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Research Article

Parasitological Evaluation of the Purifying Performance of Wastewater Treatment Plants in Kirkuk, Iraq

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A B S T R A C T

Background: Water is an important resource for the survival of living organisms on our planet. Recently, some countries around the world have been suffered from water scarcity due to the increase in population and decreasing of annual rainfalls in the past few years, these reasons have led to a shortage of fresh-water resources. Hence, purification of wastewaters has been adopted for agricultural and aquaculture purposes. The current study aimed to determine the presence of protozoan (oo)cysts and helminth ova in both raw and treated water in two municipal wastewater treatment plants (MWTP^s) in Kirkuk province; and the validity of treated wastewater for irrigation according to global standards as well, using $ZnSo_4$ solution floatation and filtration protocol. One hundred and sixty wastewater samples were collected from selected two MWTP^s in Kirkuk province and investigated for the presence of pathogenic parasites.

Results: Different parasites had been detected form the two MWTP^s such as protozoan (oo)cysts and helminth ova, where protozoa; Entamoeba spp., Giardia, and Toxoplasma (oo)cysts, and hookworms, pinworms and whipworm; Ascaris, Enterobius, Strongyloides, and cestodes; Echinococcus and Hymenolepis were extracted. Protozoan (oo)cyst and helminths ova L^{-1} of raw wastewater ranged from 14-82 and from 11-38, respectively, while in the treated wastewater ranged from 2-12 and form 0-4, respectively. The data exhibited that the highest count was Entamoeba histolytica cysts of 82 L^{-1} (23.7%) followed by Entamoeba coli cysts of 78 L^{-1} (22.54%), while the least count L^{-1} was exhibited by Enterobius vermicularis ova of 1 L^{-1} (0.29%). The removal efficiencies of protozoan (oo)cyst and helminths ova by MWTP^s was about 90%.

Conclusion: The finding in the current study revealed that the efficacy of removal of total parasitic profile in the final effluent of Kirkuk's municipal wastewater treatment plants, is in compliance with the recommendation of Iraqi and WHO parasitological guideline required for unrestricted farmlands' irrigation.

Keywords: Parasitic helminthes, Protozoa, Wastewater Treatment Plant, Irrigation, Kirkuk

Introduction

All over the last few decennium, due to water scarcity pressure and increases in water request worldwide, reclaimed wastewater has been reused for a different purposes, such as agricultural irrigation, industrial, environmental and municipal uses.[1,2]

Like any arid and semi-arid countries in the world, Iraq "Mesopotamia" suffers from water scarcity due to the increase in population density and decreasing of annual rainfalls in the past few years, in addition to the construction of dams on the Tigris and Euphrates rivers by Turkey, as well as Iran closed some water tributaries for preventing its access to Iraq. These all caused a decrease in water levels in dam reservoirs and drying up of some lakes and some marshes' basins. However, the random well drilling played a major role in the drop in groundwater levels as well. All these problems led the local government resorting to the alternative solutions, and encouraging to sanitize wastewater and reuse it into for several purposes such as irrigate farmlands, football yards and in aquaculture.

Since wastewater transmitted a variable waterborne pathogenic agents 'including infectious parasites which may pose a riskiness to human health.[3,6] In addition, reuse of raw wastewater in agriculture and aquaculture might led to transmission of pathogenic organisms including oral-fecal parasites[7,10] and might emerge a variable foodborne diseases.[11,16]

Recently, several studies conducted around the world which indicated outbreak of the foodborne infections which associated to the consumption of fresh vegetables that played an important role in transmission of oral-fecal parasites, for instance in Iraq, Saudi Arabia, Egypt, Libya, Turkey, Iran, India, USA.[17,24]

Considerably, in any progression, the pathogenic agent's removal efficiency is a function of wastewater features, designing of the treatment plants criteria and experiences high disparities.[25] However, studies on protozoan cysts removal stated that wastewater treatment promoted the infectious parasites' cysts reduction, but not completely. [26,27]

