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Pathogenic Biota of Wastewater of Ganja

Sima Hasanova[1*], Nigar Agayeva[2] and Anar Huseynov[3]

[1]*Department of Ecology of Azerbaijan State Agrarian University,*
[2]*Department of Immunology and Microbiology of Azerbaijan State Medical University*
[3]*Institute of Microbiology of the Azerbaijan National Academy of Sciences, Azerbaijan*

**Corresponding author*

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A B S T R A C T

The presented work is devoted to the study of microbiological and parasitological parameters to determine the state of agrophytocoenoses irrigated with wastewater discharged from Ganja, the second largest city in the Republic of Azerbaijan. It has been established that the main factors contributing to the effective transmission of pathogens during irrigation with wastewater are: prolonged presence of the pathogen in the environment; low minimum infectious dose; weak or very low immunity; ingestion by other means, such as food and water, and poor personal or household hygiene. It has been found that there are many public health risks associated with wastewater irrigation. Thus, the lack of wastewater treatment facilities in Ganja aggravates the situation. Microbiological studies show that each intestinal microbe is found in the wastewater of Ganja. It has been clarified that parasites belonging to three classes of invertebrates: cestodes or tapeworms, trematodes or flukes and nematode or roundworms can be considered more widespread parasites in the studied waters.

Introduction

In recent years, as a result of the scarcity of alternative water resources, the need to irrigate crops in arid and semi-arid regions and increase local food production has significantly increased interest in wastewater use. With this in mind, care should be taken to provide practical suggestions when planning the value of water resources. These approaches are very interesting both in terms of protecting water and preventing surface and groundwater pollution, as well as food circulation.

In the neseccary public health protection, it is commendable to resort to such an experience. Today, in the global world, in some countries, the reuse of all wastes, mainly from sewage treatment plants, for irrigation is a state policy (Aziz *et al.,* 2014). The publication of guidelines for the protection of health by the World Health

Organization is constantly updated. The results show that, based on available epidemiological data, intestinal nematodes and helminths pose the highest risk for disease transmission, as they remain in wastewater for a long time (Flörke *et al.,* 2013).

In the subject of sanitary parasitology and related pollution the use of wastewater and sludge in agriculture is considered a topical issue for sanitary engineers. Microbiologists and parasitologists must now work together in this area.

Taking into account all this, the study of microbiological and parasitological aspects of wastewater of Ganja is set as a goal.

Materials and Methods

The research is dedicated to the microbiological and parasitological study of wastewater discharged from Ganja in the direction of Samukh district in 2019-2021. One of the main factors influencing the formation of helminth fauna is atmospheric precipitation. Humidity plays an important role in the development of helminth eggs and their reaching the invasion stage. Helminths in water and underwater have been studied by known methods.When examining helminthosis, several helminthoovoscopic, as well as helmintholarvo-scopic and helminthoscopic methods have been proposed and put into practice. The taken waste water is filtered and separated from the large particles, then water is added to it and the washing process is repeated several times until the mixture is completely transparent. Finally, the clear mixture is poured into the beaker of the centrifuge, rotated for 1 minute, then the liquid is discarded but sediment is retained and saturated zinc sulfate solution is added to it. The beaker is covered with a cover glass and re-rotated in a centrifuge. Cover glass is carefully removed, placed on the glass slide and examined under a microscope. From the strongylatoses of the gastrointestinal tract, eggs of nematodirus are surrounded by a double membrane and a small nucleus appears inside in the form of particles. Since it is not possible to separate the eggs of Haemonchus, Chabertia, Bunostomum, Oesophagostomum, they are generally calculated. The inside of their eggs is homogeneous and surrounded by a membrane. Since trichocephalosis eggs are also clearly selected, their distribution has been studied by helminthoovoscopic. Trichocephalosis eggs were also examined by the Vishniauskas method (Berberoglu *et al.,* 2013).

To determine the distribution characteristics of helminths based on imaginal helminths, helminths parasitizing in wastewater in different ecological zonesare poured into separate containers and water is added to them. After settling the mixture for 30 minutes, the top layer is filtered and washed several times in succession. The sediment is then collected in glass jars and the helminths are separated by a partial examination in a Petri dish. After settling the remaining water and discarding the upper part, the sediment is examined by eye and with a magnifying glass. When there is a lot of material, a few drops of 3% formalin solution are added to the glass jar.

Haemonchus collected from wastewater, which is filamentous and appears in a relatively twisted position, were red and in a sticky state. The collected strongylatoses of the gastrointestinal tract are white and filamentous helmints. The taken trichocephalosis was whitish-yellow, head and cervix parts looked like a hair-like form. Brown fasciolas are triangular, leaf-shaped (Ivashkin, 1989; Parasitology and invasive.., 2008).

