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Use of an electrophoresis box made with inexpensive materials to verify DNA from *Escherichia coli*

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Abstract. Experimental classes may aid to develop scientific concepts and allow students to understand how to objectively approach their reality and develop solutions for complex problems, as environmental pollution and support for high schools and universities laboratories. In this work an electrophoresis box was made by students from and acrylic chocolate box, to verify DNA extracted and amplified from *E. coli*. Disposable cell phone power chargers of 12 V capacity were used as energy source. DNA fragments migrated during the electrophoresis process, demonstrating this artisanal box may be used for molecular biology experiments in schools and universities. Beside the possibility of using inexpensive laboratory material a reduction of environmental impacts, due to disposed materials such cell phone chargers and plastic containers, is also an advantage.

Key words: Experimental Classes; Environmental Impacts; Electrophoresis; Low Cost Materials

INTRODUCTION

Experience is a natural part of life where the situation and the subject are modified, constituting a permanent construction and deconstruction process (Souza et al., 2013; Souza

et al., 2014; Goldbach et al., 2023). Within the nowadays financial context of public education institutions in Brazil, the experience in the field of molecular biology is commonly denied due to the lack of specific materials, because they are very expensive or due to the lack of public policies for education which may provide public institutions with equipment and reagents in this field of knowledge (Setúval and Berjerano, 2009; Leite, 2010; Scheid et al., 2023). Therefore, experimental classes performed with inexpensive and alternative materials may allow the students to experience molecular biology contents, or other fields within the Biological Sciences in a ludic, technological and scientific manner, surpassing obstacles such as lack of state of the art and expensive equipment (Manso and Santana et al., 2017; Lopes, 2023). Low cost materials used in experiments may be varied: boxes for objects or food, paper, plastic bottles, among others (Santana et al., 2018; Jan et al., 2023). This represents a low cost materials used in experiments may be varied: boxes for objects or food, paper, *pet* like bottles, among others (Santana et al., 2018; Jann et al., 2023). This represents a reduction on the environmental impact due to the prevention of discarding these materials. Preserving the environment where the human being leaves is also a way to preserve its well-being, protect and avoid any kind of pollution results in positive perspectives for the population and specially the environment where such facts are occurring (Silva et al., 2013; Morais and Santos, 2016; Manso and Santana, 2019).

Electrophoresis is one laboratory experience which may be performed using inexpensive materials and reducing environmental impacts. Among the laboratory experiences in molecular biology, electrophoresis is one of the most universally executed. Electrophoresis is technique used to separate molecules, DNA, RNA and proteins, it consist in the migration of those molecules through a gel during the application of an electric current (Biotium, 2019; Kasvi, 2019). Industrial electrophoresis boxes for electrophoresis come in varying sizes and are usually made of acrylic. Generally these box have resistance between 30-50 Ohms. They also have power between 300 and 500W. In the present work, bacterial DNA of *Escherichia coli* was extracted, amplified, stained with GelRed® and applied in gel in an electrophoresis recycled box made from a chocolate container. This box mobilized DNA molecules. Different materials such as cell phone chargers and acrylic box were also assembled in the experiment, reducing the environmental impacts caused by their disposal. The use of low cost equipaments can represent a support for molecular biology laboratories.

MATERIAL AND METHODS

Involvement of High Schools and University Students with the Research

The schools involved into this research were Luís Navarro de Brito and Alagoinhas Municipal High School with 30 students class, each. These public high schools are localized in the city of Alagoinhas, State of Bahia, Brazil. The students answered questionnaires before and after construction of box to examine them knowledge about electrical concepts, molecular biology and electrophoresis techniques. About 10 biology UNEB´s students participated as coordinators of activities in extraction and amplified DNA. UNEB is a public university. The electrophoresis box has been used 20 times in schools and UNEB.

Growing *E.coli* for DNA extraction

Specific bacterial strains of *E. coli* , provided by Experimental Biology Laboratory (LABEX - UNEB), were grown in Petri dishes containing Agar Mueller Hinton medium at 37°C for a period of 24 hours. Suspensions were obtained after incubation of microorganisms, using 2 mL of homogenized saline solution until attaining the desired turbidity in the standard solution of McFarland 0.5 (1.5 x 108 bacteria/mL), measured at spectrophotometer. The suspension was transferred to the petri dishes with the aid of a cotton swab, which was submerged in the prepared solution and carefully rubbed through the whole Petri dish, in order to homogenize all the solution captured.

