**Influence of Cropping Practices on the Persistence and Vertical Migration of *Escherichia coli* from wastewater in Hydromorph Soil in Wet Tropical Zone**


## ABSTRACT

Little attention is paid to the influence of cultivation practices on the persistence and vertical migration of undesirable bacteria in hydromorphic soils as they have increased the risk of crop recontamination. Therefore the objective of this study was to determine the implication of some cultural practices on the persistence and vertical migration of *E. coli* in the soil. In this study, raw sewage (single application) and stream water (multiple application) were applied on lettuce, carrot and aubergine plots. The results revealed that overall, *E. coli* persisted longer on plots with crops and were more persisted in the rainy season on all cultivated plots that had received wastewater from the sewage treatment plant until harvest. While in the dry season, it was only detected at harvest on lettuce plots. The *E. coli* rate increased gradually overtime on the plots that had received water from the watercourse. Aubergine was the only plant that significantly facilitates the vertical migration of *E. coli* to the water table. On the whole, crops favored the persistence of *E. coli* on the soil surface and therefore increase the health risk related to the use of wastewater in agriculture.



*Keywords: waste Irrigation, cultivation practices, E.coli persistence, hydromorphic soil, Dschang-Cameroon*




## 1. INTRODUCTION

The demand for water is increasingly growing and the share of water used for agriculture is by far the most important, ahead of industry and domestic needs, because of irrigation, which allows to increase productivity and thus to meet the food needs of a constantly growing population. [1, 2]. This sector consumes more than 70% of water resources, particularly in developing countries [3]. To meet this demand, wastewater is increasingly used because the nutrient content of wastewater, especially nitrogen, potassium and phosphorus, makes it possible to reduce soil fertilisation costs [4, 5]. Although this resource is a water value and a potential source of fertilizer, it can also be a source of pollution depending mainly on the characteristics of the wastewater, the degree of treatment, the method and the location of use [6]. These waters generally contain large quantities of pathogenic microorganisms that can contaminate the soil, crops and even groundwater. Indeed, soil and groundwater pollution are among the most important potential drawbacks of wastewater use in agriculture that should not be underestimated. This last aspect is somewhat neglected in Cameroon as in many developing countries.

While in developing countries, food poisoning associated with the consumption of raw vegetables has been increasing in recent years, and Salmonella and Escherichia coli pathogens are usually responsible [7, 8, 9, 10, 11]. Consumption of vegetables has considerable nutritional benefits, [12], however, despite these benefits of consuming vegetables, many studies have shown that these foods, when consumed in their fresh state, provide a favourable substrate for microbial contamination and in the chain contamination of consumers of these vegetables [13, 14, 15].

The marshy shallows of the Dschang town are numerous and are used by the poorest people as a place to live and as a site for market gardening; Carrots (root vegetables), aubergines (fruit vegetables) and lettuce (leaf vegetables) are among the main raw vegetables grown in the lowlands. Unfortunately, the sanitary quality of the water used for watering vegetables and the vegetables produced in these lowlands does not meet WHO standards [15, 16, 17]. Indeed, the city does not have any site for the treatment or disposal of sludge from the pits. The structures, built by untrained craftsmen, are poorly positioned on the land and discharge their contents into the riverbeds used downstream for watering crops. Indeed, there is considerable evidence of health and nutritional benefits associated with the consumption of vegetables [12]. Despite the benefits associated with the consumption of vegetables, numerous studies have shown that these foodstuffs consumed in their fresh state, provide an ideal substrate, favourable to microbial contamination and are therefore products known to be foods at risk of transmitting pathogenic microorganisms [13, 14]. Besides, waterborne diseases are recurrent in Dschang, and the people in these lowlands are the most affected [18, 19, 20].

The survey conducted in 2010 in the entire health district of Dschang found that out of a population of 209 055 inhabitants, 24 232 patients were diagnosed in the 51 health facilities in this health district from March 2009 to March 2010; 2000 diagnosed patients were suffering from waterborne diseases; 63% of these patients suffering from waterborne diseases were diagnosed in the dry season (November to March) [21]. This period corresponds to the period of the year when the marshy lowlands are heavily exploited, and almost all the vegetables consumed in the city come from these lowlands. The market gardeners of these lowlands use not only water of very poor microbiological quality but also fertilizers of animal origin, such as chicken manure and pig manure that contain large quantities of pathogenic microorganisms . Bacteria in irrigation water, once on the soil surface, can live there for several months, and even migrate to great depths; the direct consequence is contamination of crops and groundwater [22].The physical properties of these soils, their organic matter content and high moisture content have generally been questioned; little attention has been paid to the implication of cultivation practices on the persistence and migration of undesirable bacteria in hydromorphic soils. While they can play a role and therefore increase the risk of recontamination of crops, persistence is the determining factor in the number of microorganisms that can not only contaminate soils and crops but also migrate to the water table. Hence the need to evaluate the implication of some cultural practices on the persistence and vertical migration of bacteria (*E. coli*) in the soil. In terms of local management, the decentralization process initiated by the state in 2004 has led to the transfer of responsibility for urban services to local authorities. Thus, the highlighting of a model for the management of human pathogenic microorganisms in the agricultural environment can allow its inclusion in the specifications.



