RESEARCH ARTICLE OPEN ACCESS

Verification of potential herbicide-biodegradation by two strains of *Bacillus safensis* recovered from sugarcane cultivation soil

Carol da Costa Schulze*, Thyago Henrique Cavalcante de Sousa*, Rafael Limongi*, Andrea Farias de Almeida**, Thiago Gonçalves Cavalcanti*, Ulrich Vasconcelos*

**(Laboratório de Microbiologia Ambiental, CBIOTEC, Universidade Federal da Paraíba, Campus I, CEP- 58051-900, João Pessoa-PB*
*** (Laboratório de Engenharia de Bioprocessos, CBIOTEC, Universidade Federal da Paraíba, Campus I)*
Corresponding Author: Carol Da Costa Schulze

ABSTRACT
Herbicides are phytotoxic substances ostensibly used in agriculture and responsible for significant impacts on the soil, posing a risk to many organisms. This makes it urgent to find strategies to remove or deactivate these compounds. The aim of this work was to verify the biodegrading potential of two wild *Bacillus safensis* strains, recovered from sugarcane cultivation soil. After acclimation, the strains were inoculated into soils contaminated with 50 $mg.Kg^{-1}$ of ametryn (AMT) or hexazinone (HZN) and left for 60 days. Bioaugmention and biostimulation were associated by the addition of fertilizer and lipopeptide. Herbicide degradation was evaluated by the determination of CO_2 concentration ($mg.g^{-1}$ of soil) and pH monitoring. Both strains presented similar results. In reactors containing HZN, the maximum gas production reached about 1600 $mg.g^{-1}$, while in reactors containing AMT, the production approached 800 $mg.g^{-1}$, suggesting a higher recalcitrance of the compound. The best conditions were in the reactors to which lipopeptide and fertilizer had been added, compared to those where only lipopeptide was present. The germination index in two plants demonstrated that the strain degraded the herbicides, pointing to *Cucumis anguria* as the best indicator of toxicity.
Keywords – Ametryn, Bioremediation, Hexazinone, Substrate-induced respiration test

---

Date of Submission: 01-01-2020 Date of Acceptance: 16-01-2020

I. INTRODUCTION

Herbicides are phytotoxic substances used to eliminate or inhibit the growth of unwanted plants, especially in agriculture, where weeds commonly hinder production [1]. Although there are no reliable studies on global weed damage to agriculture, it is estimated that weeds account for more than 45% of crop loss, exceeding any group of agricultural pests, including insects, diseases, rodents, worms and other pests [2]. The indiscriminate use of herbicides has led to soil and water contamination, with toxic effects on the trophic chain throughout different systems [3, 4]. Besides, there is the fact that these compounds have carcinogenic potential or endocrine action [5, 6].

Herbicides are classified according to target, application and mechanism of action [7]. Most herbicides act by blocking the synthesis of aromatic amino acids [8], forming large quantities of reactive oxygen species [9] as well as inhibiting photosynthesis [10], to which triazines are classified.

These compounds are employed in sugarcane cultivation and act by blocking weed photosystem II [11].

In Brazil, sugarcane cultivation is one of the most important agribusinesses, making the country the world's largest producer of sugar and ethanol [12]. The Brazilian Northeast represents 30% of the country's sugarcane production and, in this region the amount of triazine consumption exceeded 10,000t in the first decade of the 21st Century, making it the third class of most used herbicides [13]. As a consequence of their wide-spread use over decades, these compounds are absorbed and accumulated in soil. Given their chemical nature, they are resistant to biodegradation. This causes high-grade impacts on the soil and also contamination to groundwater and surface water bodies as the compounds are carried by rainwater [14].

Several methods have been proposed for removing herbicides from the environment, but their cost is high, such as ozonolysis and use of catalysers [15-17]. Bioremediation stands out as one of the best strategies for soil herbicide removal, especially for

its low cost and greater public acceptance [18]. The association of bioaugmentation to biostimulation is one of the best options when the contaminants are recalcitrant. The indigenous microbiota may be enhanced by adding a myriad of compounds, from fertilizers to cosubstrates [19].

