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Assessment of the quality of soil polluted by toxic wastes after bioremediation process (Abidjan, Côte d'Ivoire)

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Abstract

The subsistence of humans and livestock depends on the protection of the soil. As a result, soil pollution by waste toxic elements is a major concern for humanity. The objective of this study is to examine the source distribution, concentration levels and spatial distribution of toxic elements and microorganisms in previously polluted soils in the district of Abidjan, Côte d'Ivoire. The total number of topsoil samples was 13 and was analyzed using ICP-OES. Heavy metals concentrations determined in soil were higher than those of control soil at all stations, but were generally less than Ivorian standard values. For organic compounds and hydrocarbons, only total sulfur exceeded Ivorian standards at all sampling sites (i.e. 1340, 1320 and 190 mg/kg for Site 1, site 2 and control of Alépé 1, respectively, while those of site 1, site 2 and control of Alépé 2 were 1720, 1630 and 190 mg/kg, contrary to 10 x 10^{-3} mg/kg as standard values). Four types of microorganisms were detected within differents stations and sampling sites, i.e. Heterotrophic bacteria, *Pseudomonas sp.*, *Bacillus sp.*, *Escherichia coli* and Intestinal enterococci in remediated and control soils. Finally, these results confirm the positive impact of remediation with the possibility to reuse these sites for agriculture or other purposes.

1. Introduction

Soil pollution occurs when some natural or anthropogenic constituents exceed the maximum allowable limits in natural soil environments [1]. Chemical pollutants are often discharged into environment, increasing concentration of toxic chemicals in soil, air and aquatic ecosystem. It is well known that these chemical pollutants (petroleum hydrocarbons; heavy metals; pesticides and solvents) may affect humans and also others animals living in these matrixes [2,3]. Most of these chemical pollutants are xenobiotic coming from industrials process residues and discharged through accidental pollution. Once present in these matrixes, they represent potential health risks for living organisms at low or high concentrations depending on the type of the pollutant. Among these consequences, the

appearance of chronic diseases (e.g. cancer) in humans and animals, thereby leading to their death [4]. However, the management of the toxic pollutants is very complex and depends on the type and also the matrix concerned. In the case of chronic pollution, consequences are observed after a long time of exposure except the molecules that exhibited high level of toxicity causing instantaneous death [5]. The accumulation and the transfer of the toxic molecules in the environment (water, air and soil), as well as in human bodies after exposure are difficult to monitor. Then, the most common strategies used to detect and warn of potentially detrimental and hazardous events is the surveillance of the different matrixes by regular monitoring [6]. Then, the most common strategies used to detect and warn of potentially detrimental and hazardous events is the surveillance of the different matrixes by regular monitoring [7]. In spite of regular monitoring studies and the various laws limiting the release of toxic pollutants into the environment, there are always chronic or accidental pollutions [8]. To improve monitoring, several methods have been used, but they are costly. For this reason, some micro-organisms are often used as indicators to monitor aquatic systems and soils. These indicators can help detect change in an environment and indicate potential causes of pollution. They are used in the treatment of soils and waters through bioremediation processes that have shown acceptable efficiencies [9]. Recently in Côte d'Ivoire, the oil tanker Probo Koala spilled hundreds of tons of toxic waste at numerous sites in the city of Abidjan. Soil and water courses around the district of Abidjan were impacted by this accidental chemicals pollution [10]. In the days and weeks that followed, thousands of people showed signs of poisoning. Analysis of the waste revealed the presence of toxic chemicals such as mercaptans and hydrogen sulfide. A soil bioremediation technology was conducted to ensure the physical, chemical and biological quality. Also, in order to proceed with a wide dissemination of information on the impact of the soil treatment technology, an assessment of the soil quality which were used different parameters were implemented. This study aims to examine previously polluted soils in two places (i.e. Alépé 1 and Alépé 2) and one bioremediation site (Erymakoudjé) in order to understand the effect of depollution. Especially, this involves identifying the micro-organisms which are bio-indicators of pollution, as well as the heavy metal content and some chemical components of the soil.