There are different parasites removing protocol during wastewater treatment progressions.[28] The most efficient techniques are sedimentation, filtration, absorption and immotilization, and deactivation.[25,29] It is noteworthy that the quantification of parasite in the raw and treated wastewater is very important to further more evaluate of the affection of the wastewater treatment approaches on the protozoan (oo)cysts and/or helminthes ova occurrence. However, to minimize the risk of pathogen exposure in receiving environments the appropriate control technologies are continuously required having simple operation necessities, low principal investment and maintenance costs, and high competence for removal of pathogenic agents 'including parasitic helminthes' in order to protect public health.[30,32]

To our best knowledge, there is no previously published studies in the literatures dealing with the evaluate of the purifying performance of municipal wastewater treatment plants (MWTP^s) in Kirkuk. Therefore, this study has been carried out to find out the efficiency of MWTP^s in eliminating parasitic contamination, as well as the validity of treated wastewater for irrigation according to international standards.

Materials and Methods

Study Area

The current study was conducted on the wastewater samples which were collected from two MWTP^s in province of Kirkuk -Iraq, these are Shoraw located at Northeast of Kirkuk, and Al-Rasheed at Southwest Kirkuk, both operate as Sequencing Batch Reactors (SBR). Kirkuk province geographical coordinates: latitude: 35°47'05" N; longitude: 44°39'31" E; Elevation above sea level: 350-400 m; population: about 1.8 million. Due to security conditions; the third MWTP^s station "UNAMI" was excluded during the experimental period due to its location "in Al-Hurryah military air base" and it was required a superior permitions.

Sample Collection

A total of 160 wastewater samples were collected from two MWTP^s and investigated for the presence and concentration of pathogenic parasites. The samples were collected in plastic carboys with tight covers. Samples of raw untreated (5L each) from influent, and treated (5L each) from effluent of MWTP^s were collected once each month, from September, 1st, 2020 to April, 30th, 2021. the samples were labeled In situ with date and site of collection. The collected samples were transported directly to the Biology Department Advance Laboratory in College of Education/ 'Al-Hawija; recently, for Women', University of Kirkuk as sequential batches for subjected to the investigations. The samples were stored at 4 ºC until further used. Each batch was completed within a week.

Sample Investigation

The collected wastewater samples were investigated for parasite protozoan (oo)cysts and helminth ova by direct method and by sedimentation and floatation techniques, as described in.[33] To determine the incidence of parasites in the water samples, slides were prepared and examined under a low and high-power fields of compound microscope.

Data Statistical Analysis

The obtained data were statistical processed using the IBM SPSS software package (v. 22, USA) for windows 10. One-way analysis of variance 'ANOVA', paired t-test

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and statistical comparison of averages analysis was used to compare the parasites particles, related to the characteristics of the subjects. To identify the significant variables; Probability (p-values) of <0.05 were considered as the level of statistically significance.

Results

The collected wastewater samples from two MWTPs were investigated for the presence and concentration of pathogenic parasites. It has noticed that the concentrations were higher in the influent samples than the effluent samples. A total of 346 L^{-1} of (oo)cysts, ova and larvae of protozoa; Entamoeba histolytica, Entamoeba coli, Giardia lamblia, and Toxoplasma gondii (oo)cysts, and hookworms, pinworms and whipworm; Ascaris lumbricoides, Enterobius vermicularis, Strongyloides stercoralis, and cestodes; Echinococcus granulosus and Hymenolepis nana ova were recorded within the sampling period of the investigation, the distribution was 237 cysts/oocysts, 41 ova and 68 ova/larvae, respectively. The results are summarized in Table 1. Generally, protozoan cysts were the most frequency among the investigated MWTP^s samples.