*Bacillus safensi*s is a Gram-positive, aerobic, mesophilic, chemoheterotrophic and spore-forming bacterium. Cells are 0.5-0.7 µm in diameter, 1-1.2 µm wide. Optimum growth conditions are at 28ºC [20]. The species was described in the early 21st Century and was isolated by NASA's Spacecraft Assembly Facility (SAF). It is characterized by its high adaptation to extreme conditions, including ultra-clean rooms, saline deserts, oil contaminated environments and industrial effluents. Additionally, the microbe exhibits several enzymes of industrial interest, such as amylases, cellulases, proteases, lipases, xylanases, chitinases, inulinases and keratinases [21].

The potential of *B. safensis* to degrade recalcitrant compounds has been described in some studies, for example, in insecticide assimilation as a carbon source [22], for nickel removal [23] and in bioremediation [20]. The present work aimed to evaluate the biodegrading potential of two strains of *Bacillus safensis* recovered from sugarcane cultivation soil against two triazine herbicides.

II. MATERIAL AND METHODS

2.1 Herbicides

Commercial preparations of herbicides, ametryn AMT (ALTA, Curitiba, Brazil) and hexazinone-D, HZN (Nortox, Arapongas, Brazil) were purchased from commercial establishments and handled according to the manufacturer's instructions.

2.2 Microorganisms and acclimation to herbicides

Two strains of *Bacillus safensis*, C1AC5507-1 and C1C5502-1, isolated from sugarcane cultivation soil in a rural region of Santa Rita, state of Paraíba, Brazil, were used. The strains are registered in the National System of Genetic Heritage Management and Associated Traditional Knowledge (SisGen) under #A06A1E6.

The acclimation was carried out by transferring 0.5 mL of freshly grown nutrient broth into flasks containing a minimal mineral medium consisting of: K_2HPO_4 (0.5 $g.L^{-1}$), $(NH_4)_2SO_4$ (0.5 g,L^{-1}), $MgSO_4$ (0.5 $g.L^{-1}$), $FeCl_2$ (10 $mg.L^{-1}$), $CaCl_2$ (10 $mg.L^{-1}$), $MnCl_2$ (0.1 $mg.L^{-1}$) and $ZnSO_4$ (0.01 $mg.L^{-1}$) [24], added to 0.1 mL of the diluted herbicide solution (AMT 1% v/v or HZN 0.5 $g.L^{-1}$). The concentration of herbicides was gradually increased up to 20 $mg.L^{-1}$. All flasks were incubated at 30°C for 5 days. Cell viability of the strains in the presence of herbicides was identified by the visual inspection of the turbidity [25].

2.3 Soil

A sandy soil without fertilizer and herbicide contamination was used, the characteristics of which are summarized in Table 1.

Table 1 – Physical and chemical characteristics of the soil

Analysis	**Result**	**References**
Texture		[26]
Clay	0.99%	
Silt	1.29%	
Sand	95.86%	
Gravel	1.86%	
Total Organic Carbon	0.74 $mg.kg^{-1}$	[27]
Total nitrogen	31.80 $mg.kg^{1}$	[28]
Total phosphorus	3.04 $mg.kg^{1}$	[29]
pH	7.35±0.01	[30]
Humidity	0.30±0.01%	[30]

2.4 Biodegradation test

The test was carried out for 60 days in sealed polyethylene reactors with 500 mL capacity, filled with 250 g of sterile soil which was contaminated with AMT or HZN (50 mg of active ingredient per kg of soil). After homogenization, a vessel containing 25 mL of 0.5 $mol.L^{-1}$ NaOH solution was laid on the soil surface in the reactor. The degradation of AMT and HZN was evaluated under an association of bioaugmentation (addition of *B. safensis*) and biostimulation, i.e., by adding 100 $mg.Kg^{-1}$ of soil fertilizer NPK 10:10:10 (Vitaplan, Captain Leônidas Marques, Brazil), associated with the addition of 0.1 $g.L^{-1}$ lipopeptide. There were two control reactors: soil without the association of biostimulation and the abiotic control, prepared by adding a 10% (w/v) $AgNO_3$ [31].

