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Leachates From The Wild Discharge Of El Hajeb City (Morocco): Analytical Assessment Of Quality Microbiological And Sanitary Risks

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Abstract : This study aims to determine the microbiological quality of leachate from the wild dump of the town of El Hajeb (Morocco) and its sanitary risks. In this investigation, monthly sixteen samples were collected during twenty-one months (May 2015–January 2017). Counts of total coliforms, fecal coliforms and faecal streptococci were carried out by the MPN method, while E. coli, salmonella spps, staphylococcus aureus, revivable germs at 22°C and at 37°C, sulphite-reducing clostridia at 46°C, helminth eggs, vibrio cholera and protozoal cysts were examined using standard microbiological methods of health protection.

Bacteriological and parasitological results indicated the presence of high levels in pathogenic microorganisms which exceeded the limits and guide values of WHO and the current Moroccan standards,what makes these effluents extremely toxic in the case of infiltration or diffusion towards the surface waters or the adjacent groundwater which circulates at shallow depth and could consequently expose the health of the neighboring population to grave sanitary risks.

Keywords : Leachate, Quality, Microbiology, Health risks.

1. Introduction

Originally, the dumping was the technique of elimination the most used in the world, easy to implement, relatively inexpensive [1]. By the percolation of the materials they deposit, these sites are a potential source of ground and surface water contamination. There is a common misconception that since the materials placed in landfills are basically household wastes, they are relatively "safe" and would not likely adversely affect public health and groundwater quality. It is enough to consider the proposal to drink the mud which develops at the bottom of a garbage can or water used to clean a garbage can to understand that it is not desirable to have a leachate of municipal solid waste in the drinking water. Even the addition of a drop of such a liquid derived of a garbage can in a glass of drinking water, i.e. a highly diluted leachate, would not be considered desirable. Nevertheless, it is what occurs when the leachate of discharges of municipal solid waste is authorized to contaminate the water which is or could be used for domestic supply. The impact of the leachates of the discharges on underground and surfaces waters gave rise to a number of studies in recent years [2-7]. In addition, little is known about the composition of pathogenic bacterial populations in landfill leachate. However, leachates can therefore play the role of potentially dangerous microbiological agent carriers such as salmonella, cholera vibrio and parasites. The microbiological quality of leachates is determined by the search and the isolation of the bacterial germs witnesses of faecal contamination or bio-indicators of pollution, such as fecal coliforms, faecal streptococci and sulphite-reducing clostridia. Borrego and Romero [8] have used the ratio of the concentrations of faecalcoliforms (FC) and of faecal streptococci (FS) to try to differentiate the human sources of not human sources of faecalcontamination (Table 1).

Table 1: Borrego and Romero classification of fecal pollution

N°	FC/FS	Source of pollution	Observations
1	>4	Essentially humain pollution	Wastewater discharge
2	2<FC/FS<4	Mixed pollution to human dominance	/
3	1<FC/FS<2	Uncertain pollution	/
4	0.7<FC/FS<2	Mixed animal pollution predominance	/
5	<0.7	Animal pollution, including livestock	Particularly sheep

Pathogenic bacteria in immune-deficient subjects are sometimes responsible for diarrhea, wound infection or genitourinary and ocular infections [9]. The development of microbial communities in surface waters, as well as in groundwater, is linked to meteorological factors and to the physicochemical and biological characteristics of the biotope [10, 11]. In these environments the chemical elements also strongly influence the survival of the microorganisms present. Indeed the activity of nitrifying bacteria can be affected by the strong variations of the contents in dissolved oxygen [12]. Changes in temperature, pH, and NaCl concentrations of the medium may affect the growth of pseudomonas marginalis and its pectinolytic activity [13].On the other hand the activity of E. coli is inhibited by concentrations of salt superior to 9%. Heavy metals may also influencethe composition of the microbial community and therefore inhibit the decomposition of organic pollutants [14]. However despite the fact that heavy metals are acutely toxic to most microbes, there are heavy metal tolerant bacteria, long term exposure to metals favours proliferation of microbes that tolerate the existence of metals [15]. Recent studies [16, 17] have shown that the landfill leachates of the city of El Hajeb (Morocco) had an acidic pH (around 6), an anaerobic medium (O_2 dissolved<1mgO_2 L^{-1}) and a high concentration of heavy metals (>50 mg L^{-1}). Environment so was favorable for the proliferation of pathogenic bacteria, germs and parasites.