## 2. MATERIALS AND METHODS

### 2.1 Experimental site

Situated in the West Region of Cameroon, the town of Dschang, capital of the Menoua Department and the district that bears its name, extends over part of the villages of Foto and Foréké-DschangThis area is located on the South-Western slope of the Bamboo Mountains and is dominated by low plateaus strongly dissected by small valleys sometimes swampy. The climate is characterized by a dry season from mid-November to mid-March and a rainy season from mid-March to mid-November.

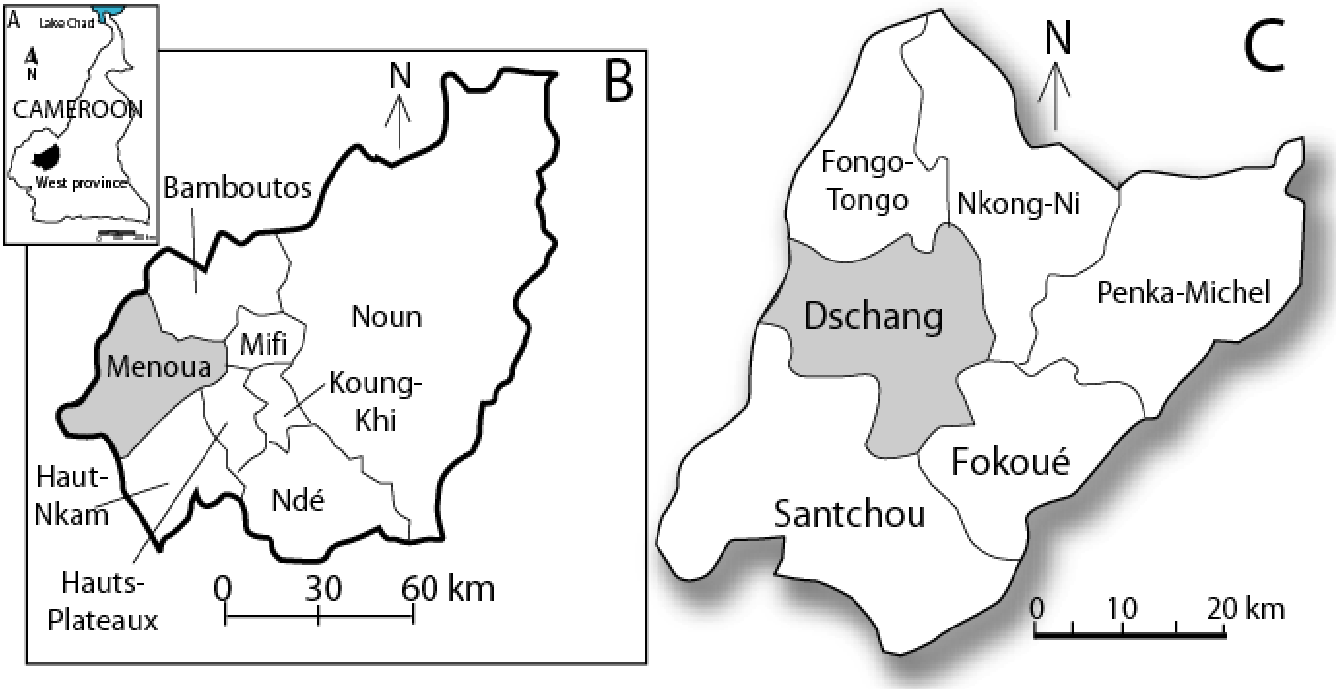


**Fig 1: Location of the study area (source: road and administrative maps of Cameroon (INC) WGS 1984, ZONE 32 N)**

**2.2 Experimental material**

The study focused on lettuce (leaf vegetable), carrot (root vegetable) and aubergine (fruit vegetable). Six randomized blocks of four planks each were laid out in a lowland area in the town of Dschang (which had been left fallow for several years) in a split-plot scheme. 3 plots of aubergine, 3 plots of carrot, 3 plots of lettuce and 3 plots without crop each received 40 liters of raw sewage water once, returning 8.60 ± 0.37 *E. coli* Log (CFU/100 mL) in the wet season and 8.80 ± 0.52 *E. coli* Log (CFU/100 mL) in the dry season before sowing. The same number of plots with the same crops each received 40 liters of stream water containing 4.20 ± 0.13 Log *E. coli* (CFU/100 mL) in the wet season and 4.10 ± 0.2 Log *E. coli* (CFU/100 mL) in the dry season, once every fortnight in the wet season and twice a week in the dry season.