The inoculum suspension in a minimum mineral medium was prepared from acclimatized strains, corresponding to 10% of the soil mass in the reactors [19]. Soil pH was monitored using method 2.1.1 [26] in soil samples suspended in distilled water at a ratio of 2:5 after homogenization and 30-minute rest.

2.5 Substrate-Induced Respiration test (SIR)

Herbicide degradation was indirectly identified by determining the CO_2 concentration every 10 days of the bioprocesses. The vials containing 0.5 $mol.L^{-1}$ NaOH were removed and replaced with new ones. Immediately, the solution was treated with 2 mL of 0.5 $mol.L^{-1}$ $BaCl_2$ solution and drops of 2% phenol red. The mixture was then titrated with 0.5 $mol.L^{-1}$ HCl until it became

colourless. The CO_2 concentration in $mg.g^{-1}$ was calculated by applying the equation:

$$CO_2 = (44 \times V_0\text{-}V_1) \div m$$

Where V_0= volume of acid spent in control soil, V_1= volume of acid spent during treatment, and m= mass in kg of reactor soil [32].

2.6 Phytotoxicity test

Soil samples taken at the beginning and the end of treatment were evaluated to determine the germination index (GI) of *Lactuca sativa* and *Cucumis anguria* seeds. The seeds were washed with distilled water and dried on filter paper. A total of ten seeds per plant were placed on filter paper in petri dishes. Then the filter paper was soaked with 8 mL of the filtered soil sample extract, prepared with 10 g of soil and 90 mL of distilled water. The control of the test was performed with distilled water. The plates were incubated at 22°C for five days. After, the GI was calculated using the equation:

$$GI = [(S_1 \times R_1) \div (S_2 \times R_2)] \times 100$$

Where S_1 and S_2= number of seeds germinating in treatment and control, respectively and R_1 and R_2= average root lengths in treatment and control [33].

III. RESULTS

Fig. 1 and 2 show the average accumulated CO_2 production over 60 days of bioprocess.

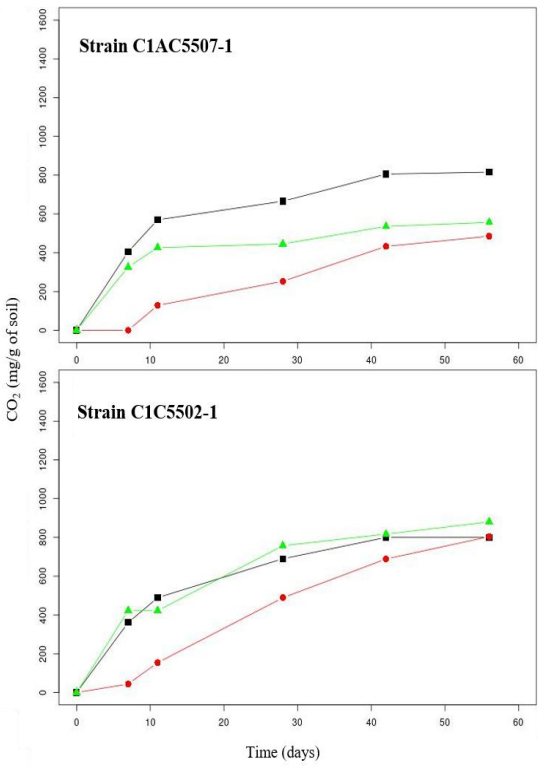

Fig. 1. Average cumulative CO_2 production by *Bacillus safensis* strains in AMT-contaminated reactors: lipopeptide (black), fertilizer (red) and lipopeptide associated with fertilizer (green)

Both strains emitted significant quantities of gas. Comparatively, a higher CO_2 production was observed in the reactors whose soil was contaminated by HZN, with maximum production of CO_2 ranging from 1000 to 1600 $mg.g^{-1}$ of soil. Gas emissions were about 50% lower in reactors whose soil was contaminated with AMT. Maximum CO_2 concentrations ranged from approximately 400 to 800 $mg.g^{-1}$ of soil under all experimental conditions, suggesting a greater recalcitrance from AMT.