In this perspective, we have been particularly interested in the identification and enumeration of some germs indicators of pollution grouping heterotrophic bacteria such as Total Coliform (TC), FaecalColiform (FC), FaecalStreptococci (FS), EscherichiaColi (E. Coli), Revivable Germs at 22°C (RG22) and at 37°C (RG37) and some microorganisms with pathogenic characters such as Salmonella spps (S), Vibrio spp (V), StaphylococcusAureus (SA),

Sulphite-ReducingClostridia at 46°C (SRC), Helminth eggs (H), and Protozoal cysts (P).

Fecal Streptococci are characteristic of animal and human excreta [18, 19]. This group is also considered a good indicator of fecal contamination. The fact that intestinal enterococci survive longer than E. coli in the natural aquatic environment may be an advantage to this group if it is sought to identify an old fecal contamination.E. coli whose growth is not affected by the molecular oxygen concentration [20] is a well-established indicator of fecal contamination in the environment. It is normally non-pathogenic, i.e. not responsible for infection, but may become so under certain conditions and some “specialized” strains of E. coli are associated with very diverse pathologies in both humans and animals; diarrhea, gastro-gastroenteritis, urinary tract infections, meningitis, septicaemia...The search for non-pathogenic aerobic micro-organisms known as "revivable" allows counting bacteria developing in usual conditions of culture. These germs have no direct health effects but under certain conditions they can cause problems. These are indicators which reveal the possible presence of a bacteriological contamination.TheSulphite-Reducing Clostridia (SRC) (especially Clostridium perfringens type a) are orally pathogenic species responsible for toxic infections. The search and the enumeration of the spores of sulphite-reducing clostridia are made with the aim of looking for a faecal contamination. Direct research of these spores can therefore be serving as a screening test for ancient fecal contamination because of the long survival of the spores in the outside environment. The staphylococcus aureus is a bacterium at the origin of numerous infections or intoxications. Those are opportunist pathogenic bacteria which can cause diverse diseases at the human beings, ranging from infections that spontaneously evolve towards healing to mortal pathologies. Vibrio cholerae, or bacillus comma, is a bacterium which is at the origin of the cholera where from it holds its naming. Salmonellae are generally considered pathogenic although their virulence and pathogenesis vary enormously. They can cause gastroenteritis, bacteremia, enteric fever, malaria, typhoid fevers, systemic salmonellosis, gastroenteritis and food poisoning. The natural hosts of salmonellae are the human population, domestic animals, poultry and livestock, and wild animals, including common birds [21]. They are also indicators of fecal contamination.Eggs helminths are the most resistant pathogenic agents of the environment of all the pathogenic agents and can in extreme cases survive during several years. TheProtozoan cysts is a set of parasites (anaerobic pathogenic amoebas), among which some not possessing a mitochondrion to allow the oxidation of organic compounds, but a different organelle called Mitosome. Some specialized strains of protozoan cysts are pathogenic to humans, capable of provoking diarrheas and secondary malnutrition which can be mortal and a deficiency of the immune system.

The objective of this work is to assess the microbiological quality of the leachates from the wild landfill of the city of El Hajeb (Morocco) and their health risks to determine in the near future its impact on adjacent ground-waters.

2. Materials and methods

Presentation of the study area

The city of El Hajeb is part of the territory of the Fes-Meknes region, this city has 36.491 inhabitants. The area is characterized by a mountainous climate with an average annual temperature and rainfall respectively of 14.8°C and of 723 mm. The region is based on a karst aquifer;its lithology is of a high permeability that could actually affect the quality of groundwater. The municipal dump, which serves this city, is located to the west and about 12 km and receives an average amount of household waste estimated at 11.000 tons per year, a daily ratio of about 1 kg/d/capita with a high organic matter content of 80%.

