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Bio-purification of *Ascaris lumbricoides* ova in a transformed *Echinochloa pyramidalis* wastewater treatment filter plant in the city of Yaounde (Cameroon)

Francine Victorine Ngono Ngono *, Synthia Danielle Atchoukeu Yonguy and Aghaindum Gideon Ajeagah

Laboratory of Hydrobiology and Environment, Department of Animal Biology and Physiology, Faculty of Science, University of Yaounde I, P.O. Box 812, Yaounde, Cameroon.

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Abstract

The reuse of wastewater is widespread across the globe, especially in regions with water scarcity. Recycled water can be utilized in irrigation but if not treated effectively can pose risk for public health owing to the pathogens present in the recycled water used for irrigation *Ascaris lumbricoides* is a major soil-transmitted helminth that is highly infective to humans. The ova of *A. lumbricoides* are able to survive wastewater treatment, thus making it an indicator organism for effective water treatment and sanitation. Hence, Ascaris ova must be removed from wastewater matrices for the safe use of recycled water. The results of the observations of *Ascaris lumbricoides* eggs indicate a general density of 88 ± 72 ova.l^{-1}. The highest density was 338 ova.l^{-1} obtained in fat and oil removal basin during the LRS. Although a density of 24 larvae/L was detected at the outlet. Overall, larvae decreased at the secondary treatment level with a general purification yield of 85%.

Keywords: *Ascaris lumbricoïdes*; Indicator organism; Purification yield; Wastewater treatment plan; Yaounde

1. Introduction

In particular, soil-transmitted helminth ova can survive for several months or years in the environment and are a concern where wastewater and sludge reuse are prominent. In 1989, the World Health Organization (WHO) focused on the helminth-associated infections that occur due to poor sanitation, poor hygiene, and inadequate water quality .Soil-transmitted helminth (STH) infections are of severe concern, affecting nearly one-third of the world's population *Ascaris lumbricoïdes* is the major STH, afflicting more than one billion people worldwide and leading to malnutrition in children, cognitive impairment, and gastrointestinal complications .As a result of their environmental hardiness, the WHO recommends parasitic helminth ova as an indicator of sanitary risk and water quality parameters. Wastewater treatment plants are a set of structures and reservoirs where waste water confluences with the aim of giving it better quality characteristics. These are indeed functional systems which, using physical, chemical and biotechnological processes, purify wastewater of various origins. Wastewater can be of domestic, agricultural, industrial and river origin. Regarding domestic wastewater, it includes gray water and black water. The first come from bathtubs, showers, sinks, sinks, washing machines. Their pollutant load consists of soaps, detergents, (kitchen) greases and also phosphates from certain laundry products [1]; the second come from sanitary facilities where urine and fecal carry. Their pollutant load consists of nitrogenous substances (urea proteins), organic phosphorus of metabolic origin and drug residues (estrogens, antibiotics, etc.) [1]. Dirty water poses risks to public health, food security, and other ecosystem services and functions [2]. It is therefore necessary to set up public and industrial services to promote the recycling and safe reuse of wastewater [3]. One of the oldest references to water treatment dates back to 2000 BC, the authors indicated how to treat water by heating it, filtering it through a bed of sand and gravel [4]. The operating principle of the stations

* Corresponding author: Ngono Ngono Francine.

is based on the succession of several treatment chains which are pre-treatment, primary, secondary and tertiary treatment.

Pretreatment is a physical process that aims to rid the water of its coarser particles for better chemical and biological treatment downstream. It thus protects the equipment from possible clogging or obstructions in the pipes. It is made up of different installation units including screening, grit removal, oil removal or degreasing [5]. Primary treatment eliminates suspended and colloidal matter of mineral and organic nature from the pretreated effluent. Primary treatment removes 30 to 50% of suspended solids and reduces BOD5 by about 40% [6]. This treatment involves natural physical and physico-chemical processes. Suspended solids settle to the bottom of the settling tank by gravity in the form of so-called primary sludge and the sludge is removed by scraping. Secondary treatment is a treatment process, most often biological, the aim of which is to eliminate carbon and nitrogen pollution [5]. The process is based on the degradation of biodegradable organic matter under the action of autotrophic and heterotrophic organisms. Indeed, plants and certain bacteria (nitrifying species) draw their energy from the oxidation of ammonia and use sources of mineral carbon and phosphorus to synthesize their own organic matter.