It was extracted a total of 9 different parasite species throughout the raw untreated wastewater from influent and treated from effluent of both MWTP^s. The overall highest average initial concentrations of the cropped parasite materials were; in the raw wastewater, protozoan species amoebae: E. histolytica was recorded a very high statistical differences, where were 82 cysts L^{-1} [10.25(%95 CI 5.63-11.87); $p < 0.0001$] this was closely followed by E. coli 78 cysts L^{-1} [9.75(%95 CI 4.91-12.34); $p < 0.001$], respectively, while both G. lamblia and T. gondii were showed significantly least variables. Simultaneously, about cestode: E. granulosus also recorded a very high significant differences 26 ova L^{-1} [3.25(%95 CI 2.01-3.49); $p < 0.0001$]; concerning to the helminthic parasites, was nematode: A. lumbricoides with high significant differences with 38 ova L^{-1} [4.75(%95 CI 1.66-7.84); $p < 0.01$], both of S. stercoralis and E. vermicularis were showed least significant differences ($p < 0.05$). Generally, it was noticed that there was notable reduction of the parasite's materials in the treated samples (Table 1).

Regarding to the percentage of the harvested parasite materials concentrations from untreated raw wastewater in influent and treated once in effluent samples, the variances are shown in (Figure 1), where it was noticed that the high frequented percentages of the protozoan parasite's cysts and oocysts in the raw wastewater were for both E. histolytica and E. coli cysts of 23.69% and 22.5%, respectively. According to the cestodes finding, it was observed significantly incidence of E. granulosus ova 7.51%, and the nematodes result shows the most abundance of A. lumbricoides ova, where its percentage was about 11%. However, the percentages of the parasite's materials were remarkably decreased in the treated specimens, and a total percentage of the abatement rate of both MWTP^s was 89.44%.

Table 1. Concentration of Pathogenic Parasites in Influents and Effluents of Wastewater of Two Municipal Wastewater Treatment Plants (MWTPs).

Parasites	Ova, Larvae & (oo)cysts/liter				μ ± SEM	%95 CI	t	p
	Influent		Effluent					
	No.	x̄	No.	x̄				
Entamoeba histolytica	82	10.25	12	1.5	8.75 ± 1.32	5.63-11.87	6.63	0.000***
Entamoeba coli	78	9.75	9	1.125	8.63 ± 1.57	4.91-12.34	5.5	0.001**
Giardia lamblia	37	4.63	2	0.25	4.37 ± 0.92	2.19-6.56	4.73	0.002**
Toxoplasma gondii	14	1.75	3	0.38	1.38 ±0.59	-0.03-2.78	2.31	0.054 (NS)
Echinococcus granulosus	26	3.25	4	0.5	2.75 ± 0.31	2.01-3.49	8.78	0.000***
Hymenolepis nana	11	1.38	0	0	1.38 ± 0.32	0.61-2.14	4.25	0.004**
Ascaris lumbricoides	38	4.75	0	0	4.75 ± 1.31	1.66-7.84	3.64	0.008**
Strongyloides stercoralis	13	1.63	2	0.25	1.38 ± 0.56	0.39-2.71	2.43	0.045*
Enterobius vermicularis	14	1.75	1	0.125	1.63 ± 0.32	0.86-2.39	5.02	0.002**

No.= (+)ve case number, x̄ = sample mean, μ = population mean
SEM= standard error of mean, CI= Confidence Interval, t= t test value,
*** = very high significant, ** = high significant, * = significant, NS = non-significant

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In the current study, data shows that the monthly quantitative assess is directly proportional with the percentage concentrations of overall finding parasites materials. Generally, in both of raw and treated wastewater, it was observed that the highest numbers of parasites materials were recorded at September and gradually decreased till February, then increased again, the details are represented in Figure[s]. 2-4.