Methodology

The sampling period of leachate lasted 22 months (May, 2015-January, 2017). They were carried out monthly and immediately transported towards two complementary laboratories, the one is that of the National Laboratory of the Studies and the Surveillance of the Pollution (NLSSP) in Rabat and the other is the Laboratory of the National Office of Electricity and Water-drinking water branch (LNOEW) in Meknes.

TC, FC, FS and E. coli were identified and counted by the MPN method. Revivable aerobic bacteria by incorporation in agar, the anaerobic sulphite-reducing clostridia (SRC) by incorporation on SPS (sulfitepolymixin sulfadiazine) medium, staphylococci aureus (SA) by filtration on cellulose membrane in Chapman-mannitol medium and cholera vibrio by filtration on membrane incubation and seeding on medium TCBS (Thiosulfate Citrate Bile Sucrose). As for salmonella, their research is carried out on the Selenite-cysteine medium and transplanting on Hektoen agar. The Bailenger technique, strongly recommended byWHO [22], was used for the research and enumeration of protozoan cysts and helmintheggs.

Statistical analysis

The data collected were seized and analyzed statistically with the IBM SPSS statistics version 19 software. The mean values of the results of microbiological analyses were presented in the form Mean ± SE. The level of statistical significance was estimated at the threshold of significance alpha = 0.050 (statistical test of Jarque-Bera). At this threshold most of the statistical results were highly satisfactory and significant.

3. Results and discussion

Table 2: Results of microbiologicalanalysis of the leachate

Leachates (n = 336)				
Microorganisms	**Mean±SE**	**Min**	**Max**	**p-value**
Total coliforms (TC)	$8.42e^{+6} \pm 7.2e^{+5}$	$1.63e^{+5}$	$4.37e^{+7}$	< 0.0001
Faecalcoliforms (FC)	$2.26e^{+6} \pm 1.6e^{+5}$	$2.68e^{+2}$	$7.13e^{+6}$	< 0.0001
Faecalstreptococci (FS)	$1.57e^{+7} \pm 1.18e^{+6}$	$2.9e^{+4}$	$6.48e^{+7}$	< 0.0001
Staphylococcus Aureus (SA)	$2.3e^{+9} \pm 1.83e^{+8}$	0	$9.23e^{+9}$	< 0.0001
Salmonella (S)	$9.75e^{+2} \pm 9.13e^{+1}$	0	$6.1e^{+3}$	< 0.0001
Revivable Germs at 37°C (RG37)	$1.87e^{+10} \pm 1.32e^{+9}$	$3.83e^{+5}$	$7.85e^{+10}$	< 0.0001
Sulphite-Reducing Clostridia at 46°C (SRC)	$2.64e^{+6} \pm 1.76e^{+5}$	0	$1.2e^{+7}$	< 0.0001
Revivable Germs at 22 °C (RG22)	$7.55e^{+10} \pm 5.83e^{+9}$	$1.5e^{+9}$	$3.18e^{+11}$	< 0.0001
Escherichia coli (E. coli)	$5.14e^{+3} \pm 2.68e^{+2}$	$3.53e^{+2}$	$1.23e^{+4}$	< 0.0001
Helmintheggs (H)	$4.3e^{+1} \pm 1.58$	0	$8e^{+1}$	0.0001
Vibriocholerae (V)	$8.26 \pm 2.6e^{-1}$	3	$1.8e^{+1}$	< 0.0001
Protozoalcysts (P)	$4.71e^{+3} \pm 1.53e^{+2}$	0	$8.67e^{+3}$	0.0009

Min: Minimal value; Max: Maximal value; Mean: Average value; SE: Standard Error.
The count of all microorganisms is expressed in CFU 100 mL^{-1} except for Helminth Eggs and Protozoan Cysts which are expressed as L^{-1}.