2. Material and methods

2.1 Sampling sites

Three sampling sites were selected, namely Alépé 1 (located Route d'Alépé) and Alépé 2 (located Route d'Alépé) with the respective UTM coordinates of 369699 / 650953, 392237 / 603155, 392367 / 603543. These sites were chosen because they have been firstly impacted by the pollution and also, they were used as monitoring sites by United Nations (UN) for environment (2017) in their evaluation study performed in order to determine their ability to be considered as safe sites after an additional clean-up process realized from 2008 to 2010. After the pollution of these sites in 2006, some sites were excavated in order to bring their soils onto the site of Alépé 1 for bioremediation. A part of maize field stored in these sites during this period was considered as potentially contaminated and was transferred to Erymakoudjé site for treatment. A total of 13 samples of soil were taken over the period from 31 May to 02 June 2018 for microbiological analyses and also for the macro-invertebrate's index evaluation. Soil samples are taken at one depth (i.e. 0.2 m) in the concerned sites. Also, a control site, which has not received any toxic waste, were selected upstream of the spill sites for comparison purposes. Then, for microbial index, fecal indicator bacteria, heterotrophic bacteria count, total bacteria count and were analyzed based on the references proposed international standards (ISO standards).

2.2 Chemical parameters analysis

Several chemical parameters were determined in the soil. Organic components, hydrocarbons and heavy metals were analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES, Agilent 700 series). Indeed, 10 g of soil were digested using 60 mL of HNO_3 and 20 mL of concentrated H_2O_2 (30 %, V/V) using a microwave digestion system for 25 min and then, diluted to 100 mL with 2 % HNO_3. Blank preparation was done in the same way. Finally, clear liquid samples were analyzed by using ICP-OES [11, 12].

2.3 Microbial index evaluation

Soils contain a large diversity of bacteria whose presence and abundance can help to understand biological processes and mechanisms in soils. Three characteristic groups of germs were investigated in this study by the culture method [13]. These are i) *E. coli* and intestinal Enterococci for soil contamination by fecal matter; ii) *Pseudomonas sp*., *Bacillus sp*., for the bioremediation process of soils due to their ability to degrade complex molecules; iii) heterotrophic bacteria, for the characterization of the mineralization processes of organic matter in soils. Quantification of microbial abundances was performed by the culture method. The culture method consists of revealing the presence and quantifying the abundance of bacterial microorganisms capable of growing on the selected culture media. Two types of culture media were used, namely "specific" culture media for the growth of particular germs and "non-specific" culture media allowing the simultaneous research of large groups of microorganisms. The culture method consists of 4 main phases: i) preparation of the culture media, ii) inoculation of the culture media, iii) incubation at temperatures ranging from 36 to 44°C for periods of 24 to 48 hours and iv) counting of the colonies and calculation of the concentrations. The culture method was followed by biochemical tests for identification and confirmation of colonies previously revealed by the culture method. The biochemical tests were performed using the API 20 E and API 20 NE gallery (Biomérieux). The first phase of the analysis of soil samples consisted in preparing stock solutions from the weighed soil mass. A sample of 1g of soil is resuspended in 10 mL of phosphate buffer. Then, cascade dilutions were performed to obtain diluted solutions with low concentrations of microorganisms. The dilution coefficients made varied from 10^{-1} to 10^{-8} using the stock solution. 1 mL samples are either filtered on a polycarbonate membrane (0,22 mm porosity) or spread (100 µL) on a specific culture medium (TBX agar, Slanetz Barley agar, Cetremide agar and Mossel agar) or on a non-specific culture medium (Plate Count Agar; EMB agar). After incubation, the desired colonies (according to the expected staining) are counted. The calculation of bacterial concentrations takes into account the number of colonies counted, the filtered or spread volume and the dilution factor. The results are expressed in colony forming units (CFU) /g soil.

2.4 Analyses and statistical tests

Tests of mean and variance were performed using R software to compare the difference between the microbial abundances observed at the control sites and the microbial abundances observed at the sites with additional sanitation.

3. Results and Discussion

3.1 Evolution of heavy metals in the soil

Figure 1 shows the heavy metals concentrations in soil at two stations (i.e. Alepe 1 and Alepe 2). Overall, heavy metals concentration were higher than those of the control at all stations. In addition, chromium and zinc represent the highest concentrations (See **Figure 1A and B**). However, all the

concentration obtained were lower than those of the Ivorian standard (i.e. 400 mg/kg for lead; 190 mg/kg for copper; 130 mg/kg for chromium; 20 mg/kg for cadmium; 37 mg/kg for arsenic; 140 mg/kg for nickel; 240 mg/kg for cobalt; 9000 mg/kg for zinc and 7 mg/kg for mercury) [14]. These results highlighted the efficiency effect of the remediation process carried out previously for metals [11]. Otherwise, the results of Adebiyi et al [11] revealed high concentrations of heavy metals in the soil containing hydrocarbons.

