

Assessment of the Effect of Light, HgCl₂ and Organic Compound on *Enterococcus faecalis* and *Escherichia coli* Cells Survive in Aquatic Microcosm

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Abstract: The aim of this study was to determine the cultivability of *E. faecalis* and enteropathogenic *E. coli* (EPEC) under different light intensities in the presence of different concentrations of mercury chloride (HgCl₂) and biodegradable organic compound (BOC). Studied light intensities were 0, 500, 1000, 1500 and 2000 lux while studied HgCl₂ concentrations were 0.001, 0.01, 0.1 and 1 µg/L. The BOC used was glucose at concentrations of 0.001, 0.01, 0.1 and 1 mg/L and at pH 7. The BOC used was glucose at concentrations of 0.001, 0.01, 0.1 and 1 mg/L and at pH 7. The duration of incubation under light was 6 h. Results showed that *E. faecalis* and EPEC bacteria are inactivated by HgCl₂ irrespective of studied concentration. The cells inhibition percentage (CIP) of *E. faecalis* varied from 94.46% to 99.53% at 0.001 µg/L, from 94.77% to 99.55% at 0.01 µg/L, from 94.92% to 99.57% at 0.1 µg/L and from 96.97% to 99.77% at 1 µg/L of HgCl₂. For EPEC cells, the CIP fluctuated between 89.87% and 98.99%, between 90.67% and 99.14%, between 92.05% and 99.14% and 93.50% and 99.25% respectively in solutions containing 0.001, 0.01, 0.1 and 1 µg/L of HgCl₂. The highest abundance was observed under 1500 lux for *E. faecalis* and 500 lux for EPEC. Exposure to light seemed to intensify the toxic action of HgCl₂ in the medium. Cells metabolism reactivations under 2000 lux for *E. faecalis* and 1000 lux for EPEC were nevertheless observed. The level of this photo-reactivation varies from one organism species to another. The oligotrophic nature of the medium could not allow studied the microorganisms to set up specific protection mechanism against HgCl₂ and light.

Keywords: *Enterococcus faecalis*, *Escherichia coli*, Light Intensity, Mercury Chloride, Biodegradable Organic Compound, Aquatic Microcosm

1. Introduction

Microorganisms have played a major role in the co-evolution of the geosphere and biosphere. The microbe-environment interactions still operate, and humans sometimes

use interactions to assess the quality of water, food and even its physical state [1]. The undesirable presence of microorganisms in an environment is a pollution index. This is often evidenced by indicators of faecal contamination, particularly the bacteria *Escherichia coli* and *Enterococcus faecalis*. These

microorganisms, which are normally endo-commensally to the gastrointestinal tract, may prove to be deleterious to man's formidable in terms of health, given that they are responsible in some cases for acute gastroenteritis, urinary infections, nosocomial infections and endocarditis [2, 3].

Several processes to reduce or remove bacterial pollutants from water have been developed: 1) chemical disinfectants such as chlorine and ozone, 2) SODIS (Solar Water Disinfection) and SOPAS (Solar Pasteurisation) methods, using sunlight radiation, which in the presence of certain chemicals or organic Biodegradable compounds, may become ineffective [4, 5]. Biodegradable organic matter serves as a nutrient and energy source for heterotrophic bacteria, while autotrophs use mineral compounds.

Aquatic bacterioplankton known to be sensitive to sunlight radiation, especially to short-wavelength fraction of UV radiation. Solar UV radiation (UVR, 100 to 400 nm) causes cellular damage on different cell targets [6]. Because bacteria are considered to be too small to develop efficient photoprotection against UVR [7], UV radiation can significantly affect the in situ single-cell activity of bacterioplankton and naturally dominating phylogenetic bacterial groups have different sensitivity to natural levels of incident solar radiation [8].

Some cellular forms, including spores, are UV-resistant. This resistance is not explained by a reduction in the number of DNA damages but by difference in their nature allowing a very effective repair in the future. Thus, due to the particular conditions inside the spore (high dehydration, presence of proteins modifying the conformation of the DNA, high concentration of dipicolinic acid, among others), the absorption of a UV photon by DNA does not lead to the appearance of 12 different photoproducts as in all other cells but to the formation of only one type of photoproduct. Spore germination thus requires only a single repair enzyme, *spore photoproduct lyase*, present in large quantities in the spore, to repair its DNA [9].