In order to examine the soil for nematode larvae, samples are taken from several areas at a depth of 5-6 cm and mixed. Then 5-6 g of average sample is taken from it and examined. To do this, the soil sample is wrapped in gauze and put on a metal filterplaced in a glass funnel in the Berman apparatus. Water is poured into funnel at a temperature of 50^0. A glass jarof physiological solution is placed through a rubber tube at the bottom of the funnel. After 3 hours, the nematode larvae in the soil pass into the water and settle to the bottom of the glass jar. The sediment at the bottom of the jar is examined for nematode larvae

(Mammadov, 1986). For microbiological examination water samples were taken from the same station in February, April, July and September. During the determination of this station, opinions of researchers who have previously conducted physical and chemical pollution studies in the area, factors such as the distances to residential and industrial settlements, and various wastes were taken into account.

The station where water samples were taken is located in the part where wastewater of Ganja city is discharged into the irrigation channel of Samukh district. Water samples were taken in sterile containers and brought to the laboratory within 24 hours (provided it is stored in the refrigerator) and analyzed. Membrane Filter Method, which is accepted and recommended all over the world and is often used in our country, was used in the examination of samples.

In some samples, the dilution method was used. Filtration was performed using 0.45 µm filters in the Millipore membrane filter unit. The membrane filters through which the sample passes are placed in an appropriate elective nutrient medium used to differentiate each microorganism after the filtration process. Finally, the Petri dishes are closed and placed in a thermostat upside down. The appropriate temperature is selected for each type of microorganism. At the end of the incubation period, the colonies are counted (Alexakis *et al.,* 2012; APHA-AWWA-WEF, 2005).

In order to determine the total number of coliform bacteria, filter papers were placedin Lauryl tryptose broth and Brilliant green lactose brothnutrient mediumand kept in a thermostat at 35±0.5 C^0 for 24 ± 2 hours. The coliform group of bacteria was found to grow in the form of dark red colonies on filter paper. Fecal coliform bacteria were kept in a mFC (m-fecal coliform) agar medium at a temperature of 44.5 ± 0.2C° for 24 ± 2 hours. At the end of the waiting period, fecal bacteria were found to grow in the form of blue colonies of 0.5-2.5 mm. Fecal streptococci were kept in a Slanetz-Bartley medium at 41 ± 0.5°C for 48±2 hours and Salmonella in Endo broth at 37 ± 0.5°C for 48 ± 2 hours in thermostat (Alexakis *et al.*, 2012).

Results and Discussion

Population growth, social and economic development have become a serious problem for water scarcity in the world. For this reason, scientists are trying to find a major alternative source of water and ways to use wastewater in agriculture to compensate for water shortages.

The use of wastewater for irrigation has a long history of development and has gone through different stages in developing countries, including developed ones. It has been proven that untreated wastewater can cause a number of environmental problems.

Studies have shown that the identification of helminth eggs is a fairly easy field to study, given that it can be easily achieved using a microscope. The following table provides information on the general microscopic view of helminths (Table 1).

The most common parasitic helminths belong to three classes of invertebrates: cestodes or tapeworms, trematodes or flukes and nematode or roundworms. The names of helminths found in the wastewater of Ganja city are shown in Table 2.

Some of the helminths shown in Table 2 are spread not only in wastewater of our country, but also in the wastewater of other countries. *Ancylostoma duodenale*, *Necator americanus*, *Schistosoma intercalatum*, *Schistosoma mansoni*, *Taenia saginata* and etc. can be shown. The species were identified based on the literature (Ivashkin, 1989).

The presence of helminths in wastewater is unavoidable and they are easily passed on plants. People can become easily infected if not washed clean or in direct contact with wastewater. There are many public health risks associated with wastewater irrigation. The main factors that contribute to the effective transmission of pathogens through wastewater irrigation are:

Prolonged stay of the pathogen in the environment;

Low minimum infectious dose;

Weak or very low immunity;

Ingestion by other means, such as food and water, and poor personal or household hygiene

The epidemiological characteristics of the main groups of intestinal pathogens are summarized by the above 4 factors. The following table provides a simplified theoretical basis for groups of pathogens due to their potential for disease transmission during wastewater irrigation. On this basis, helminth diseases appear to be transmitted in wastewater irrigation. Theoretically, pathogens can be listed as follows (Table 3).