Ascaris lumbricoides is the microorganism responsible for ascariasis which is a human and cosmopolitan parasitosis. Indeed, it is the most common in the world, affecting a quarter of the world's population. It predominates in developing countries where various factors contribute to its transmission such as heat, humidity and fecal peril [6]. *Ascaris lumbricoides* is a round worm, 12 to 30 cm long by 2 to 4 mm in diameter for the male, 20 to 35 cm by 3 to 6 mm for the female. It lives in the male jejunum but can reach the liver, bile and pancreas. The number of hosted worms is variable, possibly exceeding a hundred. In the environment (water or soil), it is materialized by the presence of oval and symmetrical eggs of 50 to 80 µm by 35 to 55 µm, with a thick and hilly dark brown outer shell, with a smooth, colorless and very thick, when fertilized [6]. Infertile eggs with smooth outer shells measure 90-95µm by 40-45µm (sometimes 80-105µm by 45-55µm) [7].In wastewater, the persistence of Ascaris eggs is evaluated from a few months to more than a year [8], in the soil, up to 5 months on plants and it can resist up to at 7 years old. This resistance is dependent on parameters such as water temperature, pH and the presence of other microorganisms responsible for the predation phenomenon. The internal structure of the egg has three layers from outside to inside: the protein layer, the chitinous layer and the lipid layer. Electron microscopy of the outer layer of the egg shows a granular surface made of a regular organization of protein fibers [9] whose arrangement confers its rigidity to the Ascaris egg. The chitinous layer is an extremely hard layer which confers great resistance to the egg in the external environment. The helical arrangement of the chitin fibers would be responsible for the resistance of the egg to strong acids and bases as well as to various enzymes. Unlike the other so-called protective layers, the lipid layer allows gas exchange between the external environment and the egg [10].The life cycle of *Ascaris lumbricoïdes* is simple, direct and without an intermediate host. Fertilized females lay eggs that are eliminated in the external environment where they embryonate under conditions of high temperature (28 to 32°C) and high humidity. Eggs ingested, with drinking water, vegetables, soiled fruits, soil, see inhaled mixed with dust, release larvae which cross the intestinal wall. These eggs then reach the liver through the portal vein, then the right heart, the artery and the pulmonary capillaries in 3 to 4 days. Then, after a week, they cross the alveolar-capillary wall, pass through the tracheo-bronchial tree, are swallowed and arrive at the level of the jejunum where they are transformed into adult worms. Six to eight weeks later, the females begin to lay eggs [6]. Ascaris reproduction is characterized by ovular proliferation where egg laying is estimated at 200,000 eggs per day and more [11]. The cycle lasts a total of 60 to 90 days. The high rate of ascaris infection is favored by defecation in the open air and discharges of domestic wastewater used for the fertilization of agricultural areas [12].The presence of these eggs in the environment through wastewater constitutes a real public health risk. The present work aims to limit the risks of contamination by providing information on the purification efficiency of the water treatment plant (WWTP) of the «Cité verte», Yaounde.

2. Material and Methods

2.1. Material

The study took place in the city of Yaoundé, capital of the Center region. This urban agglomeration is the political capital of Cameroon. The city's annual population growth rate was nearly 5.3% between 2001 and 2015 and 5.7% between 2015 and 2020 [13]. (complete the level of sanitation of the city and the figures on the population of Yaoundé) the capital had 2,809,712 inhabitants with 775,911 dwellings in 2020.The city of Yaoundé is located on the western edge of the South Cameroon plateau, in the equatorial zone, between 3°45' and 3°59' North; 10°94' and 11°58' East [14] and 250 km from the Atlantic coast [15]. Qualified as "city of seven hills", Yaoundé develops in a site of hills and valleys. The valley area varies between 600 and 700 meters in altitude, crowned by rounded mountain ranges culminating between 700 and 1200 meters [16].