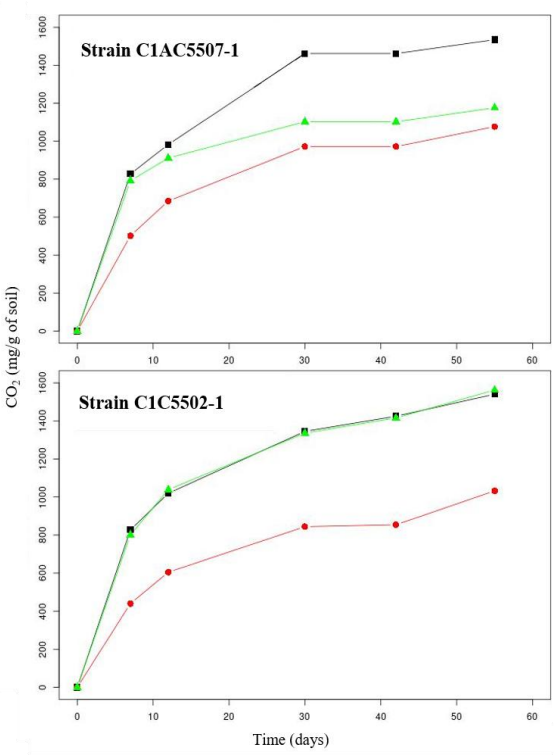

Fig. 2. Average cumulative CO_2 production by *Bacillus safensis* strains in HZN-contaminated reactors: Lipopeptide (black), fertilizer (red) and lipopeptide associated with fertilizer (green)

The results also revealed that there were differences between the treatments isolated with fertilizer and lipopeptide, compared to that obtained with the combination of the two treatments. Among all, the gas production in the reactors with fertilizer addition had the lowest values. However, in reactors whose soil was contaminated with AMT, the difference was less evident for the fertilizer variable.

The highest values of gas emission were obtained in the reactors with lipopeptide addition. However, the combination of this treatment with the fertilizer guaranteed similar results between the C1C5502-1 and C1AC5507-1 strains, respectively, against AMT and HZN.

During the test the pH varied on average in 7.2±0.1 in the reactors containing lipopeptide and in 5.0±0.1 in reactors containing fertilizers. With respect to ecotoxicity tests, Table 2 summarizes the results.

Table 2. Seed germination index in soil extracts

Strain	Contaminant and treatments	Plants	
		L. sativa	***C. anguria***
CIAC5507-1	**AMT**		
	No treated soil	80.3±0.1	81.3±0,8
	Addition of lipopeptide	125.8±0.1	117.0±0,8
	Addition of fertilizer	98.6±0.1	143.2±0,8
	Lipopeptide + fertilizer	112.9±0.1	131.4±0,8
	HZN		
	No treated soil	84.2±0.1	83.7±0,8
	Addition of lipopeptide	71.5±0.1	125.7±0,8
	Addition of fertilizer	87.7±0.1	107.3±0,8
	Lipopeptide + fertilizer	111.7±0.1	106.1±0,8
CIC5502-1	**AMT**		
	No treated soil	80.3±0.1	81.3±0,8
	Addition of lipopeptide	121.0±0.1	77.0±0,8
	Addition of fertilizer	128.0±0.1	133.0±0,8
	Lipopeptide + fertilizer	105.0±0.1	102.0±0,8
	HZN		
	No treated soil	84.2±0.1	83.7±0,8
	Addition of lipopeptide	87.5±0.1	84.2±0,8
	Addition of fertilizer	105.1±0.1	85.9±0,8
	Lipopeptide + fertilizer	102.0±0.1	99.7±0,8

According to the classification proposed by literature [34], GI can be subjectively classified as high (GI <50%), moderate (50% <GI <80%), or null (GI> 80%). Both herbicides at the initial concentration of 50 mg of the active principle per kg of soil, were not toxic, even though they were close to moderate toxicity. After treatment, the rates increased significantly.

For *Lactuca sativa* and *Cucumis anguria*, the latter proved to be more sensitive in the test, especially when analysing the GI obtained from the CIC5502-1 treatment in HZN-contaminated soils. In contrast, GI after treatment in AMT-contaminated soils were mostly above 100%, corroborating with SIR test data, even with lower gas emissions than those obtained in reactors containing HZN as a contaminant.

