

Tertiary Treatment of Secondary Effluents from the East WWTP of Tizi-Ouzou (Algeria) with the Aqueous Extract of Ceratonia Siliqua Green Pod

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Abstract. The antibacterial action of the carob tree offers the possibility of using it for the disinfection of treated wastewater in biological process wastewater treatment plants. This paper reviews studies conducted to investigate the effect of carob tree on the reduction of indicator bacteria of fecal contamination that cannot be removed after the secondary stage of biological treatment. The aim is to use the wastewater safely in irrigation and expand the list of irrigable plants with this unexploited resource. Physico-chemical and bacteriological parameters were used to determine the optimal conditions and evaluate the treatment's efficiency. A contact time of 1 h and a dose of 1 mL of disinfectant reduced *E. coli* and fecal coliforms from 5.105 CFU to 1 237 CFU (99.75%) and from 7.106 CFU to 1 505 CFU (99.98%), respectively. For the same contact time, the fecal streptococci were reduced from 4.105 CFU to 59 CFU (99.99%). According to WHO standards, this dose and contact time are sufficient to use this water for irrigation. A minimum contact time of 2 h with a dose of 1 mL of the aqueous extract of the green pod of the carob is required to obtain a reduction of *E. coli* and fecal coliforms from 5.105 CFU to 5 254 CFU (99.95%) and from 7.106 CFU to 340 CFU (99.99%) to meet the Algerian standards of reuse of treated wastewater being more stringent. The results show that the same conditions reduce fecal streptococci from 4.105 CFU to 5 CFU (99.99%). This elimination level shows that green carob juice can be used for disinfection with the necessary doses for each bacterial load and sufficient contact time.

Keywords. Disinfection, Carob tree, Fecal contamination, Contact time.

I. INTRODUCTION

In the next decade, the reuse of treated wastewater will become an unavoidable practice in the next decade [1] to cover the water shortage during the drought periods caused by climate change in many parts of the world in general and in the semi-arid regions of the Mediterranean basin in particular [2]. The large volume of wastewater discharged each year constitutes an alternative resource to replace irrigation water [3]. It allows to meet the farmer's growing needs and protect the receiving environments from eutrophication caused by nutrients and pathogens contained in treated wastewater [4].

Therefore, eliminating pathogens in irrigation is important for developing this sector. The reuse of treated wastewater in agriculture is hindered by the bacteriological quality of the water leaving the treatment plants, which does not always comply with the standards for reuse. The reuse of treated wastewater in agriculture in Algeria is limited to 16 treatment plants to ensure human health and protect the environment. The volume does not exceed 12 million m³/year for 5 380 million m³/year of treated water for 2019 [5].

The biological activated sludge process is used to treat collected urban wastewater. This method guarantees a satisfactory elimination of carbon, nitrogen, and phosphorus. Reducing pollution parameters is beneficial for the environment, but the bacteriological quality of the effluents leaving the treatment plants must be guaranteed. Many attempts have been made to implement new technologies to disinfect secondary effluents [6]. Disinfection is achieved by physical processes using ultraviolet light chemical processes using chlorine or ozone [7]. The shortcomings recorded in these disinfection processes have encouraged researchers to find alternatives such as using natural products with antibacterial effects, such as carob tree. The use of products of plant origin for tertiary disinfection treatment is of great ecological and environmental importance [8].

Ceratonia siliqua, better known as the Carob tree, is a tree that belongs to the family of ever green legumes. It is present in most countries of the Mediterranean basin. The fruit of the carob tree is called a pod and is composed of two parts: the seeds (10%) and the pulp (90%) [9]. The carob pod contains many biologically active compounds, including polyphenols,

sugars, cyclitols, amino acids, fiber, and minerals. Due to its rich chemical composition, carob has strong antioxidant activity and has several interesting therapeutic functions and antibacterial pharmacological activities [10, 11, 12, 13]. The present study shows the disinfectant power of the aqueous extract of the green pods of *Ceratonia siliqua*, according to different doses and contact times. The aqueous extract was tested on a secondary effluent of the East wastewater treatment plant of Tizi-Ouzou.