The microbiological quality results of the leachates showed a high load in Total Coliform (TC) with an average of $8.42e^{+6} \pm 7.2e^{+5}$ CFU 100mL^{-1}, value almost 168.4 times higher than the Moroccan standard [23] set at $5e^{+4}$ CFU 100mL^{-1} (Table 2). Precisely all samples have exceeded this limit, indicating that these leachates do not comply with the rejection standards in surface and ground waters. The median symbolized by a horizontal line in the Tukey box calculated at $1.74e^{+6}$ CFU 100 ml^{-1}, was well below the average symbolized by a cross in Tukey box (Fig.1-A).

For Fecal Coliforms (FC), they showed concentrations that varied between 2.68e+2 and 7.13e+6 CFU 100ml-1 with a large range of $7.13e^{+6}$ CFU 100ml^{-1} (Fig.1-B). Precisely 93.4% of the samples exceeded at least 1.4 times the limit set by Moroccan legislation [23] set at $2e^{+4}$ CFU 100mL^{-1} and the mean of $2.26e^{+6} \pm 1.6e^{+5}$ CFU 100ml^{-1} exceeded 113 times the same standard (Table 2). The highest recorded level for Total Coliforms was $4.37e^{+7}$ CFU 100mL^{-1} and $7.13e^{+6}$ CFU 100ml^{-1} for Fecal Coliforms could be attributable to the elimination of the human and/or animal domestic feces contained in the municipal waste.

The population of FaecalStreptococci fluctuated between a minimum of $2.9e^{+4}$ and a maximum of $6.48e^{+7}$ CFU 100mL^{-1}with an average of $1.57e^{+7} \pm 1.18e^{+6}$ CFU 100mL^{-1}(Table 2).This mean surpassed 1570 times the national standard [23] set at $1e^{+4}$ CFU 100mL^{-1}. It should be noted that 94% of the samples exceeded this standard. The figure 1-C indicated that

FS have an atypical concentration of 6.48e+7 CFU 100mL-1. On the other hand The FC/FSratio varied from $1e^{-4}$ to $2.46e^{+2}$ with an average, of $2.16e^{+1} \pm 3.45$, greater than 4. According to Borrego and Romero [8], the origin of fecal contamination was mainly human (household garbage and hospital waste).

Other bacterium, E. coli was examined. The measured values were shown in Fig.2-A. It varied of 3.53e+2 at 1.23e+4 CFU 100mL^{-1}with an average of $5.14e^{+3} \pm 2.68e^{+2}$ CFU 100mL^{-1}, concentration slightly lower than its median concentration.Thelimits ofE. coli, fixedon the one hand by the French sanitary recommendations [24] relative to the disinfection of urban waste water at $2e^{+3}$ CFU mL^{-1} and on the other hand by the Canadian standard [25] concerning the leachates rejection fixed to 275 UFC 100mL^{-1} were exceeded by 100% of the number of samples. That of the French standard [26] relative to the quality of waters intended for the irrigation ($1e^{+4}$ UFC 100mL^{-1}) was also exceeded by almost 19% of samples (Fig. 2-A).