An aquatic environment sometimes contains heavy metals in varying concentrations. Within normal bacterial cells, most metals at lowest concentrations are essential as a cofactor in enzymes, but also available in cells to undergo many protective reactions. The metal's solubility and the reduction potential correlate with the toxicity within the cell [10]. It has been noted that only a few amino acid alterations near the metal-binding region is required to disable function of an enzyme [11]. The metal ions often oxidize residues and lead to a formation of carbonyl groups, and proteins with these marker groups are designated for intracellular degradation [12]. In bacteria, a family of Fe-S dehydratases for example is extremely vulnerable to such Reactive Oxygen Species-mediated oxidation [13]. Metal ions must first enter the cell in order to have an effect [14]. They can be transported by fast, unspecific, constitutively-expressed transporters. Some cations can competitively inhibit the assimilation of essential ions by binding to these transporters [15]. For example, Cr (IV) reduces sulphate uptake since chromate exhibits molecular mimicry, causing a decrease in intracellular S, and

a deficiency in this essential bioelement [16].

It has been hypothesized some metals as copper and cadmium can cause lipid peroxidation by increasing mutations that increase the number of unsaturated bonds in fatty acids [17], affecting the integrity of the cytoplasmic membrane. Another method of metal toxicity is through dissipation of the chemiosmotic force or siphoning electrons. Silver in *Vibrio cholerae* have been found to introduce "leakage" of protons across the membrane [18], while other metals are reduced by the quinone molecule and steal electrons away from respiration [19].

Reactive oxygen species that was previously created by the Fenton reaction can disrupt DNA replication and lead to cell death [20]. While Cr(IV) for example is a very potent mutagen, lab situations have suggested many additional cations that cause DNA damage [21]. Furthermore, copper has been correlated to the destruction of extracellular DNA following cell lysis, and this may inhibit postmortem horizontal gene transfer of resistance via transformation [22].

Little is known about the synergistic effect of light, heavy metals and biodegradable organic matter on the cultivability of bacteria in aquatic environments. The present study aims to evaluate the impact of the simultaneous presence of biodegradable organic compound (BOC), HgCl₂ and light on the cultivability of the bacterial *Enterococcus faecalis* and Enteropathogenic *Escherichia Coli* (EPEC) strains.

2. Material and Methods

2.1. Bacterial Isolations and Identifications

The EPEC strain was provided by the Laboratory of Microbiology and Environment of Centre Pasteur of Cameroon (Central Africa). The *Enterococcus faecalis* strain was isolated from waste water sampled in Yaounde (Cameroun) and cultured on Bile Esculine Agar culture medium using membrane filter method [23]. While EPEC strain was cultured on Endo agar culture medium. The identification of both strains was done using biochemical criteria [24, 25]. Each bacterial strain was then stored in glycerol at -15°C for further use.

2.2. Experimental Setup

Two transparent containers were used, one for each cells strain. The containers walls were wrapped with aluminum foil to prevent penetration of light. In each container, 4 series (coded 1, 2, 3 and 4) of 3 beakers, of measuring capacity of 200mL each were introduced. In the first series, the BOC concentration C1 was adjusted. In the second, third and fourth, the BOC concentrations C2, C3 and C4 were adjusted. For each series contains 3 beakers because experiment was done in each case in triplicate. To simulate daylight, 2 lamps of 100Watt Tungsten filament (TESLA HOLESOVICE) were used to illuminate. Each lamp was placed at 50 cm above a container. The lamps were connected in series on a single electric cable, itself connected to a variable rotary brightness (current dimmer)

(VOLTMAN) in order to obtain the desired light intensities above the containers. The experimental setup is shown in Figure 1. The light intensities reaching the bottom of each container were measured using a Luxmeter TESTO 540. Each beaker was designed to receive 100 ml of BOC solution

and heavy metal salt previously sterilized. The BOC used was the glucose ($C_6H_{12}O_6$). The pH of the prepared BOC solution was adjusted to 7 and then it was sterilized using autoclave. The prepared $HgCl_2$ solution was sterilized using Millex membrane filters (0.22 μ m porosity).