II. MATERIALS AND METHOD

• Study Site

The purified secondary effluent comes from the WWTP (wastewater treatment plant) of Tizi-Ouzou treating urban wastewater. The station is located on the city's eastern outskirts, on the left bank of the Oued Sebaou, 200 m in front of the Bougie bridge on the Wilaya road linking Tizi-Ouzou to Béjaïa. The WWTP has an area of 35 591 m² and adopts an environmental management method. It was put into service in August 2001[5].

• Sampling

A sampling campaign of the liquid secondary effluent of the Tizi-Ouzou wastewater treatment plant was carried out with a frequency of one sample per week during the period from May 17 to June 7, 2021. The study was performed on a total of five samples during 19 days. The secondary effluent samples were collected in filled glass bottles for physic-chemical analyses and sterilized glass bottles filled one-third full for microbiological analyses. The samples were kept cool in a cooler and then transported to the water treatment laboratory of the University Mouloud Mammeri of Tizi-Ouzou (UMMTO) [14], where they underwent the decontamination test with the *Ceratonia siliqua* aqueous extracts (C.A.E.) using the green pods.

• Studied Parameters

Some physico-chemical parameters such as pH, temperature, and redox potential (E.D.) were measured *in-situ* with a pH meter (HANNA Instruments model Hi 2209). Conductivity, salinity, and Total Dissolved Solid (T.D.S.) were analyzed in the laboratory with a conductivity meter (HANNA Instruments model Hi 2314) according to the standard method of the American Health Association [15]. Parameters indicating fecal contamination (*E. coli*, fecal coliforms, and fecal streptococci) were tested. *E. coli* and fecal streptococci were determined on chromagar agar, while fecal coliforms were determined on Tergitol agar. These indicators were quantified by the membrane filtration method and enumerated according to the protocol described by Rodier *et al.* (2009) [14].

After the necessary dilutions for the samples with distilled water, 100 mL were filtered through a sterile cellulose nitrate filter of 0.45 µm diameter, using a vacuum pump. The filtration membrane was then removed and deposited on the appropriate culture medium layer. Incubation occurred between 24 h and 48 h at 37 °C for fecal streptococci and *E. coli* and 44.5 °C for fecal coliforms. After incubation, the colonies and the total number of bacteria were counted. The removal efficiency was estimated for each parameter measured using the following formula:

$$\text{CFU} = \frac{\text{Number of colonies counted}}{\text{Volume of sample filtered (mL)}} \times 100 \text{ mL Removal (\%)} \\ = \frac{C_e - C_t}{C_e} \times 100 \quad [16]$$

Where, CFU represents the colony-forming unit for 100 mL, C_e is the concentration of the parameter before treatment with C.A.E., and C_t represents the concentration of the parameter after treatment with C.A.E.

• Preparation of the *Ceratonia Siliqua* Aqueous Extracts (C.A.E.)

At the beginning of the experiment, the green pods of *Ceratonia siliqua* (carob) were harvested in the region of Makouda in Tizi-Ouzou (May2021). They were stored in a cold and dark place. The extraction of the pure juice from the pods was done with a previously sterilized centrifuge. The juice was recovered in sterile glass bottles used directly in the experiment while the molasses were thrown away.

• Principle of Manipulation

In this study, the antibacterial properties of *Ceratonia siliqua* were used for the decontamination of the secondary effluent of the WWTP. These properties were obtained after the composition of the green carob pod in flavonoid [9,17] and polyphenol [18, 19]. The method used to extract the active principle of carob powder in the treatment of water and the study of its antibacterial effect consists of using a cold solvent [8,11]. In our case, using the aqueous extract (juice) of green pods without solvent allowed us to recover flavonoids for their antibacterial actions and polyphenols having an action against the growth of bacteria and a coagulant effect [10, 20].

The effluent analysis before treatment with C.A.E. was conducted to determine its physical-chemical and microbiological quality. A process consisting of different doses of decontaminant (C.A.E.) with different contact times was applied. Different concentrations of C.A.E. (1mL, 2mL, and 3mL) were added to one liter of water (secondary effluent of WWTP). The mixing of water and C.A.E. was done according to the principle of jar-test in two phases: fast at 200 rpm for 5 min then slow at 50 rpm for 20 min [16]. Afterward, all the samples were left to rest. The supernatant's physico-chemical and bacteriological characterization was carried out following the standard protocol [15].