To avoid contamination between plots, the blocks were separated from each other by a space of two meters. The boards in the same block were separated from each other by a space of one meter. Each board had a surface area of 4 $m^2$. Lettuce plants with 5 to 6 leaves and aubergine plants with 4 to 5 leaves were transplanted with a spacing of 30 and 40 cm respectively. The carrots (0.2g per row) were sown in rows spaced 25 cm apart. Soil bacteriological analyses were done before, at mid-crop (after 4 weeks for lettuce, 6 weeks for carrot and 8 weeks for aubergine), and crop maturity at three depths (0-10, 20-30, 40-50 cm).

The soil was tested for *E. coli* using the method of Malkawi & Mohammad [10]: 20 mg of soil is taken and added to 180 ml of sterile distilled water. The mixture was stirred for 2 minutes. 0.1 ml of the suspension was seeded onto agar medium (TTC-lactose agar with Merck tergitol 7) and incubated at 44 °C for 24 hours. After culture, five yellow colonies surrounded by a yellow halo (presumptive E. coli) were recoated on standard non-selective Plate Cound Agar (PCA) agar medium sloping into the test tubes for confirmatory biochemical tests, i.e. indole test, lactose fermentation and negative citrate effect, mannitol mobility, glucose fermentation and gas production.

**2.3.Data analysis**

The data were subjected to simple descriptive statistical analysis and analysis of variance (ANOVA), at the 0.05 probability level, using SPSS software version 12.0 for Windows.

**RESULTS**

The physical and the biochemical properties of soil used as an experimental site are shown in Table 1. The soil profile is characterized as "Fairly organic hydromorphic soil" of French classification, (Humaquept of soil taxonomy, Humic Gleysol of FAO classification). The rusts spots, corresponds to iron in the oxidized state. The soil is saturated with water in the rainy season and the water level drops to a few centimetres in dry season. Its pH is acid and it is very rich in nitrogen and organic matterdue to the accumulated years of fallow. However, this organic matter is of poor quality due to the C/N ratio This soil is deficient in major mineral elements P, K, Ca, and Mg. Its content in cation exchangeable capacity (CEC) was average, and the rate of exchangeable bases was very low.

**Table 1: Physicochemical and microbiological characteristics of the soil at the experimental site**

| Parameter | **0-10 cm** | **20-30 cm** | **40-50 cm** |
|---|---|---|---|
| Sand (%) | 17.31 | 32.34 | 30.15 |
| Silt (%) | 31.96 | 17.96 | 13.35 |
| Clay (%) | 50.73 | 49.70 | 56.50 |
| pH water | 5.33 | 6.54 | 6.71 |
| pH KCl | 4.46 | 6.40 | 6.42 |
| CEC (cmol+/kg) | 4.19 | 3.70 | 1.55 |
| Assimilable phosphorus (mg/kg) | 4.19 | 1.46 | 1.21 |
| Organic carbon (%) | 4.48 | 4.52 | 3.61 |
| Total Nitrogen (%) | 0.32 | 0.26 | 0.20 |
| C/N | 13.86 | 17.33 | 17.71 |
| *E. coli* (CFU/g MS) | 0 | 0 | 0 |

**3.1 Effect of crops on the persistence of *E. coli* on the soil**

**3.1.1 Effect of crops on the persistence of *E. coli* on the soil of plots that have received raw sewage only once**

On plots with lettuce that received raw sewage during the rainy season, the *E. coli* rate initially rises in the middle of the growing season and falls at harvest (Fig 2). In contrast, on plots without lettuce, there is an almost gradual decrease over time. In the dry season, a gradual decrease in the *E. coli* rate was observed over time, while the pollution rate of plots with lettuce remained higher. The plots without lettuce showed a statistically lower contamination rate ($p < 0.05$) than the plots with lettuce.

In the rainy season, on plots without carrots, there was a gradual decrease in the *E. coli* population over time. In the plots with carrots, on the other hand, the *E. coli* population at harvest is initially reduced and then increased. At harvest, the *E. coli* rate in plots with carrots is higher than in plots without carrots. In the dry season, the *E. coli* population gradually declines and is canceled at harvest on plots with and without carrots. In the rainy season, a gradual decline in the *E. coli* population was observed over time. However, at harvest, plots with aubergine showed statistically higher contamination ($p < 0.05$) than plots without aubergine. In the dry season, on plots without aubergine, the *E. coli* disappeared from the mid-crop. On the other hand, on plots with aubergines, they were not detected at harvest time.