IV. DISCUSSION

Herbicidal photosystem II blockers have been the most commonly used crop protection products since simazine was first introduced to the market in 1956 [35]. The triazines belong to this class of herbicides and their action occurs when they irreversibly bind to the D1 subunit of photosystem II, blocking the binding of plastoquinone, preventing electron transport as well as the photosynthesis process, resulting in the death of weeds [36].

AMT (triazine) and HZN (triazinone) are classified by the Brazilian regulatory council as moderately toxic in the case of acute exposure. For chronic exposure, both herbicides may promote biomagnification with consequences not yet fully understood [37-39]. Both compounds were the most used triazines, over the 14 years prior to the isolation of strains in the sugarcane soil used in this study.

Removal of persistent and/or recalcitrant compounds may require the use of a cosubstrate, i.e., a better assimilated secondary source of carbon, which, along with its uptake, consumes fewer biodegradable compounds and may be consumed as a carbon and energy source by the microbes [40]. A previous study by our research group on the use of *Pseudomonas aeruginosa* to remove hydrocarbons from petroleum in soil [19] reported a value closer to the accumulated gas concentration over 30 days of processing, in comparison to this study. On the other hand, it was reported a maximum value of 35 $mg.Kg^{-1}$ over the same period during organic waste mineralization, 5 times lower than that observed with *B. safensis* [32].

This underscores the potential of the two *B. safensis* strains evaluated, especially since there was no addition of cosubstrates. However, it is noteworthy that from the 30^{th} day, the CO_2 production reduced and, graphically, the curve was similar to that observed in a regular microbial stationary phase. This suggests an inhibition of cells, especially by the accumulation of toxic metabolites [41, 42]. This caused us to think that, regarding the addition of a cosubstrate, the maximum accumulated CO_2 might have been higher than recorded.

An interesting fact was observed in the herbicide biodegradation test. The maximum concentration of CO_2 obtained in the reactors with AMT-contaminated soil was about half of that reached by the two strains in the reactors containing HZN-contaminated soil. In these reactors, this level,

about 800 $mg.g^1$, was reached around the 10^{th} day of the bioprocess, in the reactors where the association of lipopeptide and fertilizer was used, suggesting greater bioaccessibility to HZN compared to AMT.

The degrading potential of microorganisms can be reproducibly determined by SIR. Introduced by Anderson and Domsch [43], the methodology can estimate cell biomass in a sealed container by measuring CO_2 inside and the results compared to CO_2 obtained abiotically. The correlation of CO_2 over abiotic levels and cell mass is also helpful in confirming and monitoring cell activity.

The variation in pH in a bioprocess is also an important indication of the use of xenobiotics by microbial agents. An acidic environment is not suitable for some *B. safensis* strains [20], which is possibly the reason for the lower CO_2 production in soil treatments with fertilizer. In addition, reactors enriched with chemical fertilizer may suffer a significant change in the C:N ratio in the medium. The concentration of nitrogen, however, was not inhibitory, considering that the soil already contained a significant amount of nitrogen and an initial C:N ratio about 10:3.

The increase of GI after treatments may help in the understanding that the herbicides were consumed by the *B. safensis* strains. In this test, *C. anguria* was the plant most sensitive to the metabolites. Similar results have been also reported in the literature [44, 45]. The increase in GI suggests the presence of less toxic metabolites than the initial contaminant, and corroborate with the values obtained from the CO_2 concentration, as well as the pH reduction in the reactors. The higher plant toxicity does not appear to be due to lipopeptide, since in the reactors in which it was present, the GI were higher than those determined at the beginning of the test. Additionally, the lipopeptide was produced by the strain itself. This is similar to surfactin, a bioactive molecule produced by *Bacillus safencis* [46] and other species of *Bacillus* [47].

It is important to say that since AMT appeared to be more recalcitrant or even less available to *B. safensis* strains, we expected a greater amount of less toxic metabolites to be produced during HZN consumption, thus justifying the results obtained in GI. On the other hand, the 60-day bioprocess may not have been sufficient for AMT degradation, considering that both strains were perfectly acclimated to both compounds.