III. RESULTS AND DISCUSSION

- *Physico-Chemical Characterization of the Secondary Effluent of the East WWTP of Tizi-Ouzou Before and After Treatment with the C.A.E.*

Table (1) summarizes the physico-chemical parameters of raw water (without treatment with C.A.E.) such as T.D.S., pH, salinity, and conductivity. These physico-chemical parameters meet the Algerian standards [14] and those recommended by the World Health Organization [1]. Based on the results listed in Table (1), it can be noted that the pH value of the secondary effluent of the WWTP is in the range recommended by the Algerian standard and that of the WHO for irrigation with purified wastewater. The pH value of the secondary effluent of the WWTP after treatment with C.A.E. is close to neutrality. It is 7.64 for a minimum dose of 1 mL of C.A.E. and 7.52 for a maximum dose of 3 mL of C.A.E. Polyphenols cause this slight decrease, specifically gallic acid, contained in immature carob tree (green pods) [9,19]. These results show that the use of carob as a decontaminant causes only a slight change in the pH.

TABLE 1. Effect of treatment with aqueous extract of the carob tree (C.A.E.) on the physico-chemical characteristics of the treated wastewater of the WWTP.

	Before Treatment	After Treatment with C.A.E.			Reference guidelines for effluent discharge	
		1 mL	2 mL	3 mL	IANOR ^a	WHO ^c
ED (mV)	69	67	64	61	NA	NA
TDS (mg/l)	422	387	410.5	425.4	NA	450 - 2000
pH	7,95	7,64	7,57	7,52	6.5 - 8.5	6.5 - 8.0
Temperature (°C)	21	21	21	21	NA	NA
Salinity(‰)	0.43	0.40	0.41	0.42	NA	0.7 - 3.0
conductivity (µs/cm)	848	780	827	839	3000	NA

NA: Not available

^a Algerian guidelines, Algerian Institute for Standardization [21]

^c Guidelines for the safe use of wastewater, excreta and grey water in agriculture, World Health Organization [1]

- *Effect of Different Doses of Decontaminant (C.A.E.) and Contact Time on the Bacteriological Characteristics of the Secondary Effluent of the East WWTP of Tizi-Ouzou*

The results show (Fig. 1) a clear reduction of the indicator germs of fecal contamination in the secondary effluent of the WWTP treated with C.A.E., compared with the untreated effluent, which is a function of the contact time of the dose of decontaminant. By analyzing the graphs of Figure (1), it can be noted that the results obtained with the doses of 1 and 2 mL of decontaminant concerning the pathogens indicators of fecal contamination in CFU/100 mL are conformed with the Algerian standards but beyond 2 h of contact. Whereas, for the dose of 3 mL, at the first hour of contact, the sum of the values of the pathogenic germs in CFU/100 mL already meets the standard. For *E. coli*, the maximum elimination was obtained for a dose of 3 mL of carob extract and a contact time of 3h. After these 3 hours, 99.99% of *E. coli* are eliminated. This elimination is explained by the composition of the green carob extract, which is rich in gallic acid [18]. Shao (2015) [22] reported that gallic acid affects the growth of *E. coli*.

For fecal coliforms, a dose of 1 to 2 mL of disinfectant with a contact time of 1h is not sufficient to reach the maximum value recommended by the Algerian standard, which must be less than 1000 CFU/100 mL. The dose of 3 mL for a duration of 3h ensures a total elimination with a rate of 100%. This dose can therefore be taken as a reference for optimal disinfection. The action of the C.A.E. on eliminating the fecal streptococci is visible with the dose of 1mL and a contact time of 1h. It remains approximately stable with the change of the dose of decontaminant and a greater contact time. Several studies have shown the effect of carob extract on bacteria of the genus streptococcus. However, the non-eliminated part of these bacteria might be insensitive to the components of carob extract.