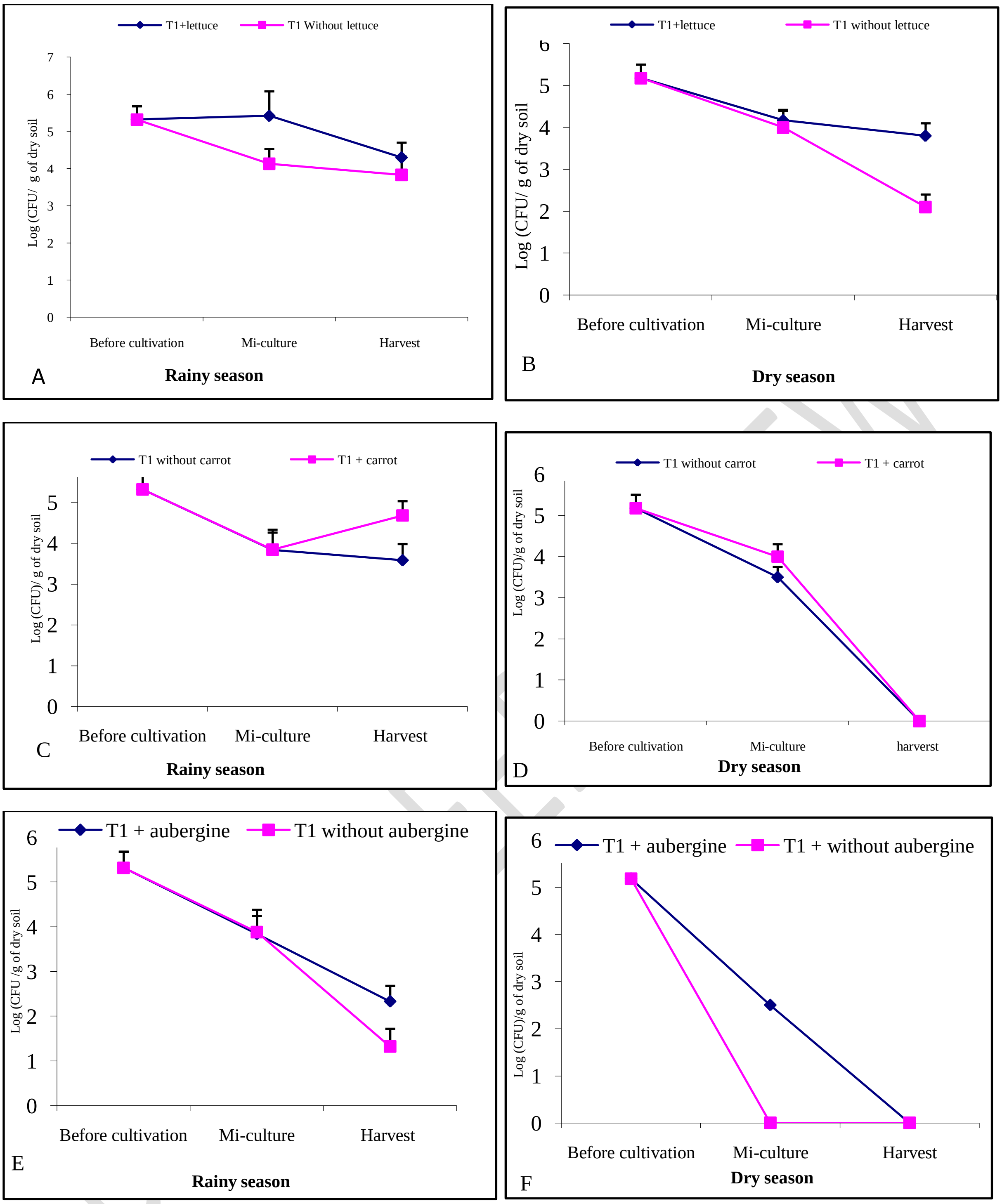


**Fig 2: Effect of crops on the persistence of *E. coli* on plots that received raw sewage water only once, T= Standard deviation, T1= Treatment with raw wastewater**

### 3.1.2 Effect of crops on the persistence of *E. coli* on the soil of plots that have received several times the water from the watercourse

For plots that had received water from the stream several times (Fig 3), a gradual increase in *E. coli* was obtained over time. This increase was more pronounced on plots with lettuce. Water supplied every fortnight in the rainy season and twice a week in the dry season would be an additional supply of *E. coli*, and of elements that enhance the survival of *E. coli* on the soil surface. In the dry season, the contamination rate of plots with lettuce differs from plots without lettuce at harvest time. At harvest, in the dry season, the pollution rate of lettuce plots that have received water from the river is statistically ($p < 0.05$) higher than that of lettuce plots that have received raw sewage water.