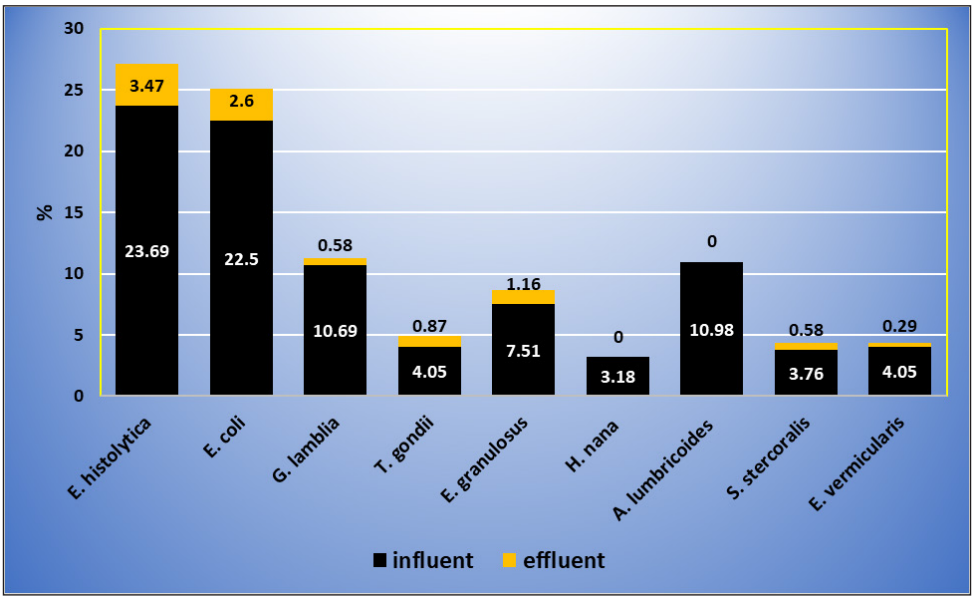

Figure 1.Overall Percentage Concentrations of Parasitic Materials in the Influent and Effluent Wastewater