V. CONCLUSION

SIR test contributed to indicate the potential use of AMT and HZN as a carbon and energy source by the *Bacillus safensis* C1AC5507-1 and C1C5502-1 strains, especially when the association between bioaugmentation and biostimulation was used. The treatment resulted in high CO_2 concentrations, especially in the HZN-contaminated soils, posteriorly reflected in the germination of *L. sativa* and *C. anguria*. The latter proved to be the most sensitive plant in the ecotoxicity test. The results indicated that both strains showed potential for use in future studies, in the elaboration of bioremediation protocols of soils contaminated with AMT or HZN, as well as to possible other herbicides of similar chemical classes.

ACKNOWLEDGEMENTS

Financial support was provided by the National Council for Scientific and Technological Development (CNPq), grants #40766/2013-3 (Edital MCTI/CNPq/FNDCT - Ação Transversal - Redes Regionais de Pesquisa em Ecossistemas, Biodiversidade e Biotecnologia n° 79/2013).

The English text of this paper has been revised by Sidney Pratt, Canadian, MAT (The Johns Hopkins University), RSAdip - TESL (Cambridge University).

REFERENCES

[1]. Barbosa FP, Vieira EG, Cumba IR, Paim LL, Nakamura APR, Andrade RDA, Carmo DR. (2019). Voltammetric techniques for pesticides and herbicides detection – an overview. International Journal of Electrochemistry Science, 14: 3418 – 3433, doi: 10.20964/2019.04.60.

[2]. Abouziena HF, and Haggag WM. Weed contron in clean agriculture. A review. (2016). Planta Daninha, 34(2): 377-392.

[3]. [3]Albanito L, Lappano R, Madeo A, Chimento A, Prossnitz ER, Cappello AR, Dolce V, Abonante S, Pezzi V, Maggiolini M. (2015). Effects of atrazine on estrogen receptor and G protein–coupled receptor 30– mediated signaling and proliferation in cancer cells and cancer-associated fibroblasts. Environmental Health Perspectives, 123(5): 493–499.

[4]. Grillo R, Santos NZP, Maruyama CR, Rosa AH, de Lima R, Fraceto LF. (2012). Poly(ε-caprolactone) nanocapsules as carrier systems for herbicides: physico-chemical characterization and genotoxicity evaluation. Journal of Hazardous Materials, 231-232: 1-9. doi:10.1016/j.jhazmat.2012.06.019.

[5]. Delancey JO, Alavanja MCR, Coble J, Blair A, Hoppin JA, Austin HD, Freeman LEB. (2009). Occupational exposure to metribuzin and the incidence of cancer in the agricultural health study. Annals of Epidemiology, 19(6): 388–395.

[6]. Maclennan PA, Delzell E, Sathiakumar N, Myers SL, Cheng H, Grizzle W, Chen V, Wu XC. (2002). Cancer incidence among triazine herbicide manufacturing workers. Journal of Occupational and Environmental Medicine, 44(11): 1048.

[7]. Gupta PK. Toxicity of herbicides (2012). doi: 10.1016/B978-0-12-385926-6.00065-X.

[8]. Schonbrunn E, Eschenburg S, Shuttleworth WA, Schloss JV, Amrhein N, Evans JNS, Kabsch W. (2001). Interaction of the herbicide glyphosate with

its target enzyme 5-enolpyruvylshikimate 3-phosphate synthase in atomic detail. Proceedings of the National Academy of Sciences, 98(4): 1376–1380.

[9]. Youngman RJ, and Dodge AD. (2014). Mechanism of paraquat action: inhibition of the herbicidal effect by a copper chelate with superoxide dismutating activity. Zeitschrift für Naturforschung C, 34(11): 1032–1035.

[10]. Barry P, Young AJ, Britton G. (1990). Photodestruction of pigments in higher plants by herbicide action: The effect of DCMU (diuron) on isolated chloroplasts. Journal of Experimental Botany, 41(2): 123–129.

[11]. Silva KS, Freitas FCL, Braga DF, Dombroski JLD, Santos AFB. (2016). Ametryn leaching in soils from the sugarcane region in Northeastern Brazilian. Planta Daninha, 34(4): 839-848.