E. coli DNA extraction and amplification

Extraction of DNA was achieved using the Qiagen extraction kit, following the manufacturer instructions. The total DNA was extracted from an *E. coli* sample with 180 µg. The amplification of *E. coli* DNA was performed by PCR. Initially the PCR tubes were labeled with the number or the initials of samples. The primers used comprehended the V6/V8 region of the 16S rDNA. The sequence of primers for the V6-V8 region was:984F5'GCClamp1378RAACGCGAACCTTAC3'e5'CGGTGTGTACAAGGCCCGGG AAC3' (Heuer et al., 1997; Santana et al., 2016). All PCR reactions used a mixture containing 1.25 U of Taq DNA polymerase (Invitrogen), 5µL reaction buffer 5X, 200 µM deoxyribonucleotides, MgCl2 3.0 mM and 2 µL DNA and sterilized Milli-Q water, to a final volume of 25 µL. The final concentration of the PCR reaction was set with 0.2 mM of each primer, 0.2 mM DNTP, 0.03 U/µL Taq DNA polymerase (Promega), 100 ng of DNA, 3 mM MgCl2 and buffer 1X. The amplification was performed using an automatic thermal cycler (Mastercycler Eppendorf). Tubes were then set in the thermal cycler plate and the desired program established, with the quantity and conditions of cycling desired. After the cycling period, 35 cycles, samples were removed from the thermal cycler and conditioned in tubes at -20ºC in a freezer.

Preparation of agarose gel

To prepare the agarose gel for artisanal box, aiming to verify the extracted DNA, 800 mg of agarose were weighed in a digital balance. Then, these 800 mg of agarose were mixed with 100 mL of TAE1X and poured into a 250 mL Erlenmeyer, resulting in an 0.8% agarose gel. Then, the mixture of agarose and buffer was heated in microwave oven for 1 minute, until all the agarose was liquefied. The agarose was set in an acrylic mould, sealed with with rubbered parts on the sides to avoid spilling the gel. This is important, once when DNA samples are applied these are prevented to overflow underneath the gel. Subsequently, plastic comb (Figure 1) were set over the agarose. The comb were used for make holes in the solidified gel, but avoiding the comb prongs to touch the bottom of the mould. The agarose polymerized forming a gel with 7 cm length, 4.8 cm width and 1cm height and the comb was removed, thus forming 10 wells. The gel was set in the electrophoresis box which was completely filled with TAE1X buffer. The gel for observation the amplified DNA was of 1% (w/v). A gel is showed in the Figure 2.

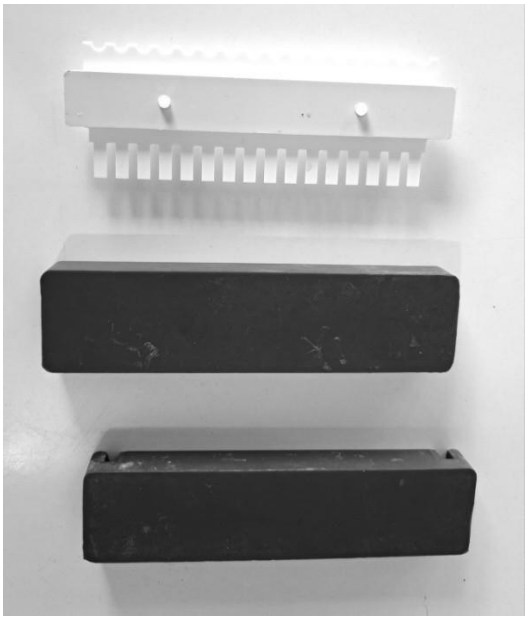

Figure 1. Comb to make holes and rubbered parts used to contain the gel.

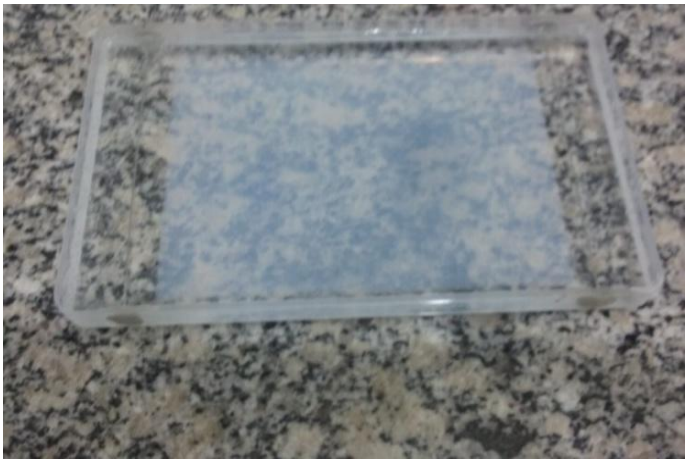

Figure 2. An agarose gel inside the artisanal electrophoresis box.