In the rainy season, on plots with and without carrots, there is an increase at mid-crop and a decrease at harvest. Despite the observed drop, the contamination rate remained higher than at the start, and the plots without carrots were more contaminated than those with carrots. In the dry season, a gradual increase was observed over time. In contrast to the dry season, in the rainy season, plots with carrots had statistically ($p < 0.05$) higher E. coli levels at harvest.

In both the dry and rainy seasons, a gradual increase in the *E. coli* population was observed over time in the plots with aubergine. Plots with aubergine showed statistically higher contamination ($p < 0.05$) at harvest.

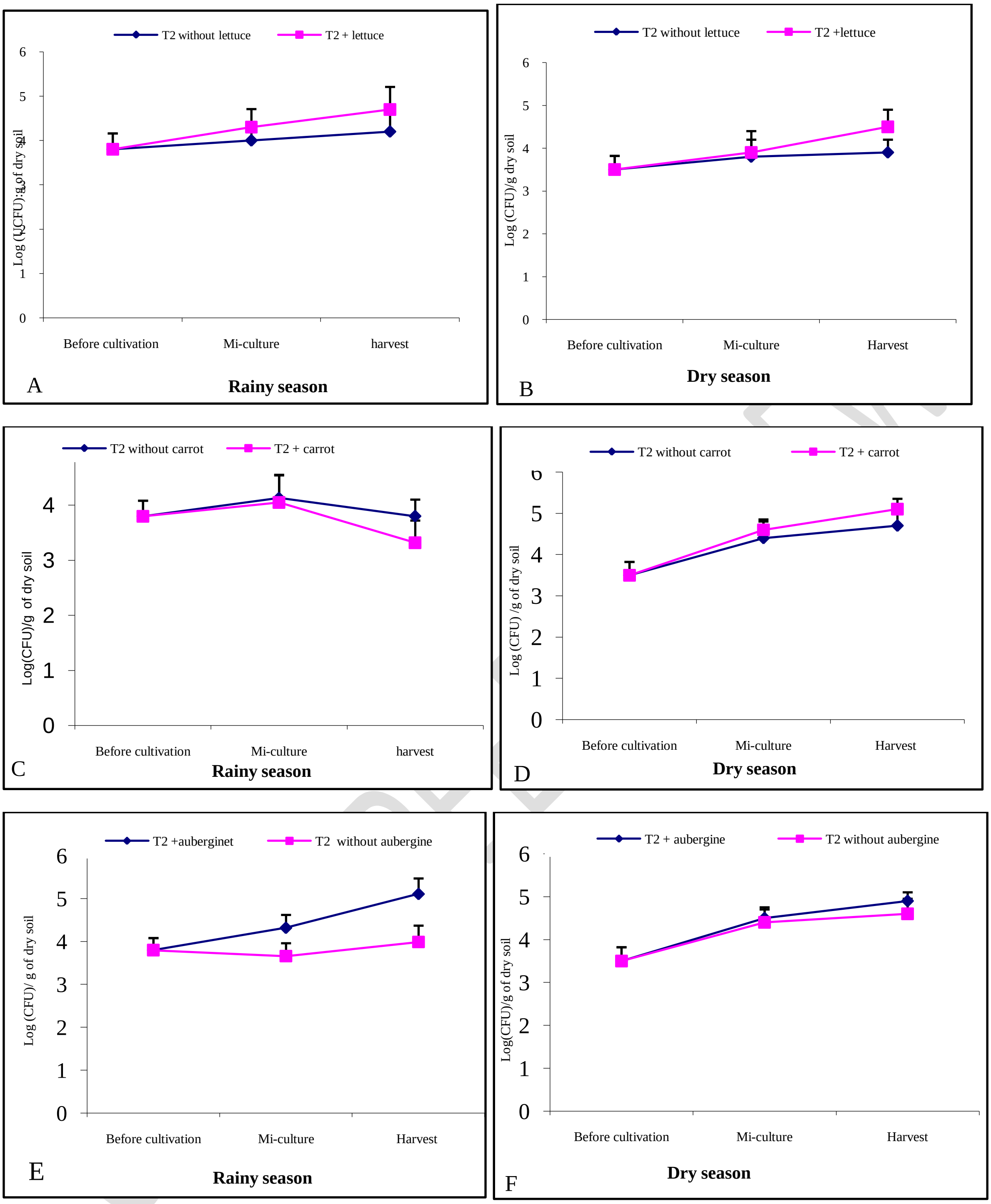


**Fig 3: Effect of crops on the persistence of *E. coli* on plots that have received several times the water from the river at the soil surface, T= Standard deviation. T2 = Treatment with stream water**