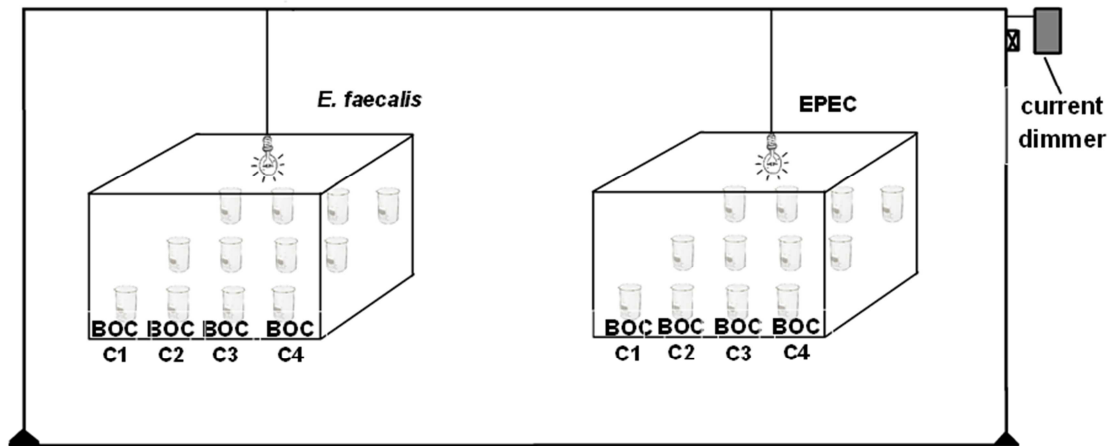


Figure 1. Experimental setup showing containers which contains beakers, a lamp which is connected on a cable, itself connected to a variable rotary brightness (current dimmer).

Prior to the experimentations, a frozen vial containing each cells strain was defrosted at room temperature. The culture (300 μ L) was then transferred into 10 mL of nutrient broth (Oxford) and incubated at 37°C for 24 hours. Cells were then collected by centrifugation (8000 rpm for 10 min at 10°C) and washed twice with sterile NaCl (8.5 g/L) solution. The sediment was then diluted in 10 mL of sterile NaCl solution. Homogenized bacterial suspension was adjusted to a density of 0.5 Mac Farland. Bacteria concentration of original suspension was about 10^8 CFU/ml. After dilution, 100 μ L of the cells suspension was added to 100 ml of BOC solution of a given concentration sterilized as indicated above, contained in a beaker. The cells concentration in each BOC solution was adjusted to 1×10^8 CFU/100mL.

The BOC concentrations were adjusted to 0.001 mg/L, 0.01 mg/L, 0.1 mg/L and 1mg/L. These different concentrations chosen are close to those encountered in most oligotrophic surface waters [23]. The light intensities used were 0 lux, 500 lux, 1000 lux, 1500 lux and 2000 lux. The light intensity that impacts bacteria cell metabolism varies the microbial nature and its physiological state on one hand, and wavelengths in the visible range on another hand [26-28]. The 0 lux condition was carried out in another tank using darkness. For each bacterial cell strain, four series experiments A, B, C and D were carried out. For the series A, the $HgCl_2$ concentration was adjusted to 0.001 μ g/L. For the series B, C and D, the $HgCl_2$ concentrations were adjusted to 0.01 μ g/L, 0.1 μ g/L and 1 μ g/L respectively. These different concentrations were chosen to also approximate those often recorded in water systems, and microbial sensitivity toward heavy metals [19, 29]. The whole aquatic microcosm condition was maintained in each case for 6 hours incubation. Thereafter, bacterial analyses were done in triplicate using Bile Esculine Agar and Endo agar culture media respectively

for *E. faecalis* and EPEC. The plate count technique was used. The Petri disks were then incubated for 24 hours at 37°C for *E. faecalis* and 44°C for EPEC. The mean abundances of bacterial cells were expressed as the number of colony forming units (CFU) per 100mL.

The experiments in each case were carried out using cells belonging to a single bacterial species.

2.3. Data Analysis

The variations of cell abundance as well as cells inhibition percentages after 6h with respect to light intensities, and $HgCl_2$ and BOC concentrations, were illustrated using histograms. The cells inhibition percentages (CPI) were calculated according to the following formula:

$$CIP = \frac{N_0 - N_n}{N_0} \times 100$$

N_0 = cells abundance before incubation; N_n = cells abundance after incubation. The Spearman correlation test "r" was used to assess the relationship between the considered parameters. Comparison among both bacteria strain abundances was carried out using the test H of Kruskal-Wallis and U of Mann Withney. This data analysis was done using SPSS version 16.0 program.