Construction of the electrophoresis Box

The electrophoresis box was constructed from a chocolate's crystal acrylic box with 25 cm length, 10 cm width, 0.15 cm thickness and 2 cm height. The donated materials used in the fabrication of the electrophoresis box were: epoxy resin, stainless steel wires (Figure 3), chocolate box, clips and cell phone chargers. On each side of the box a stainless steel wire (0.7 mm, previously heated) was introduced in order to serve as an electrode. The holes made by the introduction of wires in the box were sealed with epoxy resin. This is important in order to avoid leakage points in the box. Each wire had an extremity left outside of the box, where the electric current was connected using wired clips. These wired

clips were connected to an electric power supply with 12V, CA/CC, Model F10L1050200SPAE-U, in: 100 -240V/50-60 Hz , 0.3A and out: 5V – 2A (Flex Industries), specifically in this case a cell phone charger. The box was powered by two 12V sources in parallel. During the electrophoresis run the energy sources remained connected to electric sockets. The resistance of the electrophoresis box was measured with aohmimeter. The amperage was measured with a ammeter. To calculate the power running in box, it was used the mathematical formula P = V2/R. For running electrophoresis, the box was filled with TAE1X buffer. All materials to construct the box were donated and therefore without expenses for the University and Schools. The electrophoresis box is showed in Figures 1 and 2, open and closed, respectively. An industrial electrophoresis box is showed in Figure 3, to compare design and wire configuration.

Figure 3. Stainless steelwire used to serve as electrode in artisanal electrophoresis box.

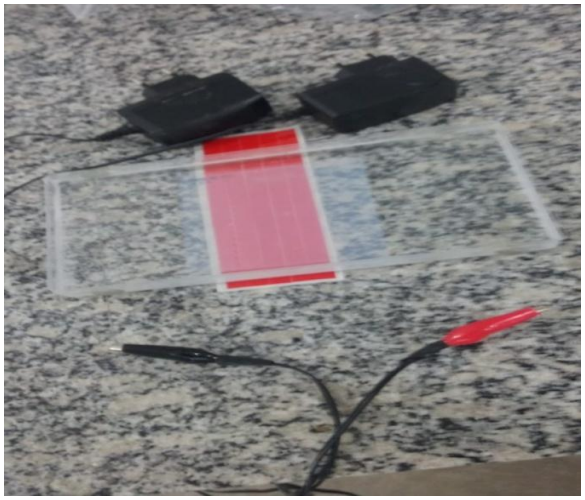

Figure 4. Opened electrophoresis recycled box showing wired clips, energy sources and

Electrophoresis running and observation of extracted and amplified DNA

Samples of *E. coli* DNA in the tubes were removed from the freezer and set to rest in a stand. After slight defrosting the samples were placed into ELISA plates, along with other reagents used in electrophoresis runs, with the following volumes: 1µL GelRed dye, 1µL bromophenol blue and 3µL DNA, with a total of 5µL per sample. The DNA extracted from *E. coli* was stained with GelRedTM, diluted at 1:1000 (1µl GelRedTM for 1000 µl miliQ water). Bromophenol blue allows visualizing and following the migration of DNA. The samples prepared for the electrophoretic run were then applied on the wells, using micropipette. Then, the box cover was mounted and the red (positive) and black (negative) cables were attached to the extremities of the stainless steel wires. The power suppliers, cell phone chargers of 12 V, were connected to an 110V power outlet. The extracted DNA migrated for 20min, while the amplified DNA migrated for 30min. At the end of the electrophoretic run the power source was disconnected, the box opened, the gel removed and placed into a transilluminator Loccus, to observe the presence of bands. The transilluminator emits ultraviolet waves which excite the GelRed dye linked to the DNA and fluoresces, enabling to visualize the bands. Due to the emission of UV waves, it is necessary to use protecting goggles during the equipment operation. After visualization bands were photographed with a Smartphone A53.