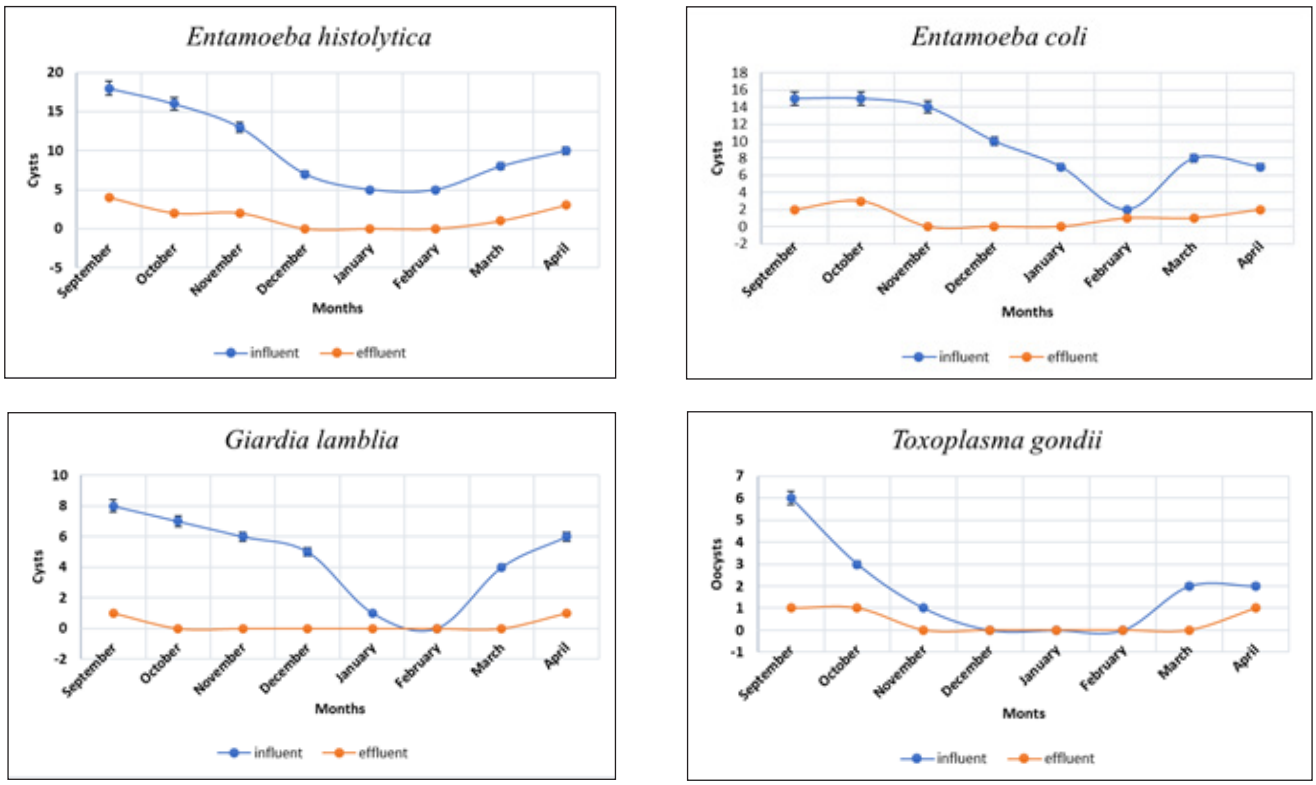

Figure 2. Monthly Quantities Variation of the Protozoan (Oo)cysts in Both Influent and Effluent Wastewater.

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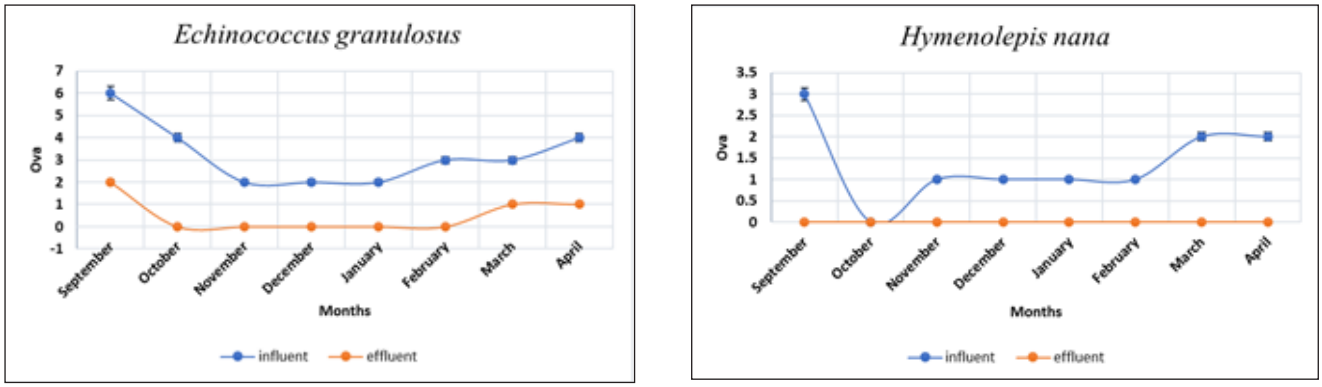
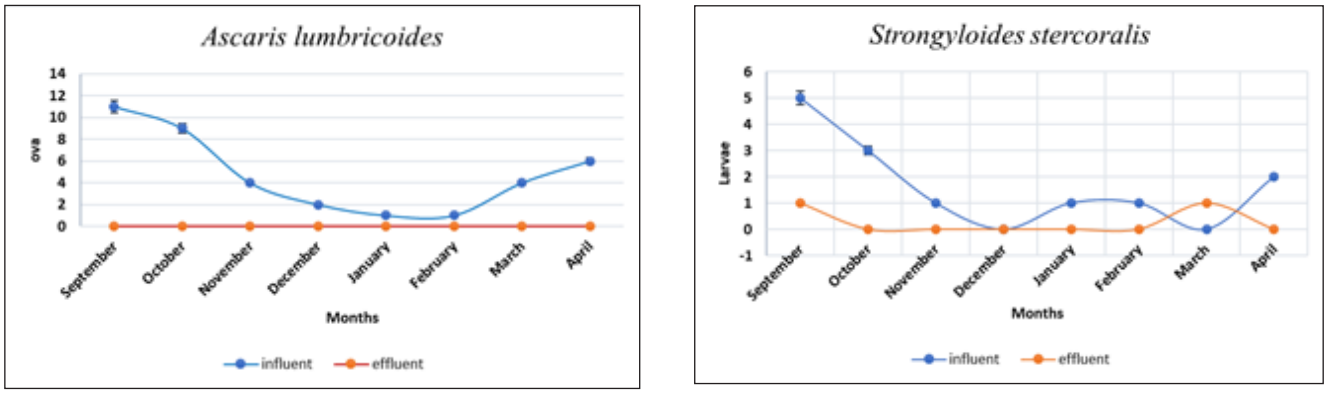