3. Results and Discussion

3.1. Variation of Cells Abundances

It is observed that at each $HgCl_2$ concentration and under each light intensity, cell abundance after 6 hours of incubation increase with the increase in the OBM concentration (Figure 2). The abundances of EPEC ranged from 10×10^5 to 101×10^5 CFU/100mL in solution containing

0.001µg/L of HgCl₂, from 8x10⁵ to 93x10⁵ CFU/100mL in that containing 0.01µg/L of HgCl₂, from 8x10⁵ to 79x10⁵ CFU/100mL in that containing 0.1µg/L of HgCl₂ and from 7x10⁵ to 65x10⁵ CFU/100mL in that containing 1µg/L of HgCl₂ (Figure 2).

The abundances of *E. faecalis* fluctuated between 5x10⁵ and 55x10⁵ CFU/100mL in solution containing 0.001µg/L of HgCl₂, between 4x10⁵ and 52x10⁵ CFU/100mL in solution containing 0.01µg/L of HgCl₂, between 4x10⁵ and 50x10⁵ CFU/100mL in the presence of 0.1µg/L of HgCl₂ and between 3x10⁵ and 30x10⁵ CFU/100 mL in the presence of 1µg/L of HgCl₂ (Figure 2). For each concentration of HgCl₂, the highest abundances of *E. faecalis* were recorded under

intensities 0 and 2000 lux. They were relatively weak under intensities 1000 to 1500 lux (Figure 2).

For EPEC, it is noted that at all HgCl₂ concentrations, the cells abundances were relatively highest when the light intensity was 1000 lux. The lowest abundances were recorded under the intensity 500 lux. It can be observed that the profile of evolution of EPEC abundances under all of the 5 ranges of light intensity considered has a convex appearance. On the other hand, the profile of evolution of the *E. faecalis* abundances under all of the 5 ranges of light intensity has a concave shape with the concavity at 1000-1500 lux (Figure 2).