### 3.2 Effect of crops on the vertical migration of *E. coli* in the soil

#### 3.2.1 Effect of crops on the vertical migration of *E. coli* in the soil of plots that have received raw sewage only once

On lettuce plots that have received raw sewage in both the dry and wet seasons (Fig 4), there is an absence of *E. coli* at the 40-50 cm depth. *E. coli* was detected at a depth of 20-30 cm at mid-crop and persisted there until harvest. *E. coli* levels at this depth were not statistically different.

In both the wet and dry seasons, *E. coli* were detected at mid-crop and harvest depths of 20-30 cm, at harvest depths of 40-50 cm, with the highest levels, but not significant in the carrot plots.

In the rainy season, *E. coli* were detected between 20-30 cm at mid-crop and persisted until the end of the study, then between 40-50 cm at harvest. Levels were consistently higher in plots with aubergines. In the dry season, *E. coli* were detected at a depth of 40-50 cm on plots with aubergines.

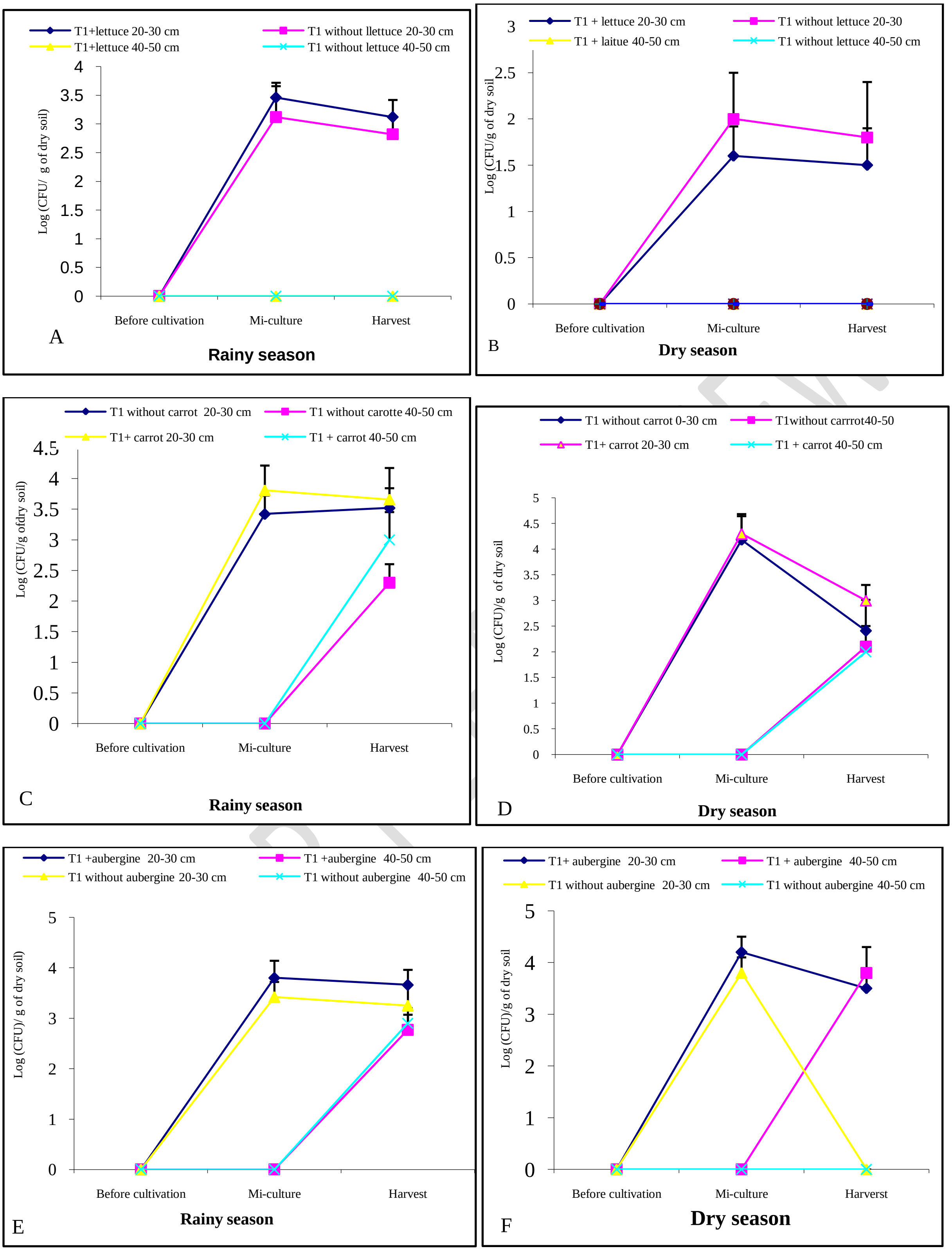


**Fig 4: Effect of crops on the vertical migration of *E. coli* on plots that have received several times the water from the river at the soil surface; T= Standard deviation. T1 = Treatment with raw sewage water**