RESULTS AND DISCUSSION

Public Schools and Universities involvement

The students of schools participated together with university students in assembling the electrophoresis equipment, learning about DNA structure, electrophoresis technique and the structural and electrical characteristics of the box. Also, the students applied and visualized the DNA samples on the gel with a transilluminator. Before experiments, only 30% of students answered the questions about electrophoresis, basic eletrical concepts and molecular biology properly (Figure 5). The evaluation of their comprehension about molecular biology and experimental activities totaled 95% (Figure 6) after experimental classes. So, beyond the machine assembling, the university students make classes for public students too.

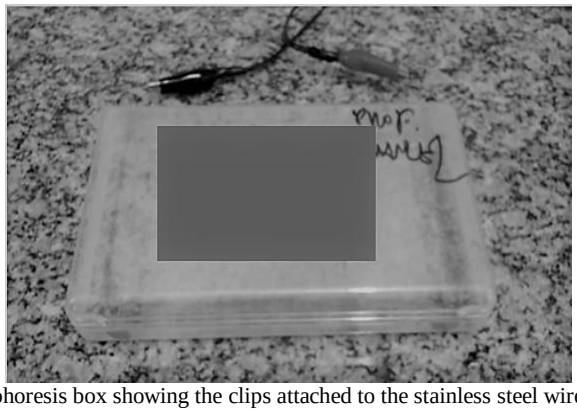

Figure 5. Closed electrophoresis box showing the clips attached to the stainless steel wires.

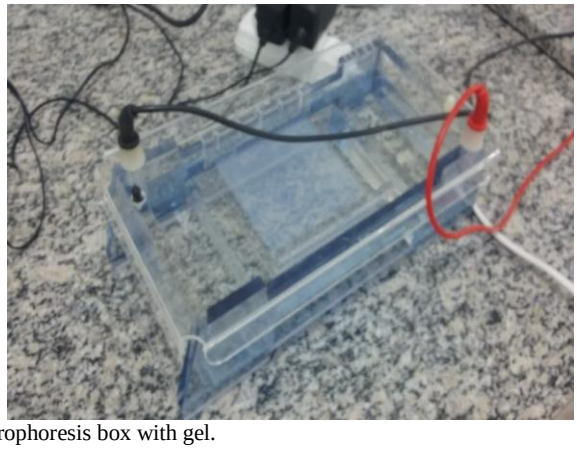

Figure 6. Industrial electrophoresis box with gel.

Agarose Gel and Electrophoresis Box Characteristics

The electrophoresis box was tested 20 times each school, demonstrating durability and resistance. The gel was set in the electrophoresis box which was completely filled with 300 mL TAE1X buffer, differing of the 700 mL of industrial device. The agarose gel has 7 cm length, 4.8 cm width and 1cm height. The comb was removed and forming 10 wells, but only 5-6 was used. As depending of comb used, the number of wells can be change.

The gel was compared to what one would obtain in a commercial device, as the same kind. The resistance measured in the electrophoresis box was 40 Ohms, differing of 50 Ohms of industrial device. The calculated power of artisanal box, assuming P = V2/R was 7.2W. The amperage measured was 0.6A. The electrophoresis equipment made of inexpensive material did work properly, promoting slower migration of bands than commercial device in the prepared gels. The Voltage, amperage and power measured during the running electrophoresis. As the objective was only to verify extracted and amplified DNA, the experiment was successful. The proper functioning of the electrophoresis box shows that it may be used in experimental classes, in schools and universities. Once it has no cost, it is accessible equipment and may be constructed in different versions by students as part of the learning contents in the field of molecular biology. During the experimental activities students may be motivated to perform practical activities or work with chemical reagents. This results from the fact that the simple handling of equipment and materials involved with the practical activity constitutes a form of interaction between the student and the object of knowledge, which may become a joyful activity for the student. The execution of practical experiences transfers to the student the responsibility to construct the results, and in order to become a successful activity requires some attitudes and behaviors which may not be experienced in an expositive class (Jann and Leite, 2010). The educational function may be attained when observing the cooperation mood between the students and the researcher or professor. Students may improve their knowledge and develop their vision regarding the subjects related with biology. This may prove the methodology adopted was efficient in the learning process. Even if the materials used in the fabrication of the electrophoresis box would not be donated, the cost will still be minimum if compared with the costs of a industrial electrophoresis box. When considering the costs of materials: epoxy