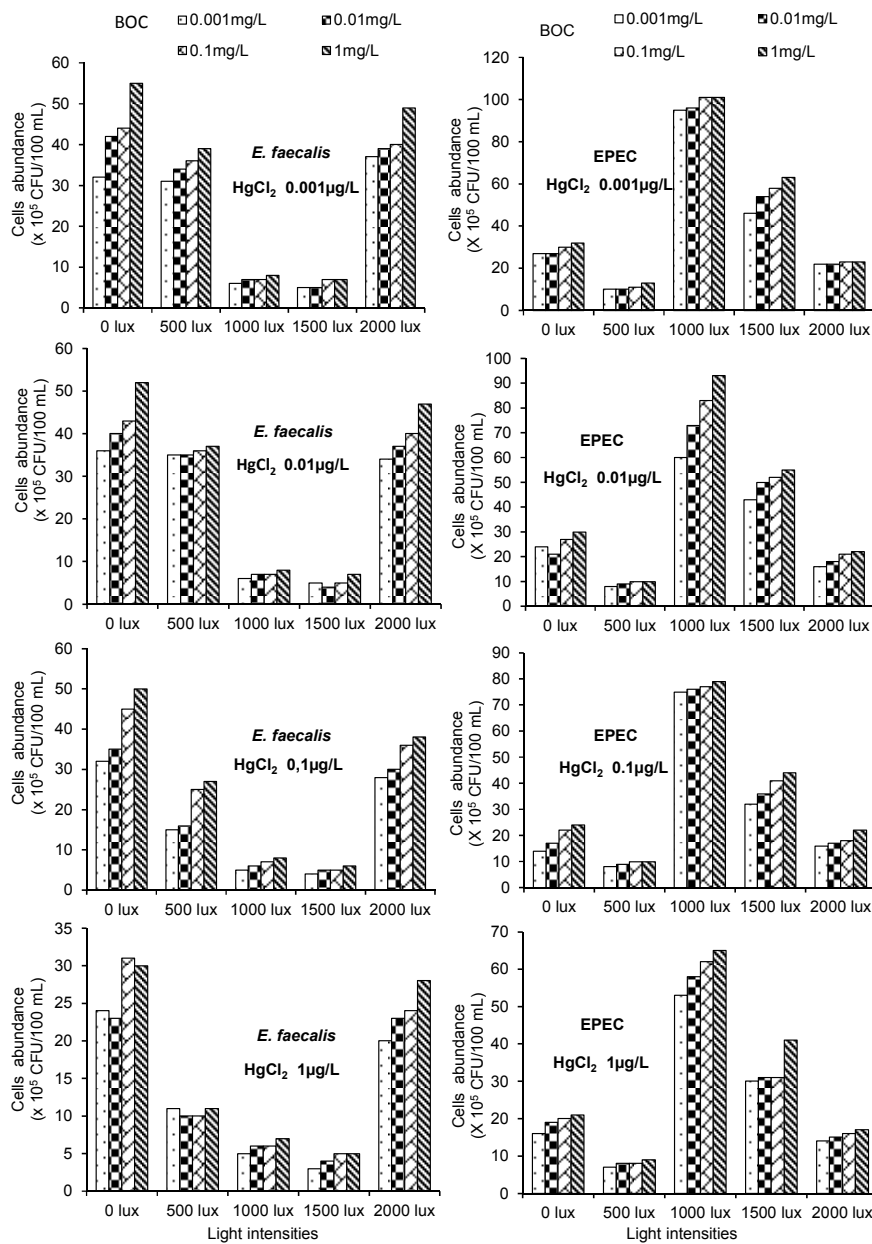


Figure 2. Variations in the abundance of *Enterococcus faecalis* (*E. faecalis*) and EPEC in the presence of different HgCl₂ and BOC with respect to the light intensities.

Cells abundances fluctuated with respect of the applied light intensities. In most cases, there was firstly a gradual

decrease in bacterial abundance, followed by a sudden increase under 2000 lux for *E. faecalis* and 1000 lux for EPEC. This would be due to cells metabolism reactivation under these light intensities. It has been indicated that some microorganisms can indeed repair their DNA by photoreactivation, a process that aims to directly eliminate lesions formed by dimers using photolyase. This photoreactivation can allow, for example, the enterococci and thermotolerant coliform bacteria to reactivate and to increase their growth up to a factor of 1 log unit [30, 31]. This reactivation would be due to repair of damaged DNA upon exposure to light. The level of photoreactivation would vary from one organism to another and this variation is a function of the number of molecules of photolyases [30, 31].

The variation of cells abundances with respect to the BOC concentration under each light intensity is also observed

(Figure 2). In the aquatic environment, solar radiation can indirectly affect the activity of heterotrophic bacteria by photochemically modifying the dissolved organic matter. The assimilation of irradiated dissolved organic matter can lead to a decrease in cell production, a decrease very strongly related to UV-B radiation and moderately linked to Ultraviolet-A radiation [32]. UV-C is absorbed in the early stratosphere and does not reach the earth's surface. In water system, with increasing water depth, there is less UV radiation to inactivate bacteria; high turbidity substantially reduces UV-B transmittance in water causing decreased elimination efficiency [33]. However, the impact of UV on the bacterium can be modified if the environment is eutrophic and UV-B-modified cells may return to normal size and relive after a stay in the darkness [32].