Figure 3.Monthly quantities Variation of Cestodes Ova in Both Influent and Effluent Wastewater



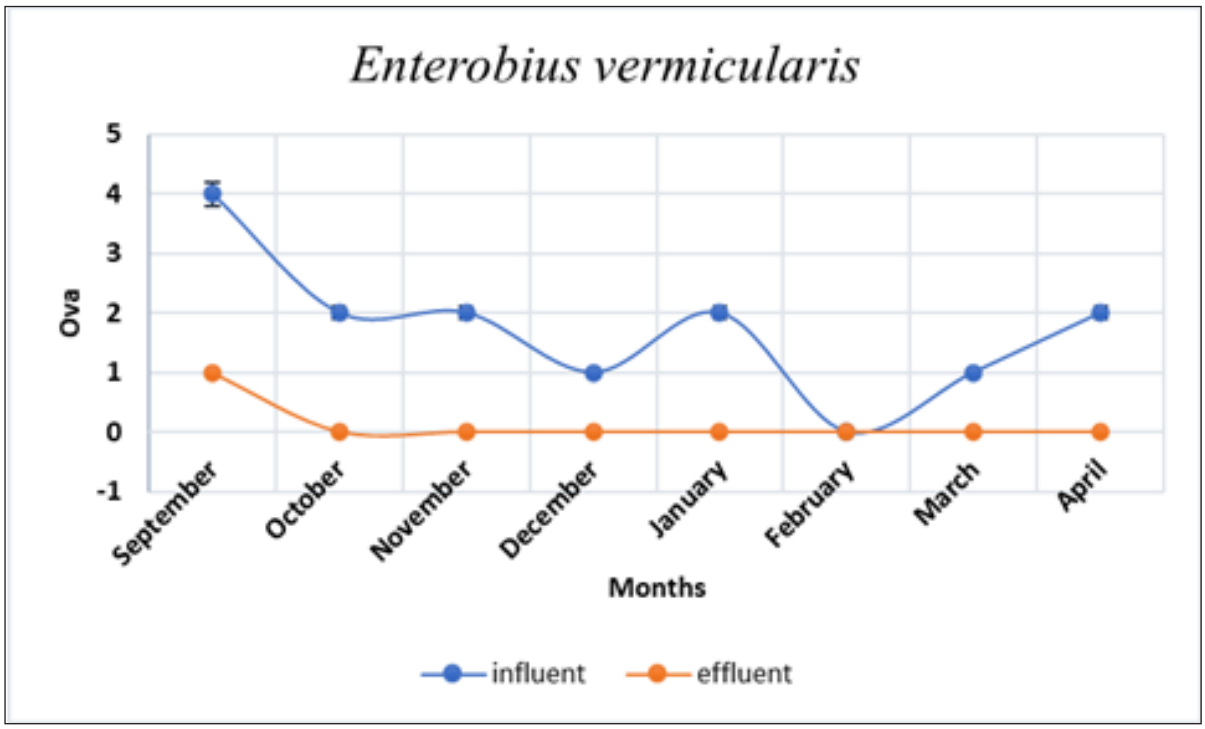


Figure 4.Monthly quantities Variation of the Helminth's Ova/Larvae in Both Influent and Effluent Wastewater

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Discussion

It is known that potential sources of water contamination by the parasites are the introduction of protozoa into the water system, including human and animal waste.[34] However, most gastro-intestinal protozoan parasites can be transmitted to humans by contaminated water and food with oral-fecal path, and transmission will be continued by cycling of both zoonotic and an anthroponotic.[35,36] Several studies had been conducted worldwide on this vital subject to spotlight on this vital affair and attempt to solve it permanently. Hence, the current study established for focusing on the occurrence of the parasitological profile in the MWTP^{s}.