resin, stainless steel wires, chocolate box, clips and cell phone chargers, the equipment will cost about R$ 105.00 (21.00 US$). At them present moment, an industrial electrophoresis box costs about R$ 1200.00 (240.00 US$) and R$ 4000.00 (800.00 US$), depending if it is specifically for migration of DNA and RNA or proteins, besides the issue of the equipment size. This demonstrates that low cost materials may be used to complement or even substitute expensive equipment. The reutilization of these materials enables to assign them new functions, assisting in the structuration of schools and universities laboratories. Innumerous pollution factors produce changes in the water and soil quality and in the organisms depending on them, once these materials may content substances with mutagenic, genotoxic and cytotoxic properties which affect the genetic integrity of organisms (Kasper et al., 2018). The recycling of disposed objects and materials reduces environmental impact, facilitating the preservation of human and other living organism's health. To maintain a molecular biology laboratory can be so expensive with use of high cost machines. So, low cost materials, like electrophoresis box, can be a solution.

DNA Extraction and Amplification

The DNA extracted from *E. coli* was verified in all the five lines of the gel where samples were applied. Other bands were also observed, under the extracted DNA, probably of RNA (Figure 7). As the RNA is composed by only one strip, it migrates faster that the total DNA extracted from *E. coli*. The amplified DNA was observed in all four lines were it was applied (Figure 8). The total DNA extracted from E.coli (line 5) was used to compare the amplified DNA. The amplified DNA was observed in all four lines were it was applied (Figure 5). The total DNA extracted from E.coli (line 5) was used to compare the amplified DNA.

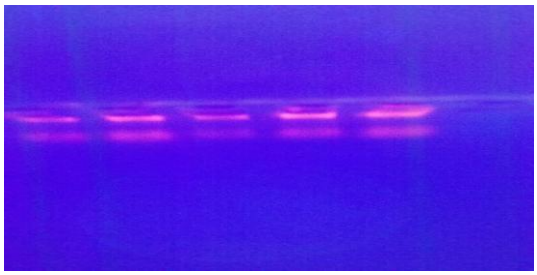

Figure 7. DNA extracted from *E. coli* and stained withGelRed.

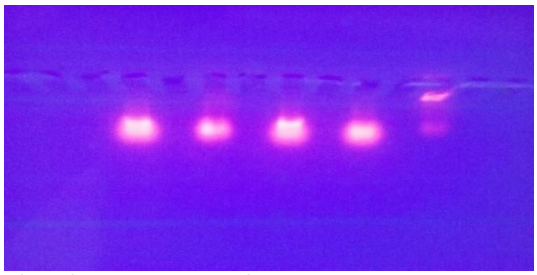

Figure 8. Amplification profile of the 16S rDNA of *E. coli*, stained with GelRed, using primers for regions V6/V8.

Final Considerations

Experimentation or development of practical classes in the classroom is important in the teaching-learning process. It is possible to construct machines which may help the students in this process by using inexpensive materials. Such electrophoresis box maybe used for the instrumentation of teaching institutions. Disposed objects may represent a serious harm to the environmental equilibrium, harming health through pollution. The use of disposable materials represents a way to reduce environmental impacts. The difficulty to maintain a molecular biology laboratory can be solved with use of low cost equipaments, as an electrophoresis box.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Biotium, Inc. GelredTM. GelRed® Nucleic Acid Gel Stain. Disponível em: https://biotium.com/product/gelred-nucleic-acid-gel-stain.Acess in April 2, 2023.

Goldbach T and Macedo AGA. Produção científica e saberes escolares na área de ensino de Genética: Olhares e Tendências. In: VII Jornadas Latino-Americanas deEstudos Sociais das Ciências e das Tecnologias, Rio de Janeiro. 2008. Available in: http://www.necso.ufrj.br/esocite2008/trabalhos/36294.doc. Acess in April, 2023.

Heuer H, Krsek M, Baker P, Smalla K, et al. (1997). Analysis of actinomycete communities by specific amplification of genes encoding 16S rRNA and gel electrophoretic separation in denaturation separation in denaturing gradients. *Appl. Environ. Microbiol.* 63: 3233-3241.