The sampling points on the green city water treatment plant are named CV1 (collector), CV2 (grit removal), CV3 (fat and oil removal), CV4 (Decanter), CV5 (manhole), CV6 (rectangular biological basin), CV7 (circular biological basin), CV8 (outlet). The green city SIC camp water WWTP is located in the second district of the city of Yaoundé instead - said MECC crossroads. It receives part of the domestic wastewater from the SIC camp of the green city which is located in the district of Yaoundé 4. At the last general population and housing census of 2019, the population of the SIC camp of the green city was estimated at 10,000 pe. The plant has a planted filter hybrid system with a capacity of 805 m^3/d and was designed for 5000 HA. The biological treatment is carried out by the joint action of a geotextile filter and plants of the species *Echinochloa pyramidalis*. The water resulting from the treatment flows into the Abiergué basin. The sludge collected on site is dried on site before being used as organic fertilizer for crops. Indeed, the interior of the site is the subject of a vegetable garden with plants such as guavas, cocoa trees, flowers. The sampling points selected for theanalyzes at the «Cité verte» water treatment plant are: the collector, the oil separator, the inspection chamber and the outlet:

2.2. Methods

The study took place in two phases: a preliminary phase from September to December 2020, devoted to prospecting for functional water treatment plants in Yaoundé. The second phase took place from January to November 2021 following a bi-seasonal step in order to collect physico-chemical and biological data in the two treatment plants selected. On behalf of this study, the samples relate to the water sector and are taken at the level of the collector and at the end of the various levels of treatment: pre-treatment, primary treatment and secondary treatment during the long dry season (LDS), Small dry season (SDS), long rainy season (LRS), small rainy season (SRS). The measurements of the physico-chemical parameters took place both in the field and in the laboratory following the recommendations of APHA [17] and Rodier and collaborator*s* [18].

Sampling of water for the identification of larvae and helminth eggs was done at collectors and at the end of each level of treatment including pre-treatment, primary treatment and secondary treatment. After gentle shaking to resuspend the particles, the water was immediately withdrawn using sterile 1 L polyethylene bottles and fixed in 10% formalin then transported to the laboratory [19]. These samples were allowed to stand at room temperature for 24 hours for sedimentation, then the supernatant was poured out and the volume of the pellet collected was measured. The techniques of Kato-Katz and diphasic formaldehyde-ether allowed us to concentrate the parasitic elements in order to guarantee a better enumeration. These samples were brought for direct examination [20] under an Olympus CK2 brand microscope with a 40X objective. Eggs of human intestinal parasitic helminths were identified using the WHO plates. The measurements of the dimensions were made using the micrometer on one of the eyepieces of the microscope. The number (X) of parasitic helminth eggs in 1 L of sample is assessed using the following formula [21]:

$$X = \frac{y.Vx}{Vy}$$

With: Vx= pellet volume in 1 L of sample, Vy= pellet volume used for observation, y= number of eggs observed in Vy.

The parametric one-factor ANOVA test was used to verify the spatial and seasonal significance of differences (or similarities) in variances of physico-chemical parameters and densities of biological variables, relative to the distribution of observed organisms. The purification yields were evaluated by the formula below [22].

$$R_2 = \frac{F_{tot,in}^{Eau} - F_{tot,out}^{Eau}}{F_{tot,in}^{Eau}} \ [\%]$$

$F_{tot,in}$: flux de substances admis

$F_{tot,out}$: flux de substances rejeté

3. Results

The minimum, maximum, annual average and standard deviations of the physico-chemical and biological parameters measured during the study period, as well as those of the purification yields are presented by sampling station in Appendix 1. Spatio-temporal variations (per station and per season) and seasonal monthly values of physicochemical parameters are presented below.

3.1. Spatial and seasonal variations of physical parameters

Spatial and seasonal variations in temperature, turbidity, SS and water color values are shown in figure 1. The water temperature varied from 24°C to 27.85°C during the study period; with a thermal amplitude of 3.85°C and an average of 26.15 ± 1°C. Suspended solids are abundant in the wastewater treatment plant. They range from 2816,5 mg/l in the decanter (CV3) during the SRS to 9 mg/l at the outlet (CV4) during the LDS. At this WWTP, the general average at the collector is 191 ± 94 mg/l and that at the outlet is 55 ± 39 mg/l. Water turbidity values are high at all sampling points. It varied from 147 FTU (CV4) during the LRS to 5.5 FTU (CV8) during the LDS. The average turbidity obtained during the study at this station is evaluated at 146.37 ± 43 FTU at the collector and 55.25 ± 39 FTU at the outlet. As regards the color contents, a peak is recorded at CV2 2296.5 during the LDS. The smallest values 97 Pt.co are obtained during the SDS. The «Cité verte» collector presented an average of 606.25 ± 419 Pt.co and the average at the outlet is 318.75 ± 181 Pt.co. Statistical analyses show seasonally significant difference in temperature and water color parameter ($p \leq 5\%$).