The obtained data showed that ova of intestinal parasites commonly occur in wastewater produced by obvious mentioned municipal sewage treatment plants in Kirkuk province, where all investigated raw water samples (100%) were contaminated with intestinal parasites. It was recorded variable parasites (oo)cysts, ova and larvae, as listed in Table 1.

In the current study, E. histolytica was the most frequented intestinal parasitic protozoan cysts with very high significant ($p<0.0001$) than others in the crude sewage sludge of MWTPs followed by E. coli and G. lamblia; with percentage of 23.69%, 22.5% and 10.69%, respectively. The present findings are close as to those of.[37,40] Also, the present findings are comparable with those of [36,41,43] who stated that Giardia sp. And E. histolytica, the pathogenic protozoa to humans and animals, were the most abundance in raw wastewater samples. Generally, the finding data in the present study were lowest than results from a number of studies conducted in Italy[35,44,45] Scotland, UK[46] France[47] Finland[48] Japan[49] Tanzania[50] and Jordan.[51]

However, these findings mean were observed that they reduced in the treated plants samples significantly but not at all, where limited ratios of (oo)cysts were remained in the mentioned samples. The latter results indicate that such kinds of parasite (oo)cysts may be due to their high resistance via treatment protocol, thus they get ability to survive, as described by.[52]

Concerning to occurrence of the helminthes ova, it was detected the highest mean of A. lumbricoides ova (10.98%) with high significant than others in the investigated MWTP^{s} samples as shown in Table 1, this was followed by E. granulosus ova (7.51%) while each of H. nana, S. stercoralis and E. vermicularis were exhibited the least percentages with 3.18-4.05%, respectively. These findings are similar with the studies of.[38,53,54] Simultaneously, the present results are compatible with those of[42,55,56] who reported that A. lumbricoides ova were the most abundant in the MWTP^{s} samples. Generally, the conventional activated sludge system and oxidation ditches were showed to be efficient in the removal of A. lumbricoides and H. nana ova with removal efficiency of 100%.

Through reviewing the obtained data, it was noticed that the abundance mean of parasites were determined for all investigated MWTP^{s} samples which collected in September. However, E. histolytica cysts was found in most abundance followed by E. coli cysts and A. lumbricoides ova respectively. T. gondii, H. nana, S. stercoralis and E. vermicularis ova were found with low density. Generally, it is observed that the mean of all parasites in the MWTP^{s} samples were decreased significantly through November till February, then they had re risen again from beginning of March till the end of April.

It is noteworthy that the treated water had not treated completely, parasites' (oo)cysts, ova, larvae still existed in some treated samples as it shown in Figures.[2,4] The harvested results are compatible to the standard WHO guidelines which stated that treated wastewater should contains ≤ 1 egg of helminth L^{-1} to be appropriate for agriculture uses[57,58] this indicated that the efficiency of the stations was satisfied in reducing the water pollution with parasite ova.

Eventually, it is noticeable in the current study that the parasitic contamination in the investigated MWTP^{s} was lower than that in the other studies. This may be attributed to the water samples volume which was rather small, and this led to decrease chances to detect more parasitic contamination. Moreover, the prevalence of parasites in the influent of a MWTP^{s} depend on the served community density as well as the percentage of infection among the population, transmission rates, economic status of the society, climatic conditions and geographical location.[59,60]

In conclusion, the obtained data in the current study are encouraging. Generally, it does achieve a total parasitic profile removal in accordance with the standards for wastewater discharge which recommended by Iraqi and WHO guidelines. Consequently, these reductions were sufficient and recommended for safe reuse for farmlands' irrigation of the final effluent for both monitored MWTP^{s}.

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