[12]. Bordonal RO, Carvalho JLN, Lal R, Figueiredo EB, Oliveira, BG, La Scala Jr, N (2018). Sustainability of sugarcane production in Brazil. A review, Agronomy Sustain Dev, 38:13. doi:10.1007/s13593-018-0490-x.

[13]. Azevedo DA, Silva TR, Knoppers BA, Schulz-Bull D. Triazines in the tropical lagoon system of Mundau-Manguaba, NE-Brazil. (2010). Journal of Brazilian Chemistry Society, 21(6): 1096-1105.

[14]. Ferenczi J, Ambrus A, Wauchope RD, Sumner HR. (2002). Persistence and runoff losses of 3 herbicides and chlorpyrifos from a corn field in the Lake Balaton watershed of Hungary. Journal of Environmental Science and health B, 37(3):211-224.

[15]. Cacho C, Turiel E, Martín-Esteban A, Ayala D, Pérez-Conde C. (2006). Semi-covalent imprinted polymer using propazine methacrylate as template molecule for the clean-up of triazines in soil and vegetable samples. Journal of Chromatography A, 1114(2): 255–262.

[16]. Robert D, and Malato S. (2002). Solar photocatalysis: a clean process for water detoxification. Science of The Total Environment, 291(1): 85–97.

[17]. Muller TS, Sun Z, Kumar G, Itoh K, Murabayashi M. (1998). The combination of photocatalysis and ozonolysis as a new approach for cleaning 2,4-dichlorophenoxyaceticacid polluted water. Chemosphere, 36(9): 2043–2055.

[18]. Vidali M. (2001). Bioremediation: An overview. Pure and Applied Chemistry, 73(7): 1163–1172.

[19]. Cavalcanti TG, Souza AF, Ferreira GF, Dias DSB, Severino LS, Morais JPS, Sousa KA, Vasconcelos U. (2017). Use of agro-industrial waste in the removal of phenanthrene and pyrene by microbial consortia in soil. Waste and Biomass Valorization. doi:10.1007/s12649-017-0041-8.

[20]. Lateef A, Adelere IA, Gueguim-Kana EB. (2015). The biology and potential biotechnological applications of *Bacillus safensis.* Biologia, 70(4): 411–419.

[21]. Satomi M, Duc MTL, Venkateswaran K. (2006). *Bacillus safensis* sp. nov., isolated from spacecraft and assembly-facility surfaces. International Journal of Systematic and Evolutionary Microbiology, 56(8): 1735–1740.

[22]. Abdelbagi AO, Wady AIA, Ishag AESA, Hammad AMA, Abdalla MAO, Hur J-H. (2018). Biodegradation of fenthion and temphos in liqud media by *Bacillus safensis* isolated from pesticides polluted soil in the Sudan. African Journal of Biotechnology, 17(12): 396–404.

[23]. Motesharezadeh B, and Savaghebi-Firoozabadi GR. (2011). Study of the increase in phytoremediation efficiency in a nickel polluted soil by the usage of native bacteria: *Bacillus safensis* FO.036B and *Micrococcus roseus* M2. Caspian Journal of Environmental Sciences, 9(2): 133–143.

[24]. Palittapongarnpim M., Pokethitiyook P., Upatham ES, Tangbanluekal L. (1998). Biodegradation of crude oil by soil microorganisms in the tropic. Biodegradation, 9(2): 83–90.

[25]. Del'Arco JP, and França FP. (1999). Biodegradation of crude oil in sandy sediment. International Biodeterioration and Biodegradation, 44(2): 87–92.

[26]. ABNT. NBR 7181. Solo – análise granulométrica. Rio de Janeiro: ABNT, 1984.

[27]. USEPA. Method 9060. Total organic carbon. Washington: US government printing office, 1996.

[28]. USEPA. Method 351.2. Determination of total Kjeldahl nitrogen by semi-automatic colorimetric. Washington: US government printing office, 1993.

[29]. USEPA. Method 365.3. Phosphorus, all forms (colorimetric, ascorbic acid, two reagents). Washington: US government printing office, 1978.