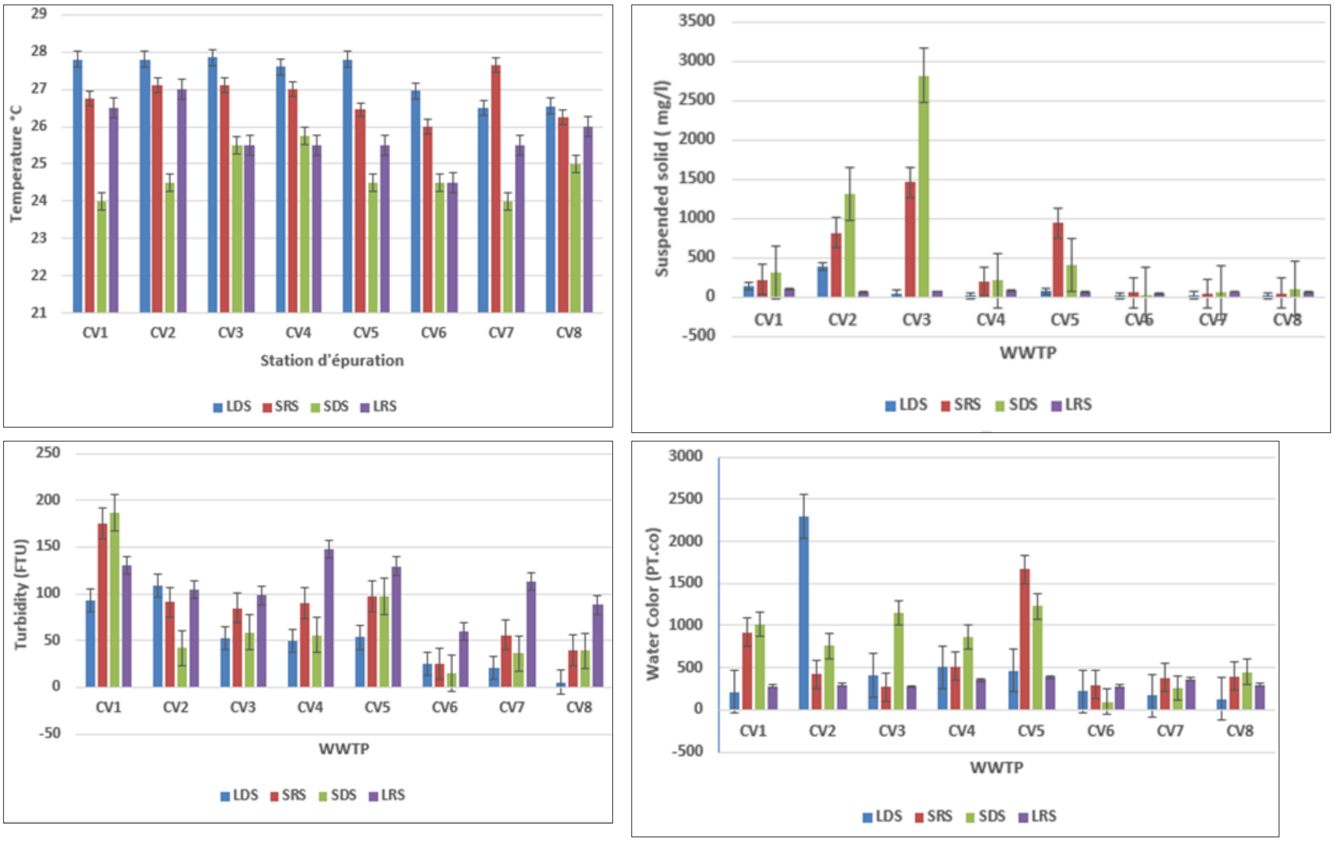

Figure 1 Spatial and seasonal variations of temperature, turbidity, SS and water color