### 3.2.2 Effect of crops on the vertical migration of *E. coli* in the soil of plots that have several times the water from the watercourse

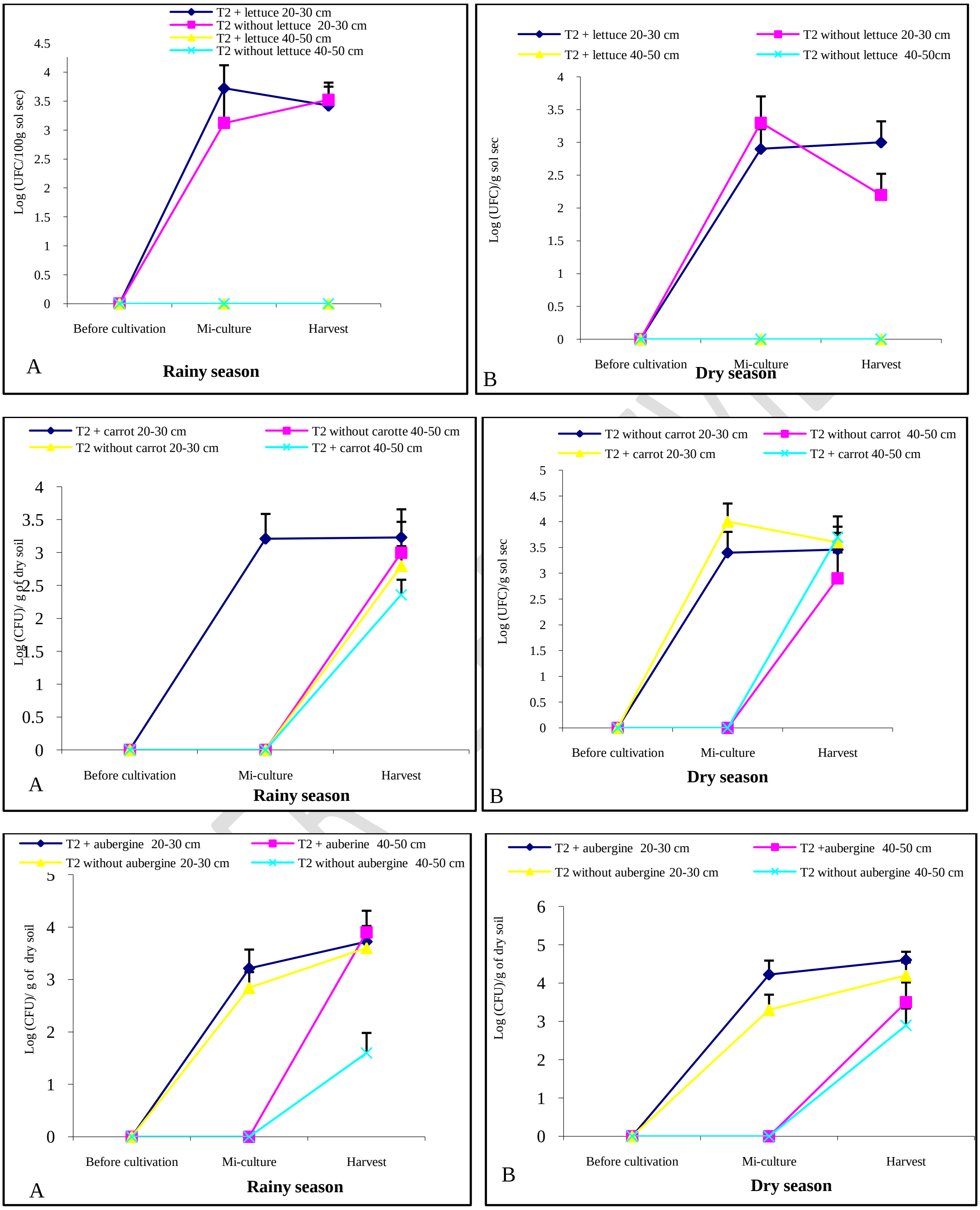


**Fig 5: Effect of crops on the vertical migration of *E. coli* on plots that have received several times the water from the river at the soil surface, T= standard deviation. T2 = Treatment with stream water**