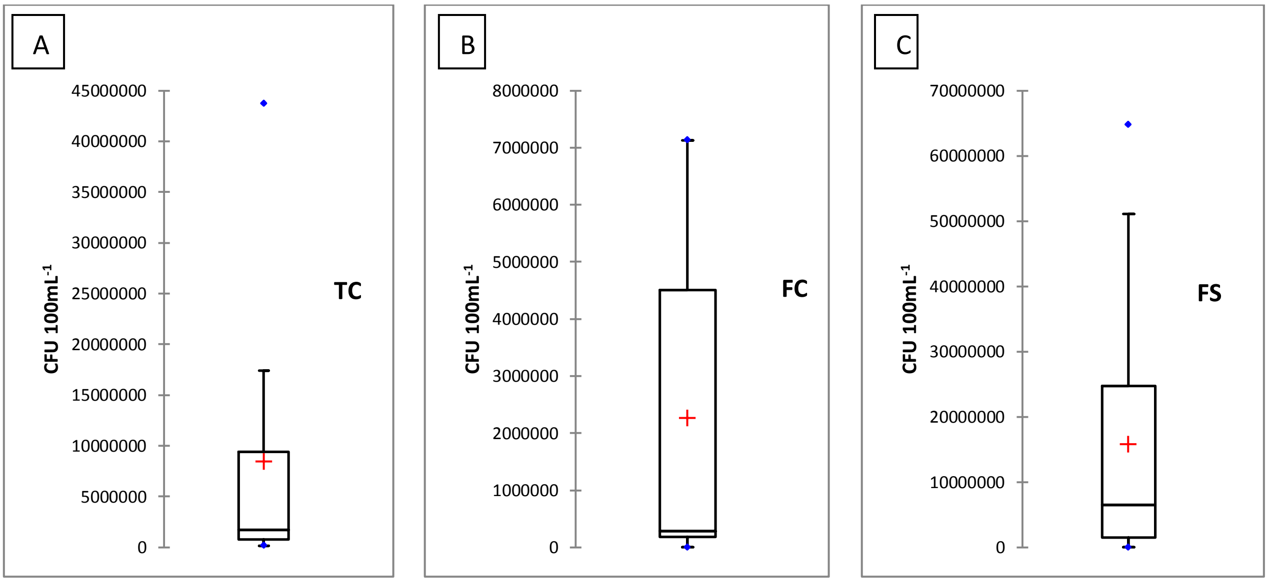

Fig. 1.Tukey-Box representations of Total Coliform (A), Fecal Coliform (B), and Fecal Streptococci (C) concentrations in leachates.

The quantitative analysis of the populating of the Revivable Germs at 22°C (RG22) in these leachates has allows to highlight strong concentrations varying between $1.5e^{+9}$ and $3.18e^{+11}$ CFU $100mL^{-1}$ with the widest range calculated at $3.16e^{+11}$ CFU $100mL^{-1}$ (Table 2). Besides box ofTukye concerning these germs showed on one hand that the detected average, was superior to the median and on the other hand a single atypical concentration equal to $3.18e^{+11}$ CFU $100mL^{-1}$(Fig. 2-B). Other controlled germs were the Revivable Germs at 37°C (RG37). These germs showed a range of $7.84e^{+10}$ CFU $100mL^{-1}$ and an interquartile range of $2.71e^{+10}$ CFU$100mL^{-1}$(Fig. 2-C). On the other hand, although 100% of the samples showed high concentrations, on the whole, the values $7.55e^{+10}$±$5.83e^{+9}$ CFU 100 ml^{-1} in RG22 and $1.87e^{+10}$±$1.32e^{+9}$ CFU 100 ml^{-1} in RG37 did not provide any information in the absence of guide and/or imperative value relating to these two kinds of germs in landfill leachates. Nevertheless, these averages surpassed widely the EEC standard [27] on water intended for human consumption, which stipulates maximum concentrations of $1e^{+4}$ CFU $100mL^{-1}$ in RG22 and $1e^{+3}$ CFU $100mL^{-1}$ in RG37 not to be exceeded.