3.2. Cells Inhibition Percentages

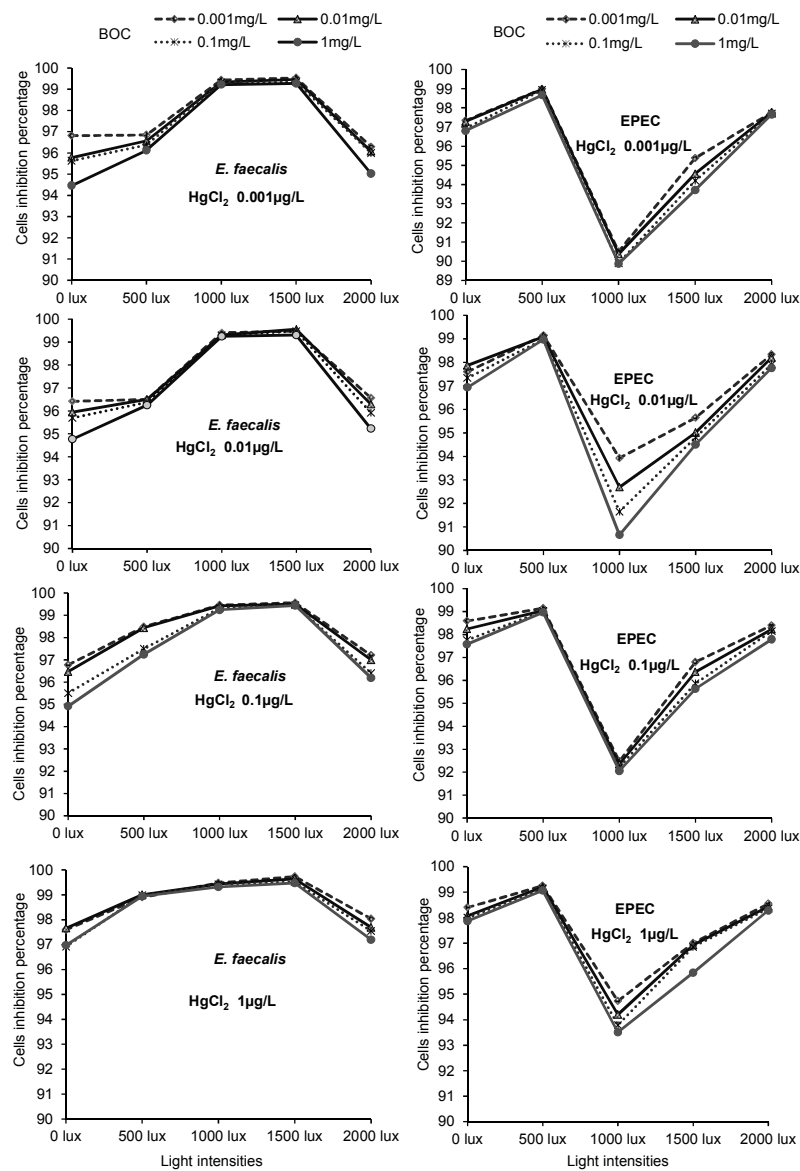


Figure 3. Variations of the cells inhibition percentages for *Enterococcus faecalis* (*E. faecalis*) and EPEC with respect to the light intensities in the presence of each $HgCl_2$ and BOC concentration.

The cells inhibition percentages (CIP) were calculated for each bacterium. For all the experiments carried out, the CIP seems to increase with the increase in HgCl₂ concentration and decrease with the increase in the glucose content. It is also noted that the percentages of EPEC inhibition are relatively lower than those of *E. faecalis* (Figure 3). The lowest CIP were noted under light intensities 0 and 2000 lux for *E. faecalis* and under 1000 lux for EPEC. The highest CIP were observed under light intensity 1500 lux for *E. faecalis* and 500 lux for EPEC (Figure 3).

The CIP of *E. faecalis* oscillated between 94.46% and 99.53% in the solution containing 0.001µg/L of HgCl₂, between 94.77% and 99.55% in that containing 0.01µg/L of HgCl₂, between 94.92% and 99.57% in that of 0.1µg/L of HgCl₂ and between 96.97% and 99.77% in the presence of 1µg/L of HgCl₂. In the solutions containing the EPEC cells, the CIP fluctuated between 89.87% and 98.99%, between 90.67% and 99.14%, between 92.05% and 99.14% and 93.50% and 99.25% respectively in solutions containing 0.001, 0.01, 0.1 and 1µg/L of HgCl₂ (Figure 3). It was observed the CIP of EPEC cells under 1000 lux is relatively low at all of the concentrations of HgCl₂ used (Figure 3). EPEC cells would resist to both stressful factors. Resistance mechanisms of microorganisms towards toxic metal is efflux or sequestration and its reduction to less toxic state. Metal toxicity differs from antibiotics mostly because there are no components to modify [14], but there are still a couple of ways the cell can defend itself. Cations can be pumped out of the cell with low ATP expense, or sequestered by chelates like thiol-containing compounds at approximately 16 ATP, an option that is only cost-effective at low metal concentrations [19]. Some cations can be reduced to a less detrimental state if its reduction potential is within physiological conditions (421 to 808mV), but sometimes the reduced state may be more toxic or insoluble [14]. Extracellular electron transfer has also been hypothetically linked to nanowires, and is an area of active research [19].