## 3. DISCUSSION

### 4.1 Crops promote the persistence of *E. coli* on the soil surface.

The use of highly polluted raw sewage as "fertilizer" (single application before sowing), or the use of stream water (diluted water) and therefore less polluted (several applications) for watering crops presents more or less serious risks depending on the season and the type of crop. *E. coli,* contained in the raw sewage used as "fertilizer" (single application before sowing), persists on aubergine, lettuce, and carrot plots throughout their cultivation cycle in the rainy season. The carrot, lettuce and aubergine plots are progressively covered by the leaves of these plants and eventually cover the plots completely. This creates a cover that prevents sunlight from reaching the soil, and consequently, the bacteria find a favourable environment for their growth, because under the leaves of these crops, the humidity is low. Indeed, the crops, low solar radiation, low temperatures and high soil moisture at this time of the year would have been the favorable factors for the persistence of *E. coli* on the soil [17]. The nature of the soil would also have contributed. Indeed, the soil at the study site has more than 50% clay, which is a favorable condition for the survival of E. coli. Studies have shown that clay contains many micro-habitats, enough water, and many protective sites [23, 24]. However, these bacteria would have found a micro-habitat beneath the crops that were favorable for their survival and growth at a given time, and therefore increased the risk [25]. Indeed, the plots with crops were generally more contaminated. In the rainy season, this practice presents risks for carrot (direct contamination), lettuce and aubergine crops (splash contamination).

In the dry season, on the other hand, this bacterium was only detected on lettuce plots at harvest. Lettuce has a short cycle (45 to 60 days). However, *E. coli* from wastewater can persist for more than 16 weeks on the surface of hydromorphic soil in the rainy season and 9 weeks in the dry season in humid tropical zones [22], hence their presence on plots with and without lettuce at harvest. Thus this dry season practice would only present risks for short-cycle crops such as lettuce. As for the plots that received water from the river, the risk is higher for long cycle crops in the dry season, due to the progressive accumulation of *E. coli* on the plots caused by the increase in the frequency of watering which, in our case, went from two to eight times a month in the dry season. Fecal bacteria have a high survival capacity on the soil surface as they can survive in unfavourable ecosystems by developing various systems, such as biofilm formation or, much more precariously, entering a viable non-cultivable state [26]. *E. coli* persists for more than two months on hydromorph soil in the dry season. However, crops, in general, would have favored the survival of *E. coli* by protecting it from certain environmental stresses at some point in its development. Aubergine, for example, a few weeks before harvest is a shrub with branches covering the whole plant bed. Therefore, any bacteria that could have resisted up to this invasion phase would find a favorable shelter for its growth under these aubergines. However, it is important to note that the use of wastewater as a "fertilizer" for growing aubergines, from a bacteriological point of view, presents no risk during the dry season. In the rainy season, it presents a risk that can be minimized, if gardeners manage to tie the branches with support so that no fruit can contact with the ground.

### 4.2 Vertical migration of *E. coli* is influenced by the type of crop.

Hydromorphic soils are conducive to the survival and vertical migration of bacteria in the soil; E. coli injected into the soil surface was detected at 20-30 cm on day 14, and 40-50 cm after 70 days and persisted for a long time [ 22, 27]. Aubergine is the only plant that significantly influenced the vertical migration of *E. coli*. Aubergine has a root system that develops gradually. At some point, this root system would have facilitated the vertical migration of *E. coli*. Lettuce, on the other hand, has a poorly developed root system; the harvested carrots were at most 15 cm long and therefore could not leave a favorable passage for the bacteria to circulate to the water table. The use of these two types of water (raw wastewater and river water) for crops with a developed root system is likely to cause serious public health problems due to the ease with which the root system allows the pathogenic microorganisms contained in these waters to reach the groundwater table. In fact, groundwater is the main source of drinking water for many families; the contamination of this water by pathogenic microorganisms will also lead to the contamination of the consumers of this wate.

## 4. CONCLUSION

The use of wastewater in agriculture is widespread and even encouraged because of its fertilizing power. But these waters generally contain many pathogenic microorganisms. The persistence of *E. coli* on the plots cultivated according to the treatment has been tested. *E. coli* persisted in the rainy season on all cultivated plots that had received wastewater from the sewage treatment plant until harvest and can therefore contaminate the crops. In the dry season the use of raw sewage only once before sowing presents risks only for short-cycle crops such as lettuce. On plots that have received water from the river, pollution increases with time, and the risk is higher in the dry season and for long cycle crops. The aubergine, with its root system developed at harvest, is the only crop that significantly facilitates the circulation of *E. coli* to the water table.



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