3.2. Spatial and seasonal variation of chemical parameters

The spatial and seasonal variations in carbon dioxide, Dissolved oxygen, Nitrate and hydrogen potential are grouped in the figure 2. The carbon dioxide content varied little during the study period. Nevertheless, a peak (343.2 mg/l) is observed in the CV2 basin during the long dry season. At the «Cité verte» WWTP, the CO_2 content is assessed at 71.72 ± 24 mg/l at the collector and at 61.66 ± 20 mg/l at the outlet. The hydrogen potential varied around neutrality throughout the study period with an average of 7.36 ± 0.34 U at the «Cité verte» WWTP. The values of nitrate are varied of 170.15 $mg.l^{-1}$ CV1 during the SRS at $5 mg.l^{-1}$ CV6 LRS with an average of the percentage of dissolved oxygen saturation varied from 7.93% (CV3) in LRS to 1.65% (CV8) in SRS. Statistical analyses show seasonally significant differences for the nitrate parameter ($p \leq 5\%$).

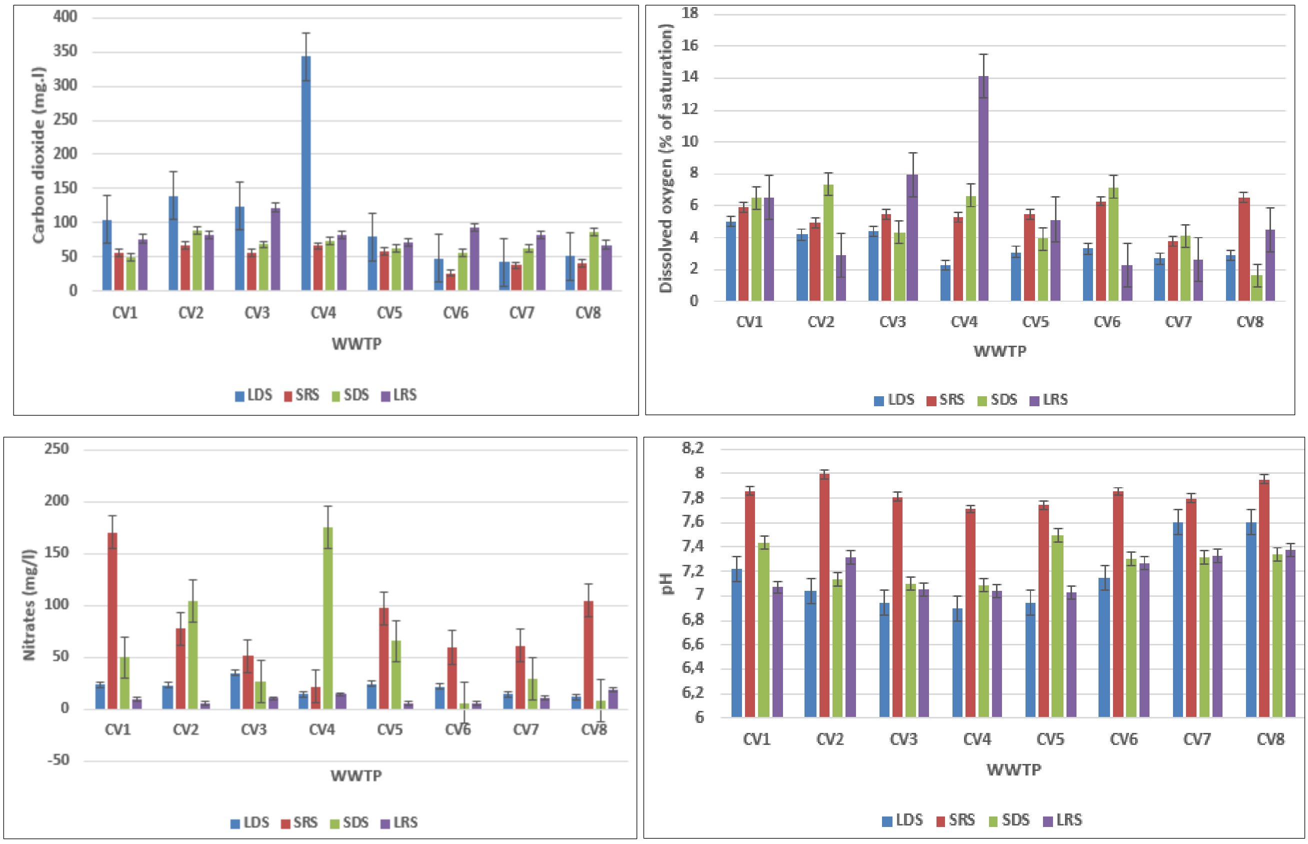

Figure 2 The spatial and seasonal variations of carbon dioxide, Dissolved oxygen, Nitrate and hydrogen potential

3.3. Spatial and seasonal variation of *Ascaris lumbricoïdes*