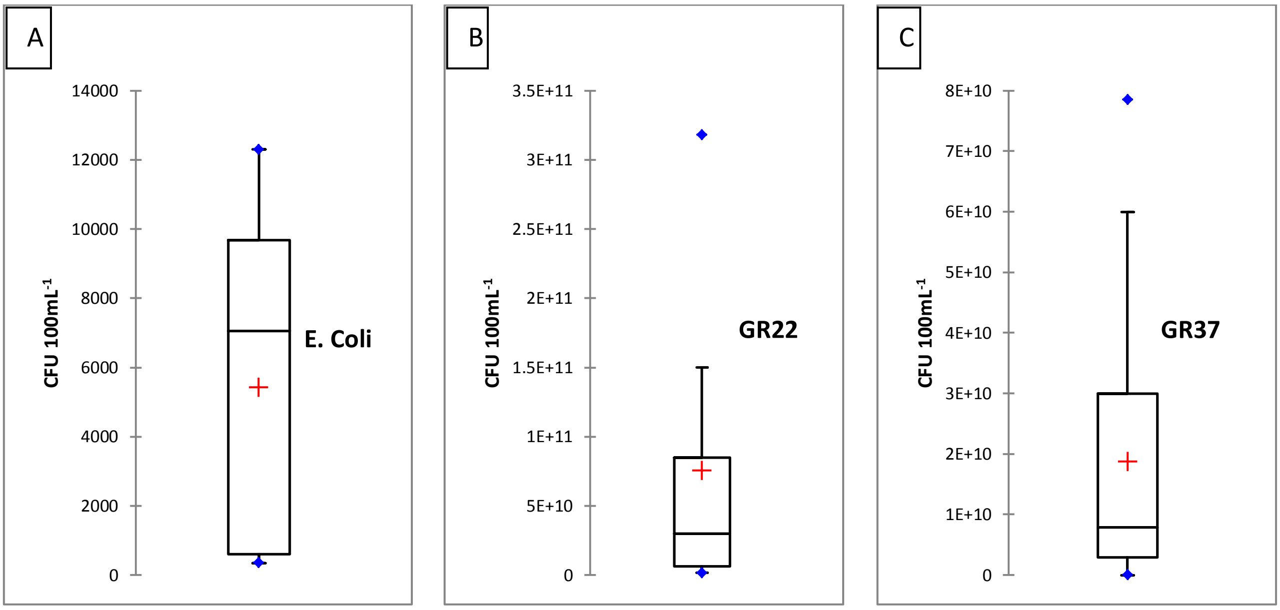

Fig. 2.Tukey-Box representations of Escherichia Coli (A), revivable germs at 22°C (B), and revivable germs at 37°C (C) concentrations in leachates

Other bacterium examined wasSulphite-Reducing Clostridia (SRC),their levels ranged from 0 to $1.2e^{+7}$ CFU 100ml^{-1}. The mean value that was $2.64e^{+6}$±$1.76e^{+5}$ CFU 100ml^{-1}(Table 2) indicated the non-compliance of these effluents with the directives of the EEC standard [35] that require maximum concentrations of $1e^{+1}$ CFU 100mL^{-1}in SRC not to be overtake. The Tukey box (Fig. 4-A) showed on the one hand a range of $1.2e^{+7}$ CFU 100 ml^{-1}andan interquartile range of $3.1e^{+6}$ CFU 100ml^{-1}.

The bacterial population of StaphylococcusAureus (SA) in leachate under study ranged from 0 to $9.23e^{+9}$ CFU 100mL^{-1} with an average of $2.3e^{+9}$ CFU 100mL^{-1}, value much greater than the median given by the Tukey box (Fig.3-B). According to the EEC Standard [27] that requires a total absence in 100mlof water, these percolate were non-compliant.

Bacterial densities of the Vibrio spp (V) varied from a minimum abundance of 3 CFU 100ml^{-1} to a maximum abundance of $1.8e^{+1}$ CFU 100ml^{-1} with an average of 8.26 CFU 100mL^{-1}(Fig. 3-C) and a smaller range of the order of 15 CFU 100mL^{-1} (Table 2) which showed a low dispersion of results. These low recorded values were related to the environmental conditions (acidic pH and low salinity) not favorable to the multiplication of Vibrio, in fact the latter proliferate in basic and salty medium (pH>8 and salinity>6.5%). However, the presence of Vibrio cholerae indicated that these leachates are non-compliant with French standards [28] of swimming pool water qualities which stipulate an absence of pathogenic germs in 100 mL^{-1} of water.