Jann PN and Leite M de F (2010). Jogo de DNA: um instrumento pedagógico para o ensino de ciências e biologia. *Ciências e Cognição.* Vol. 15 (1): 282-293. Available in :https://pt.scribd.com/doc/94794020/Jogo-do-DNA-um-instrumento-pedagogico para o ensino-de-ciencias-e-biologia. Acess in April 4, 2023.

Kasper N, et al. (2018). Impact of anthropic activities on eukaryotic cells in cytotoxic test. Ambienet. Água vol. 13 no.3, 2018.

Kasvi. kasvi.com.br. o-que-e-eletroforese-e-qual-a-sua-importancia. Acess in March 27, 2023.

Leite PN (2010). Jogo de DNA: um instrumento pedagógico para o ensino de ciências e biologia. Ciências e Cognição. Vol. 15 (1). 282-293, 2010. Available in: https://pt.scribd.com/doc/94794020/Jogo-do-DNA-um-instrumento-pedagogico-para-o- ensino-deciencias-e-biologia. Acess in March 27, 2023.

Loreto ELS and Sepel LMN (2003). Atividades experimentais e Didáticas de Biologia Molecular e Celular. Cadernos de Biologia Molecular e Celular. 2ª. Ed. SBG. 2003. Acess in March 27, 2023.

Lopes, et al. (2013). Prática de Cores. Disciplina Laboratório de Ensino de Genética. Curso Ciências Biológicas. UFMG, 2013. Available in: https://youtu.be/LcCbX5f_1w8?t=2. Acess in april 5, 2023.

Manso AJC and Santana LG (2019). Impactos sócio-ambientais no distrito Vardiero. Rev. Científica Multidisciplinar Núcleo do Conhecimento. Ano 04, Ed. 03, Vol. 09, 2019. Acess in March 27, 2023.

Morais VC and Santos AB (2016). Implicação do uso de atividades experimentais no ensino de biologia na escola pública. Investigações em Ensino de Ciências. V21(1). Abr.pp.166-181, 2016. Available in https://www.if.ufrgs.br/cref/ojs/index.php/ienci/article/download/243/169.Acess in march, 2023.

Oliveira WG, et al. (2016). GelRedTM na coloração de DNA em Jatropha curcas L. (Euphorbiaceae). *Rev. Espacios*. Vol. 37, Nº 25, 2016.

Rodrigues IB, et al. (2018). Identificação de micro-organismos presents em sistemade biolixiviação de cobre a 35 e 50°C. *Rev. Eng. Sanit. Amb.* 2018.

Santana EB (2016). Effects of Phosphate-Solubilizing Bacteria, Native Microrganisms And Rock Dust On Jatropha curcas L. Growth. *GMR*.Vol 18. 2016.

Santana EB, et al. (2017). Impactos do Uso de Experimentos de Baixo Custo no Ensino de Genética em Colégios do Ensino Médio. Vol. 8. N2. 2017. Acess in march, 2023.

Scheid et al. (2005). A construção coletiva do conhecimento científico sobre a estrutura DNA. Ciência e Educação. v. 11, n. 2, p. 223-233, 2005. Available in: www.scielo.br/pdf/ciedu/v11n2/05.pdf.

Setúval FAR and Bejarano NBR (2009). Os modelos didáticos com conteúdos de genética e a sua importância na formação inicial de professores para o ensino de ciências e biologia. VII ENPEC. Florianópolis, SC, Brasil, 2009.

Silva ACR (2005). Os conhecimentos prévios no contexto da sala de aula. Revista Metáfora Educacional. 2005. World Wide Web. Available in http://www.valdeci.bio.br/revista.php.

Souza CRT, et al. (2023). Redes Sociais e educação: mapeando possibilidades. In :Ribeiro, José Carlos, Thais, Miranda, Soares, Ana Terse (orgs) Práticas interacionais em rede. (ISBN 978-85-232-1215-5). EDUFBA, Salvador. p.215-233, 2014. Acess sin March, 2023.

Souza CRT (20013). In Situ: Orientações Pedagógicas. Grupo de Pesquisa Comunidades Virtuais (GPCV). Universidade do Estado da Bahia (UNEB). Salvador, 2013. Acess in April 4, 2023.

Teixeira KRS, et al. (1998). Descontaminação de substâncias tóxicas: fenol e brometo de etídio. Embrapa. Comunicado técnico. Nº 25, p. 13. 1998. Acess in March 27, 2023.

Tortora GR, et al. (2019). Microbiology: An Introduction. 8 ed. Menlo Park, 2019.