[30]. EMBRAPA. Manual de métodos de análise de solo. Rio de Janeiro: SNLCS, 1979.

[31]. Vasconcelos U, de França FP, Oliveira FJS. (2011). Removal of high-molecular weight polycyclic aromatic hydrocarbons. Química Nova, 34(2): 218-221.

[32]. Severino LS, Costa FX, Beltrão NEDM, Lucena AMA, Guimarães MMB. (2005). Mineralização da torta de mamona, esterco bovino e bagaço de cana estimada pela respiração microbiana. Revista de Biologia e Ciências da Terra, 5(1): 15-22.

[33]. Tiquia SM, Tam NFY, Hodgkiss IJ. (1996). Effects of composting on phytotoxicity of spent pig-manure sawdust litter. Environmental Pollution, 93(3): 249–256.

[34]. Anastasi A, Coppola T, Prigione V, Varese GC. (2009). Pyrene degradation and detoxification in soil by a consortium of basidiomycetes isolated from compost: role of laccases and peroxidases. Journal of Hazardous Materials, 165(1): 1229–1233.

[35]. Heri W, Carroll B, Parshley T, Nabors JB. Production, development, and registration of triazine herbicides. In: the triazine herbicides. San Diego: Elsevier, 2008.

[36]. Oettmeier W. (1999). Herbicide resistance and supersensitivity in photosystem II. Cellular and Molecular Life Sciences, 55(10): 1255–1277.

[37]. Moura MAM, and Jonsson CM. (2016). Acute toxicity of mixture of sugarcane herbicides to tilapia fingerlings. Ecotoxicology and Environmental Contamination, 11(1): 15-20.

[38]. ANVISA. A11 Ametrina, 2006.

[39]. ANVISA. H02 Hexazinona, 2006.

[40]. Baboshin M, Finklstein ZI, Golovleva LA. (2003). A fluorene cometabolism by *Rhodococcus rhodochrous* and *Pseudomonas fluorescens* cultures. Microbiology, 72(2): 194-198.
[41]. Ziegler S, Waidner B, Itoh T, Schumann P, Spring S, Gescher J. (2013). *Metallibacterium scheffleri* gen. nov., sp. nov., an alkalinizing Gammaproteobacterium isolated from an acidic biofilm. International Journal of Systematic and Evolutionary Microbiology, 63(4): 1499–1504.
[42]. Watson JS, Jones DM, Swannell RPJ, Duin ACT. (2002). Formation of carboxylic acids during aerobic biodegradation of crude oil and evidence of microbial oxidation of hopanes. Organic Geochemistry, 33(10): 1153–1169.
[43]. Anderson JPE, and Domsch KA. (1978). A physiological method for the quantitative measurement of microbial biomass in soils. Soil Biology and Biochemistry, 10(3): 215–221.
[44]. Dourado RA, Guedes TP, Bonifácio TTC, Cavalcanti TG, Travassos RA, Vasconcelos U. (2017). Determination of microbial contaminants recovered from Brazilian petrol stations. Revista Mexicana de Inginería Química, 16(3): 984-991.
[45]. Cavalcanti TG, Viana AAG, Guedes TP, Freire ADS, Travassos RA, Vasconcelos U. (2016) Seed options for toxicity tests in soils contaminated with oil. Canadian Journal of Pure and Applied Sciences, 10(3): 4039–4045.
[46]. Abdelli F, Jardak M, Elloumi J, Stien D, Cherif S, Mnif S, Aifa S. Antibacterial, anti-adherent and cytotoxic activities of surfactin(s) from a lipolytic strain *Bacillus safensis* F4. (2019). Biodegradation, 30(4): 287-300.
[47]. Rocha MVP, Barreto RVG, Melo VMM, Gonçalves RLB. (2009). Evaluation of cashew apple juice for surfactin production by *Bacillus subtilis* LAMI008. Applied Biochemistry and Biotechnology, 155(1-3): 63-75.

Carol da Costa Schulze, et al. "Verification of potential herbicide-biodegradation by two strains of Bacillus safensis recovered from sugarcane cultivation soil" *International Journal of Engineering Research and Applications (IJERA*), vol.9(12), 2019, pp 54-60.