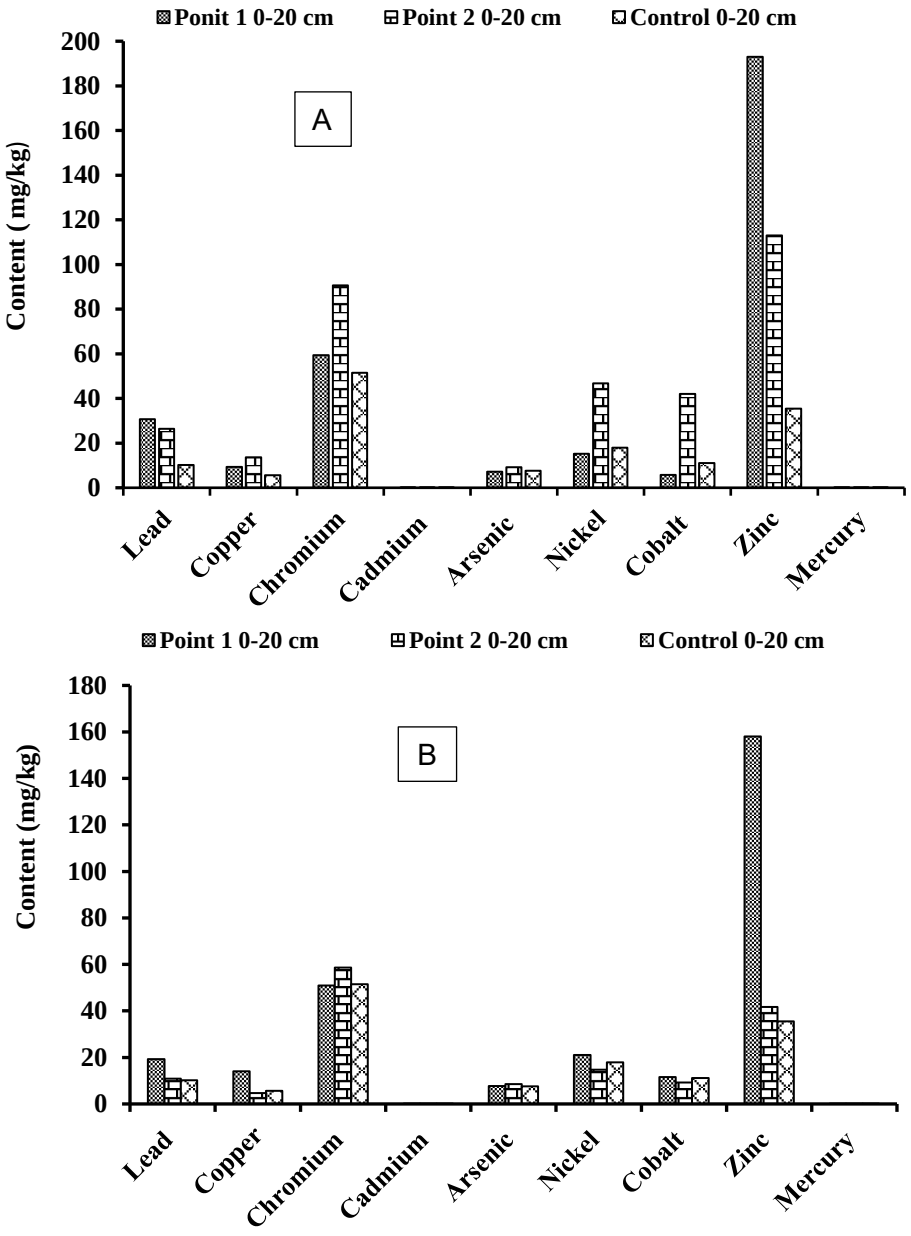

Figure 1. Content of heavy metals in the soil at two stations: A: Alepe 1; B: Alepe 2 at same depth.

3.2 Organic compounds and hydrocarbons

Table 1 presents the concentrations of organic compounds and hydrocarbons in the soil of the Alepe 1 station. Indeed, all the values obtained for the different compounds are lower than the Ivorian standard, except for total sulphur, whatever the type of soil and the sampling site (i.e. 1,340 mg/kg for site 1; 1,320 mg/kg for site 2; 190 mg/kg for the control, contrary to the 10 mg/kg as standard). These results showed that sulphur is one of the main components of the toxic waste [10].