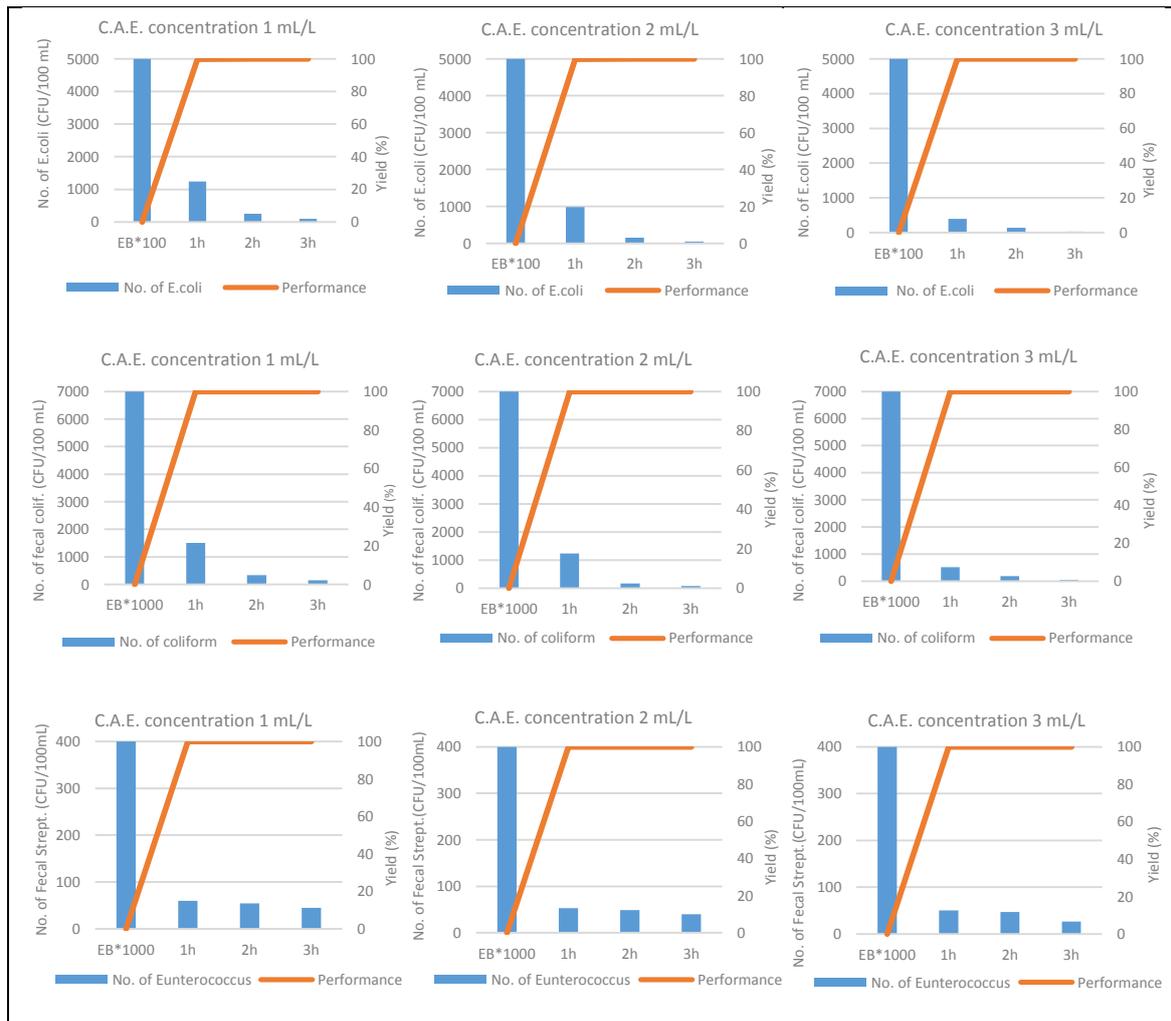


FIGURE 1. Effect of C.A.E. on the elimination of indicator germs of fecal contamination.

CONCLUSION

This study is the first to report the performance of aqueous extract of the green pod of *Ceratonia siliqua* as a disinfectant in the tertiary treatment of secondary effluents from wastewater treatment plants and for reuse in irrigation in Algeria. A new biodegradable reagent for the disinfection process was introduced. The study of the dose of the disinfectant and the contact time allowed us to note that the aqueous extract of the green pod of the carob tree allows a good elimination of the pathogenic bacteria indicative of fecal contamination.

The results show that the effectiveness of this process is proven even at small doses. A dose of 1mL of disinfectant and a contact time of 1h allows reducing *E. coli*, fecal coliforms and fecal streptococci respectively to 99.75 %, 99.98 % and 99.99 %. The elimination will be more increasing for a higher dose and a longer contact time. Moreover, it allows avoiding modifications of the physico-chemical properties of the sample. For future perspectives, the authors aim to replace certain inorganic disinfectants widely used in water treatment and have harmful effects on the environment and human health.

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