The mean values of the CIP of EPEC cells remained relatively lower than those of *E. faecalis* in most cases. This suggests that EPEC cells would be more resistant to the toxic effect of light and HgCl₂ than *E. faecalis*. Faecal enterococci (Gram +) are more sensitive to light photons than fecal coliforms (Gram -). This difference in sensibility may be due to the difference in the constitution of their cell membranes. The cell membrane of Gram- bacteria has a layer of lipopolysaccharides which acts as a barrier against singlet oxygen (O₂¹). In addition, this layer is made up of unsaturated fatty acids and proteins which in turn would constitute a trap for O₂¹ [34, 35]. The membrane of Gram+ bacteria, free of this layer, is easily destroyed by O₂¹. After the membrane is destroyed, O₂¹ enters the cell and attacks the cellular constituents; this leads to cell death. In addition, Lotavera [36] experimenting with the protein synthesis inhibitor chloramphenicol indicated that sunlight can promote synthesis of proteins preventing the dying of cells. The presence of Casamino acids is favorable for the synthesis

of protective proteins.

According to Istvan Pocs [37], the most promising targets in the genetic engineering of metal/metalloid tolerant cells include many aspects: a)-increase in secretion of extracellular metal chelator; b)-Elimination of metal transporters facilitating the uptake of the toxic metals/metalloids; c)-Overexpression of transporters pumping metals and/or their complexes out the cells or into cellular organelles, d)-Overproduction of intracellular metal chelators; e)-Overproduction of elements of the antioxidative defense system; f)-Genetic modification of the regulatory network of metal/metalloid stress defense; and g)-Interfering with the metal/metalloid-dependent initialization of apoptotic cell death. Dunkel *et al.* [38, 39], and Marie and White [40] indicated that the genotoxic potential of a compound sometimes include: induction of mutation, DNA damage, chromosomal and chromatine alterations. Mutations are detected mainly as phenotypic changes and structural alterations of DNA (deletion, insertion, substitution, frameshift, and translocation). These mutational changes are measured when cells turned out to be the most sensitive and easy to handle test microorganisms.

Lima Silva *et al.* [41], working on heavy metal tolerance by bacteria, noted that tolerance to Cr was observed mainly in Gram positive bacteria while in the case of Ag and Hg the tolerant bacteria were predominately Gram negative. Hg was the metal for which the percentage of tolerance was significantly higher. In addition, Shameer and Paramageetham [42] noted that many bacillus species have biosorption potentials towards lead, chromium, and copper and concluded that bacterial isolates could be potential agents to treat metal contamination in more efficient and ecofriendly manner.

3.3. Assessment of the Relations Among the Considered Parameters

Correlations coefficients between bacterial cell abundances and HgCl₂ concentrations at different light intensities were evaluated using the Spearman "r" test. The results show that in most cases, the increase in the concentrations of the heavy metal salt considered causes under all the light intensities used a significant decrease (P<0.05) in the abundances of *E. faecalis* and EPEC that can be cultivated Table 1. On the other hand, positive and significant correlations (P>0.05) were observed between cell abundances and BOC concentrations, in most cases with *E. faecalis* Table 2.

Table 1. Spearman correlation coefficients between cells abundances and HgCl₂ concentrations under each light intensity.

Cells species	Light intensities (lux)				
	0	500	1000	1500	2000
Enterococcus faecalis	-0.618*	-0.856**	-0.376	-0.546*	-0.812**
EPEC	-0.814**	-0.715**	-0.825**	-0.873**	-0.839**

** P< 0.01; * P< 0.05; ddl=15

Table 2. Spearman correlation coefficients between cells abundances and BOC concentrations under each light intensity.

Cells species	Light intensities (lux)				
	0	500	1000	1500	2000
Enterococcus faecalis	0.570*	0.273	0.837**	0.752**	0.521*
EPEC	0.492	0.631**	0.400	0.449	0.487

** P< 0.01; * P< 0.05; ddl=15

Comparisons among the mean abundances of *E. Faecalis* and EPEC at different HgCl₂ and BOC concentrations and under different light intensities were carried out using the "H" test of Kruskal Wallis. It resulted that mean abundances of EPEC differ significantly (P<0.05) at all light intensities. However, between 0 and 2000 lux, no significant difference (P>0.05) was observed among abundances of *E. faecalis*. The significant difference (P<0.05) was thus noted among the abundances of *E. faecalis* at all concentrations of HgCl₂ and BOC. The same observation was noted regarding EPEC abundances.

