* isolates: Several sets of primers designed to detect genes specific for *Salmonella*. These included invA gen (this gen essential for full virulence in *Salmonella* and thought to trigger the internalization required for invasion of deeper tissues) producing 784bp.

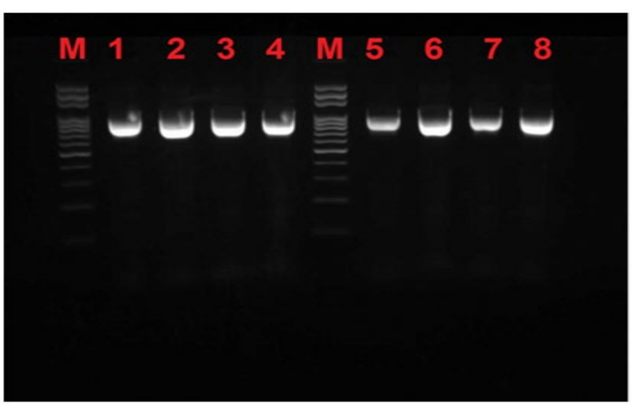

Figure 11: PCR product in gel electrophoresis 4kp Molecular weight marker; Lane 1 to Lanes 8 Salmonella isolates.

After enrichment procedure, and after their growth on specific media, randomly selected 8 isolates ideal colonies were subjected for molecular conformation using species-specific PCR primers. Agarose gel electrophoresis (2% agarose) of PCR amplified products using species-specific PCR primer sets. Lanes 1–8 are examined *Salmonella* isolates. Lane M, 4 kb DNA size marker (Figure 11) [24].

Conclusion

The aerated lagoon system at Port Said city is assessed to achieve high removal efficiencies of wastewater contaminants. The ponds demonstrate high reduction efficiencies in the physicochemical and microbiological parameters. The wastewater treatment system is effective and effluent water complies with standard wastewater management practices. Results showed that the high temperature was correlated removal efficiency of TSS, BOD_5, COD, Ammonia, TPC, TC, *Enterococcus* and *Salmonella* spp. We have worked to shed light on the *Salmonella* bacteria, as it may cause health problems for humans and animals alike, as it is one of the most important waterborne diseases *Salmonella* was counted after the various treatment stages and the efficiency ratio of each stage was measured, which in the maturation pond reached 99.73 %, biochemical tests were performed to identify the *Salmonella* bacteria, then extract the genetic material, conduct a polymerase chain reaction, and conduct electrophoresis analysis of the genetic material.

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