Table 1. Concentrations of organic compounds and hydrocarbons within Alepé 1 station soil.

	Alepé 1			
	Point 1 0-20 cm	**Point 2 0-20 cm**	**Control 0-20 cm**	**Ivoirian reference value (10^{-3}mg/kg)**
Total Sulphur	1 340	1 320	190	10
Benzene	<0.5	<0.5	<0.5	1
Toluene	<0.5	<0.5	<0.5	5
Ethylbenzene	<0.5	<0.5	<0.5	25
Xylene	<0.5	<0.5	<0.5	5
2.2.3-Trimethylbutane	<0.5	<0.5	<0.5	1 000
2.3-Dimethylheptane	<0.5	<0.5	<0.5	1 000
4-Ethyloctane	<0.5	<0.5	<0.5	1 000
Dodecane	<0.5	<0.5	<0.5	1 000
Hexadecane	<0.5	<0.5	<0.5	1 000
Heptadecane	<0.5	<0.5	<0.5	1 000
Nonadecane	<0.5	<0.5	<0.5	1 000
Eicosane	<0.5	<0.5	<0.5	1 000
Heneicosane	<0.5	<0.5	<0.5	1 000
2.21-dimethyl Docosane.	<0.5	<0.5	<0.5	1 000
Tricosane	<0.5	<0.5	<0.5	1 000
Hexacosane	<0.5	<0.5	<0.5	1 000
2 Methylhexacosane	<0.5	<0.5	<0.5	1 000
Heptacosane	<0.5	<0.5	<0.5	1 000
11-Methylnonacosane	<0.5	<0.5	<0.5	1 000
Triacontane	<0.5	<0.5	<0.5	1 000
Dotriacontane	<0.5	<0.5	<0.5	1 000
Hexatriacontane	<0.5	<0.5	<0.5	1 000

Table 2 exhibits the concentrations of organic compounds and hydrocarbons in the soil of the Alepe 2 station. Indeed, all the values obtained for the different compounds were also lower than the Ivorian standard, except for total sulphur, regardless the type of soil and the sampling site (i.e. 1,720 mg/kg for site 1; 1,630 mg/kg for site 2; 190 mg/kg for the control, contrary to the 10 mg/kg as standard). These results from the Alepe 2 station denote that toxic waste contains a large quantity of sulphur that is adverse to humans [10].

3.3. Estimation of the bacteria abundance for soil quality

The abundances of heterotrophic bacterial determined in the soils collected in the stations of Alepé 1, Alepé 2 and Erymakoudjé are presented in the **Table 3.** In these stations, the abundance of total bacteria counts in the control sample and in the polluted samples area at the same depths (i.e. 0.2 m) were in the same order of magnitude in function of bacteria type. Statistical test performed in order to compare the control samples to those from contaminated area didn't show significant difference. The abundance of total bacteria count ranged between 10^7 CFU/g and 10^1 CFU/g [15]. This indicates that the abundances observed in this study are not particularly higher or lower than those expected in the soil. All of these stations showed normal soil flora, but varied by bacterial type. The abundance of heterotrophic bacteria in the control and bioremediation area samples were also of the same order of magnitude and there was no significant difference between these abundances per bacterium. Overall, the differences observed between control and supplemental cleanup sites for each of the sampling areas considered were not significant ($p < 0.05$) [16, 17].

Table 2. Concentrations of organic compounds and hydrocarbons within Alepé 2 station soil.

	Alepé 2			
	Point 1 0-20 cm	**Point 2 0-20 cm**	**Control 0-20 cm**	**Ivoirian reference value (10^{-3} mg/kg)**
Total Sulphur	1 720	1 630	190	10
Benzene	<0.5	<0.5	<0.5	1
Toluene	<0.5	<0.5	<0.5	5
Ethylbenzene	<0.5	<0.5	<0.5	25
Xylene	<0.5	<0.5	<0.5	5
2.2.3-trimethylbutane	<0.5	<0.5	<0.5	1 000
2.3-dimethylheptane	<0.5	<0.5	<0.5	1 000
4-Ethyloctane	<0.5	<0.5	<0.5	1 000
Dodecane	<0.5	<0.5	<0.5	1 000
Hexadecane	<0.5	<0.5	<0.5	1 000
Heptadecane	<0.5	<0.5	<0.5	1 000
Nonadecane	<0.5	<0.5	<0.5	1 000
Eicosane	<0.5	<0.5	<0.5	1 000
Heneicosane	<0.5	<0.5	<0.5	1 000
2.21-dimethyl Docosane	<0.5	<0.5	<0.5	1 000
Tricosane	<0.5	<0.5	<0.5	1 000
Hexacosane	<0.5	<0.5	<0.5	1 000
2-méthylhexacosane	<0.5	<0.5	<0.5	1 000
Heptacosane	<0.5	<0.5	<0.5	1 000
11-methylnonacosane	<0.5	<0.5	<0.5	1 000
Triacontane	<0.5	<0.5	<0.5	1 000
Dotriacontane	<0.5	<0.5	<0.5	1 000
Hexatriacontane	<0.5	<0.5	<0.5	1 000