The results indicate significant and positive correlations (P<0.05) between the different BOC concentrations and the abundances of *E. faecalis* and EPEC at each light intensity. The assimilation of organic matter would induce an increase in bacterial density. In most cases, there is a decrease in cells abundances with increasing light intensities. According to Joux *et al.* [43], the effectiveness of UV light in biological inactivation arises primarily from the fact that DNA molecules absorb UV photons between 200 and 300 nm, with peak absorption at 260 nm. UV-A (wavelengths, 320 to 400 nm) causes only indirect damage to DNA, proteins and lipids through reactive oxygen intermediates. UV-B (wavelengths, 290 to 320 nm) causes both direct and indirect damage because of the strong absorption by DNA at wavelength below 320 nm.

It is noted that under the same light intensity and at the same BOC concentration, the cells abundances decrease with the increase in HgCl₂ concentration. Heavy metals have been historically used as antimicrobial agents prior to the discovery of antibiotics, and have now been incorporated into coatings, surfaces, and internally placed devices [19]. Recent evidence has shown that some metals can work synergistically with antibiotics [19], are effective against multi-drug resistant bacteria, and can disrupt biofilms [44]. There is a lot of diversity in both metals and the bacteria they affect, but in general toxicity can be broken down into various categories. However, metal toxicity differs from antibiotics mostly because there are no components to modify [14], but there are still a couple of ways the cell can defend itself. The microbial processes for bioremediation of toxic metals and radionuclides from waste streams employ living cells, nonliving biomass, or biopolymers as biosorbents and specific metabolic pathways resulting in bioprecipitation of heavy metals or their biotransformation to less toxic or easily recoverable forms have been indicated [45].

Misra [46] noted that environmental and clinical isolates of

mercury-resistant (resistant to inorganic mercury salts and organomercurials) bacteria have genes for the enzymes mercuric ion reductase and organomercurial lyase. These genes are often plasmid-encoded, although chromosomally encoded resistance determinants have been occasionally identified. Organomercurial lyase cleaves the C-Hg bond and releases Hg(II) in addition to the appropriate organic compound. Mercuric reductase reduces Hg(II) to Hg(O), which is nontoxic and volatilizes from the medium. Mercuric reductase is a FAD-containing oxidoreductase and requires NAD(P)H and thiol for in vitro activity [46]. In *Pseudomonas aeruginosa*, the metalloprotein MerR is a mercury(II) - dependent transcriptional repressor-activator that responds to mercury(II) with extraordinary sensitivity and selectivity. It's widely distributed in both Gram-negative and Gram-positive bacteria [47].

Hobman and Crossman [48] indicated that metals and metalloids can exert toxic effects in a number of different ways: by binding to or blocking functional groups in biological molecules, by displacing essential metals in enzymes, by binding to the cellular thiol pool or participating in chemical reactions in the cell that are harmful. Ultimately, the deleterious effects reported include damage to proteins, DNA and biological membranes, interference in enzyme function and cellular processes, and oxidative stress. The potential resistance strategies that bacteria can employ are limited to extracellular or intracellular sequestration of the metal, reduction in permeability, alteration of target sites, enzymic detoxification or efflux of the metal ions. These authors [48] also indicated that specific metal ion resistance mechanisms are usually characterized by a metal ion-specific response regulator, which controls the expression of structural resistance genes. The products of these genes produce a metal ion-specific efflux protein or protein complex and/or enzyme(s) that alter the metal ion into a form less toxic to the bacterial cell. This resistance is a genetic and physiological adaptation to the environment which is contaminated by the heavy metal [49]. Chang *et al.* [50] using an Engineered *Enterobacter aerogenes* and *E. coli* strains containing the artificial operon P16S-g10-merT-merP-merB1-merB2-ppk-rpsT, noted that they could accumulate the mercury in aquatic media with pH values ranging from 5 to 8 and under the temperatures ranging from 25°C to 37°C; but this depends on some chemical environmental conditions of the medium.

4. Conclusion

The aim of this study was to assess the cells bacterial cultivability under stressing effects of light and HgCl₂ in the presence of BOC. It was observed that *E. faecalis* and EPEC bacteria are inactivated by HgCl₂ at different rates. Exposure to light seemed to intensify the toxic action of HgCl₂ in the medium. Cells metabolism reactivations under 2000 lux for *E. faecalis* and 1000 lux for EPEC were nevertheless observed. The level of this photoreactivation varies from one species to another. The oligotrophic nature of the medium

could not allow the microorganisms to set up specific protection mechanisms against HgCl₂ and light.

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