Table.1 General features of helminths

Features	Cestodes	Trematodes	Nematodes
View	Straight, ribbon-like and segmented	Flat, leafy and unsegmented	Cylindrical and unsegmented
Front view	They have suckers and hooks, but do not have mouth	They have suckers	They have no scolex and hooks, but have mouth
Body cavity	None	None	Yes
Intestines	None	Yes, but no anus	Anus is visible
Sex	Hermaphrodite	Hermaphrodite Schistosoma spp. excluding	Female and male worms

Table.2 The most common parasites in the wastewater of Ganja

Scientific names	
1. *Ancylostoma duodenale*	2. *Schistosome (intestinal)*
3. *Ascaris lumbricoides*	4. *Hymenolepis diminuta*
5. *Clonorchis sinensis*	6. *Hymenolepis nana*
7. *Diphyllobothrium latum*	8. *Paragonimus westermani*
9. *Dipylidmm canssinum*	10. *Opisthorichis felineus*
11. *Enterobius vermicularis*	12. *Metagonimus yokogawi*
13. *Enterobius vermicularis*	14. *Necator americanus*
15. *Fasciolopsis buski*	16. *Schistosoma bovis*
17. *Heterophyes heterophyes*	18. *Schistosoma haematobium*
19. *Schistosoma intercalatum*	20. *Schistosome (oriental)*
21. *Schistosoma mansoni*	22. *Schistosoma japonicum*
23. *Taenia saginata*	24. *Taenia solium*
25. *Strongyloides stercoralis*	26. *Trichuris trichiura*

Table.3 Epidemiological characteristics of intestinal pathogens that cause infections during irrigation with wastewater

№	Pathogen	Sustainability of the environment	Parallel ways of infection	Infectious dose
Microbiology				
1	Bacteria	Average	Mainly associated with home, food and water	Low
2	Protozoa	Short	Mainly associated with home, food and water	Average/High
3	Virus	Average	Mainly associated with home, food and water	Low
Parasitology				
4	Helminths	Long period	Mainly ground contact and eating outside the home	Low
5	Lambliacysts	Long period	-	Average
Entomology				
6	Fly larvae and pupae	Average	Home conditions, food and soil	Average/High

Table.4 Seasonal change of pathogenic bacteria spread in the wastewater of Ganja (kol/100 ml)

Pathogenic bacteria	February	Aprel	July	September
General coliform	$23*10^3$	$68*10^3$	$21*10^5$	$12*10^4$
Fecal coliform	$12*10^2$	$19*10^2$	$26*10^2$	$21*10^2$
Fecal streptococcus	25	75	236	145
***Salmonella* spp**	0	12	65	34

Table.5 Water quality classification criteria (4)

Water quality parameters	Water quality classes			
	I class	II class	III class	IV class
General coliform	100	20000	100000	>100000
Fecal coliform	10	200	2000	>2000

Wastewater and waste sometimes contain secretions, which have their own ecological classification. Pathogenic intestinal viruses, bacteria, protozoa and helminths leave the body of infected people by secretion and are mixed in wastewater. In this case, it can be transmitted to others through vegetables irrigated with wastewater or by mouth (for example, through food), including the skin (as in schistosomes), which generally contain high concentrations of secreted pathogens. The causative agents of infections inpeople infected with diarrhea and intestinal parasites in different ways also differ. Experience should be used to determine under which conditions which infection is more prevalent or manageable during waste reuse (Madera, 2012; AWWA-WEF, 2005).

The situation is even worse in Ganja due to the lack of wastewater treatment plants. Thus, microbiological studies show that each of the pathogenic intestinal bacteria is found in Ganja wastewater (Table 4). The table shows the total number of common coliform, fecal coliform, fecal streptococcal and salmonella bacteria found in the studies carried out by seasons. According to the results obtained total number of coliforms in surface waters depending on the seasons was $23*10^3$-$21*10^5$kol/100 ml, number of fecal coliform bacteria - $12*10^2$- $26*10^2$kol/100 ml, number of fecal streptococcal bacteria - 25-236 kol/100 ml, number of salmonella bacteria - 12-65 col/100 ml. Compared to other groups of microorganisms, common coliforms and fecal coliforms are particularly high in the studied water. As a result of the study the most coliform groups were recorded in July. At the same time, the group of salmonella that causes health problems such as typhoid and salmonellosis is the least common among the shown bacteria and is more common in the summer. Temperature limits affect the spread of microorganisms in the water, especially their development.The fact that the number of coliforms which are mesophilic (20-45^{0}C) microorganisms in the measurements taken in July in the study is high can be attributed to the warming of surface water, especially in these months. In addition, another reason for the high number of coliforms in the water in July may be the mixing of feces of animals grazing around the channel, especially during these months.