The spatial and seasonal distribution of *Ascaris lumbricoïdes* eggs is presented in figure 3. The results of the observations of *Ascaris lumbricoïdes* eggs indicate a general density of 88 ± 72 ova.l^{-1}. The highest density was 338 ova.l^{-1} obtained in CV3 during the LRS. However, the lowest density is 0 ova.l^{-1} obtained at CV8 during the LDS. In addition, the analyses reveal the presence of roundworm eggs at the outlet during the LRS, SRS and SDS. Overall, larvae decreased dramaticall at the secondary treatment level. The ANOVA test applied to *Ascaris lumbricoides* egg distribution schows spatially significant differences ($p \leq 5\%$).

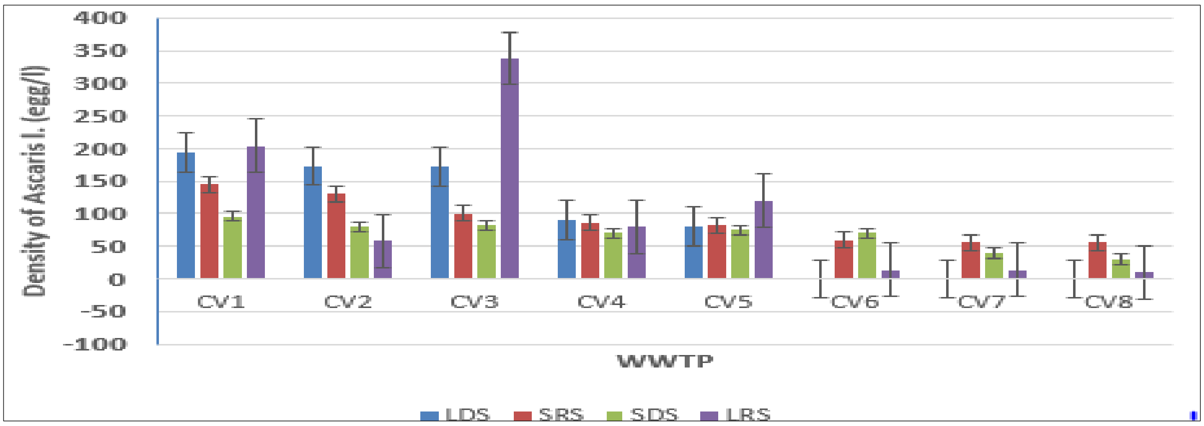

Figure 3 The spatial and seasonal variations *Ascaris lumbricoïdes* eggs

3.4. Rendements épuratoires annuels des paramètres

The purification yield of the physical, chemical and biological variables are presented in table1. CO_2, O_2, Warter color and Nitrates show low yields after treatment, while the SS, Turbidity, BD05 and *Ascaris l*umbricoides eggs show satisfactory yields. However, some parameters shows high concentrations at the outlet.