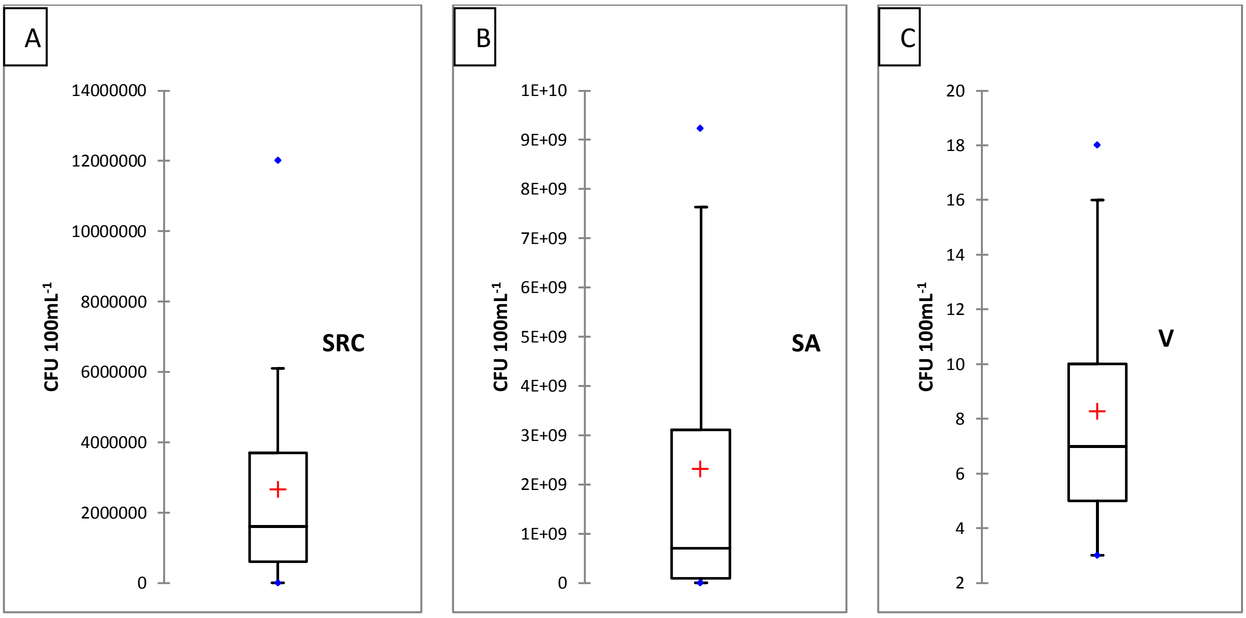

Fig. 3.Tukey-Box representations of Clostridium sulfite-reducer (A), Staphylococcus aureus (B), and Vibriocholerae (C) concentrations in leachates

The Salmonella content in the leachates, under study, fluctuated between 0 and 6.1e^{+3} CFU 100ml^{-1}(Table 2)with an average of 9.75e^{+2}±9.13e^{+1} CFU 100ml^{-1}(Fig.4-A). The presence of salmonellas indicated the non-compliance of leachates with the directives and with the guide and the values of the WHO [29,30] concerning the use of waste water and waters of excrements in agriculture and in fish farming that require the absence of salmonellas in these effluents. Neverthelessonly 22 samples of the 336 (i.e. 6.54%) showed 0CFU ml^{-1} and were therefore in accordance with these guidelines.

The populating of Helminth eggs (H) (Table 2) was weak and showed concentrations which went of a minimum of 0 L^{-1} to a maximum of 8e^{+1}L^{-1} with an average of 4.3e^{+1}±1.58 L^{-1}. Such value was widely superior to the directives of the WHO [29]andto the Tunisian standards [31]. Indeed, the WHO requires a concentration in eggs which must be lower or equal in 1 L^{-1}, while the Tunisian standard is fixed only at 10 L^{-1}. The box Tukey showed that the found average was lower than the median with a range of 8e^{+1}L^{-1} and an interquartile range of 32 L^{-1} (Fig. 4-B).