There are four different quality classes for inland surface water under the Regulation on Water Pollution Control and Water Intended for Human Consumption (WPCR) (Class I: High quality water, Class II: Less polluted water, Class III: Contaminated water, Class IV: Highly contaminated water). Table 2 gives limit values for this classification.According to the regulations applied in many developed countries of the world, there should be no coliform bacteria, streptococci and staphylococcifor any kind of use(50-100-250 ml) (APHA-AWWA-WEF, 2005; Berberoğlu *et al.*, 2013).

It has been established that wastewater in the study area has the III class of quality in terms of parameters.

According to the standards of water pollution control rules, water assessed as III quality class belongs to the category of polluted water. These waters should not be used in areas requiring quality water such as food and textiles. This type of water can be used in industry and for irrigation of agricultural areas after passing through appropriate treatment facilities (Bingül *et al.*, 2017). As a result of the research, it was found out that it is not expedient to use the wastewater of Ganja city, which is currently used for irrigation purposes, discharged into the channels located in the Samukh region, in any direction. These waters have the status of polluted waters. Detection of coliform bacteria (fecal coliform and fecal streptococci) and a large number of parasites in all seasons indicates that these waters are mixed with waste or wastewater with human and animal feces. Intensive agricultural activities, topographic structure and meteorological conditions in the region are factors determining the level of coliform bacteria. When spring and autumn rains are intense in the region surface runoff and leaks from agriculture and settlements increase the level of microbiological types. Given the fact that seasonal precipitation plays an active role in the transport of various pollutants into the irrigation channel, the presence of disease-causing pathogen species can be associated with this situation. After harvesting vegetables irrigated with such water, they should be washed several times and the eggs destroyed by keeping them in vinegar for 1 hour. Otherwise, bacteriological and parasitological diseases will increase.

References

Alexakis, D., Gotsis, D., Giakoumakis, S., 2012. Assessment of Drainage Water Quality in Preand Post-Irrigation Seasons for Supplemental Irrigation Use. Environmental Monitoring and Assessment, 184, 5051-

5063.

APHA-AWWA-WEF, 2005. Standard Methods for the Examination of Water and Wastewater, 21st edition. American Public Health Association, American Water Works Association, Water Environment Federation, Washington DC, USA.

Aziz F, Farissi M. 2014. Reuse of treated wastewater in agriculture: solving water deficit problems in arid areas (review). Ann West UnivTimis¸ oara, 17:95–110.

Berberoğlu, U., Güngör, Ç., 2013. Yüzey Suyuve Sulama Amaçlı Atık Sularda Fekal Kirlilik Düzeyleriile Helmint Yumurta ve Protozoa Kistlerinin Araştırılması. Türk Hijyen Dergisi, 70, 191-200. (in Turkish) *(Berberoglu, U., Gungor, Ch., 2013. Investigation of Fecal Pollution Levels and Helminth Egg and Protozoa Cysts in Surface Water and Irrigation Wastewater. Turkish Hygiene Journal, 70, 191-200).*

Bingül, Z., Altıkat, A., 2017. EvselNitelikliAtıksuArıtmaTesisi Çıkış Sularının Tarımsal Sulamada Kullanıla bilirliği. Iğdır Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 7, 69-75.(in Turkish) *(Bingul, Z., Altikat, A., 2017. Usability of Domestic Wastewater after Treatment in Agricultural Irrigation. Journal of the Institute of Science and Technology, 7, 69-75)*

Flörke M, Kynast E, Bärlund I, Eisner S, Wimmer F, Alcamo J. 2013. Domestic and industrial water uses of the past 60 years as a mirror of socio-economic development: a global simulation study. Glob Environ Chang, 23:144–56.

Madera C A, Peña M R, Mara D D. Microbiological quality of a waste stabilization pond effluent used for restricted irrigation in Valle Del Cauca, Colombia. Water SciTechnol, 2002; 45 (1): 139-43.

Məmmədov A. Q., Hacıyev Y. H., Şirinov N. M., Ağayev Ə. Ə. 1986. Baytarlıq-parazitologiyası. Bakı: Azərnəşr, 428 s. (in Azerbaijan) *(Mammadov A. G., Hajiyev Y. H., Shirinov N. M., Agayev A. A. Veterinary parasitology. Baku: Azerneshr, 1986, 428 p.)*

Parasitology and invasion diseases of animals. 2008. / Ed. By M.Sh.Akbaeva. M.: Kolos, 776 p(in Russian) *(Паразитология и инвазионные болезни животных / Под ред. М.Ш.Акбаева. М.: Колос, 2008, 776 с.)*

Ивашкин В. М., Орипов А. О., Сонин М. Д. 1989. Определитель гельминтов мелкого рогатого скота. М.: Наука, 242 с. (in Russian) *(Ivashkin V. M., Oripov A. O., Sonin M. D. Key to helminths of small cattle. M.: Nauka, 1989, 242 p.)*

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