Table 1 Purification performance of physical, chemical and biological parameters

parameters	Collector	Outlet	Yields
CO_2	71.72	61.65	14.03%
O_2	5.98	3.9	34.78%
Water Color	606.25	318.75	47.42%
SS	191	55.25	71.07%
Turbidity	146.375	43	70.8%
DBO5	400	50	87.5%
NitrateS	63.175	35.9	43.17%
Ascaris lumbricoïdes	160	24	85%

4. Discussion

Ascariasis is the most prevalent and widespread intestinal helminthiases. Transmission of disease occurs by ingestion or inhalation of Ascaris eggs embryonated in the soil. During the migration of Ascaris to the lungs, pneumonic symptoms may develop. The intestinal stage of the parasite can cause severe symptoms and complications when the worm burden is high. The poor standard of hygiene, the biology of the parasite (which is such that a high number of very resistant eggs are produced), and the habits of the host (such as the use of night soil) are factors causing a high prevalence of infection in many countries in Asia, Africa, South America, and Europe. The mechanism of the transmission of Ascaris varies in different communities. The peak of infection is among children aged four to 14 years. Worm burden is normally low, and only a small segment of the population harbors a high proportion of the worms present in a community. Control of infections due to Ascaris is feasible by a combination of mass chemotherapy with the effective drugs now available, safe disposal of excreta, destruction of the eggs, and health education. integrity of ova.

The purification yields of Ascaris eggs were quite satisfactory overall, i.e. 85% during the study period, although a density of 24 larvae/L was detected at the outlet. However, WHO [23] recommends ≤1 helminth/L in waters intended for reuse. The high density of eggs recorded during the main rainy season would be linked to the resuspension of colloidal particles that would have previously settled during the dry period. The purification efficiency of Ascaris eggs can be sublimated by those of SS, turbidity and BOD5 which are all as satisfactory as those of the eggs. Indeed, many studies have shown strong affinities between the levels of these parameters and Ascaris eggs. Moreover, although the yields of these physico-chemical variables are satisfactory, the levels at the outlet hardly meet the environmental discharge standards. Similarly, the system of the plant has difficulties in purifying the parameters CO_2, O_2, Nitrate and water color, hence the yields obtained. To this end, the levels recorded at the outlet do not comply with Cameroonian standards [24] for the discharge of reusable water after treatment. In addition, seasonal activity would have an impact on the purification yield of many parameters. Indeed, due to the current state of the plant, marked by the disappearance of the covers on all the basins that had them at the time of construction, the temperature, sunshine, wind and rainfall would have a full effect on the system set up at the start. This research could result in the development of a universal method suitable for wastewater and sludge samples of diverse composition and origin. Biological treatment is one of the most efficient treatment techniques for the treatment of polluted effluents [25], it can be carried out under both aerobic and anaerobic conditions according to established systems. The success of biological treatment lies in the ability of bacteria to degrade organic matter and in the proficiency of the solid-fluid disintegration process of biomass in the last phase of treatment [26]. Nevertheless under certain conditions, the biological treatment can be substituted or coupled with the physico-chemical treatment for a better yield. Still called complementary treatments, they aim to eliminate nitrogen and phosphate components as well as biological pollution. The process is generally based on the principles of nitrification-denitrification and biological or mixed (biological and physico-chemical) dephosphatation. This treatment can also be subject to bacteriological or virological disinfection via chemical (chlorination, ozonation) or physical (ultraviolet radiation) processes [5]. Tertiary treatment is mainly applied in developed countries for subsequent reuse in households, industries and plantations. Based on the principle of operation of a wastewater treatment plant, there are several purification systems, namely biological treatments, physico-chemical treatments and mixed treatments.

5. Conclusion

Wastewater treatment must cope up with the increasing demand for reusable water. Reusable water and sludge are in high demand in fields of agriculture and industries. The increasing abundance of parasites in wastewater is a raising concern. Diagnosis and treatment of helminth eggs is a major assessment to be conducted for safe reuse of water and sludge. It is necessary for researchers and scientists to understand helminth and develop necessary tools to detect and eradicate them. Proper diagnostic and treatment technologies should be adopted in water reuse schemes to stop the spread of helminth eggs in the environment. The helminth eggs should be removed before reaching the host to prevent the spread of diseases caused by them. The life cycle of helminths can be completely cut off by removing these eggs in the environment itself. The secondary system of treatment using *Echynochloa pyramydalis* presents an effective method for elimination of *Ascaris lumbricoïdes*. The collaboration between governments, researchers, scientists, and people is important in creating an efficient and effective water reuse system for every part of the world. Development of an efficient and effective water reuse system will have an immense impact ecosystem if promoted and encouraged properly.

Compliance with ethical standards

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Disclosure of conflict of interest

Author declare that there are no conflicts of interest in any form.

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