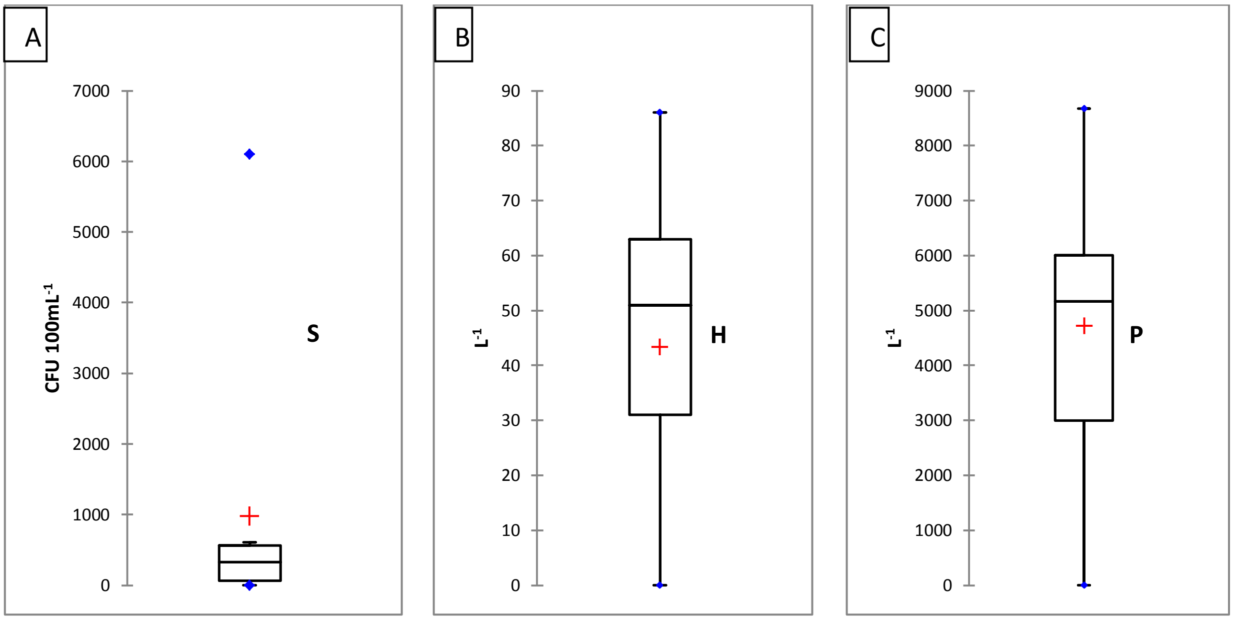

Fig. 4.Tukey-Box representations of Salmonellae (A), Eggs Helminths (B), and Protozoan cysts (C) concentrations in leachates

The enumeration of Protozoal cysts (P) indicated concentrations which varied between a minimum of 0 L^{-1} and a maximum of $8.67e^{+3}$ L^{-1} with an average of $4.71e^{+3}\pm1.53e^{+2}$ L^{-1}(Fig. 4-C). Such value non-compliant with most national and international standards, concerning the quality of waste water and waters intended for the production of human consumption waters and for the irrigation of vegetable crops raw consumables, requiring all a concentration of 0 Cysts $100ml^{-1}$. However 12.5% only of samples realized showed a content of 0 L^{-1} and was consequently in accordance with these standards.

4. Conclusion

Leachates, as waste water, are the most optimal medium for microbial proliferation. This study revealed that serious health risks could result from contamination of the aquatic environment by this percolates in case of infiltration and/or diffusion. Leachates from the El Hajeb (Morocco) landfill were found to be very loaded in heterotrophic bacteria and microorganisms with pathogenic characters far exceeding the existing standards, in contrast, these results were lower, in concentrations, than those of the literature. This was explained, doubtless, by the presence of a strong concentration of heavy metals that inhibit their proliferations. However other bacteria (case of E.coli, Staphylococcus aureus, Salmonella), due to their long-term exposure to heavy metals, became tolerant and resistant to these metals. These biological pollutants could therefore expose residents around the site (landfill), directly or indirectly, to health risks such as malaria, salmonellosis, cholera and gastroenteritis…

Consequently these effluents are extremely toxics and these results, on the one hand, will undoubtedly help at the choice of a better technique of treatment and on the other hand will be a very important tool and starting point for the next work concerning the diagnosis of the quality of the neighboring water resources.

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