Table 3. Microbial abundance in the soil of different stations in function of micro-organisms.

Micro-organisms	**Matrix**	**Alepé 1** (CFU/g)	**Alepé 2** (CFU/g)	**Erymakoudjé** (CFU/g)
Heterotrophic bacteria	Control (Depth 0.2 m)	4.20×10^7	10.2×10^7	10.8×10^7
	Soil (Depth 0.2 m)	9.40×10^7	2.30×10^7	4.80×10^7
Pseudomonas sp.	Control (Depth 0.2 m)	3.80×10^2	14.2×10^2	2.50×10^2
	Soil (Depth 0.2 m)	1.00×10^2	5.20×10^2	10.6×10^2
Bacillus sp.	Control (Depth 0.2 m)	30.0×10^3	4.40×10^3	1.44×10^3
	Soil (Depth 0.2 m)	19.6×10^3	23.0×10^3	7.10×10^3
Escherichia coli	Control (Depth 0.2 m)	1.29×10^3	4.00×10^3	10.7×10^3
	Soil (Depth 0.2 m)	2.70×10^3	-	16.0×10^3
Intestinal enterococci	Control (Depth 0.2 m)	2.10×10^2	-	1.10×10^1
	Soil (Depth 0.2 m)	1.30×10^2	-	5.60×10^3

Indeed, **Table 3** shows the abundance of Heterotrophic bacteria contrary of *Pseudomonas sp.*, *Bacillus sp.*, *Escherichia coli* and intestinal enterococci in remediated soils and the control. Heterotrophic bacteria concentrations range from 2.3 to 10.8 x 10^7 CFU/g, while those of *Pseudomonas sp.*, *Bacillus sp.*, *Escherichia coli* and intestinal enterococci ranged from 1.00 to 10.6 x 10^2 CFU/g, 1.44 to 30.0 x 10^3 CFU/g, 1.29 to 16.0 x 10^3 CFU/g, and 11.0 to 5.60 x 10^3 CFU/g, respectively. These microorganisms, known for their capacity to degrade complex pollutants are present in soils with

concentration values between values lower than 1 and 10^4 CFU/g of soil (BTEX, hydrocarbons, pesticides etc...) [18-20]. The concentration values observed are in agreement with the values observed in the previous study carried out by VAGNY LAB on the same sites. The presence/abundance of *Pseudomonas sp.* and *Bacillus sp.* in soils does not limit the potential uses of the soil (agriculture, construction etc...). However, the absence of *Escherichia coli* and intestinal enterococci in the Alepe 2 remediated soil thus emphasizes the non-fecal contamination of this site by warm blood animals or human faeces.

Conclusion

In this study, we note that the concentrations of heavy metals determined in the soil were higher than those obtained in the control soil at all stations, but were generally lower than Ivorian Norm values. Regarding organic compounds and hydrocarbons, only total sulfur exceeded Ivorian standards at all sampling sites (i.e., 1340, 1320, and 190 mg/kg for site 1, site 2, and the Alépé 1 control, respectively, while those for site 1, site 2, and the Alépé 2 control were 1720, 1630, and 190 mg/kg, contrary to the standard values of 10 x 10-3 mg/kg). Then, four types of microorganisms were detected in the different sampling stations and sites, namely heterotrophic bacteria, *Pseudomonas sp., Bacillus sp., Escherichia coli* and intestinal enterococci in the remediated and control soils at the similar range of values. Finally, these results confirm the positive impact of remediation with the possibility to reuse these sites for agriculture or other purposes.

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References

[1] K. N. Palansooriya, D. C.W. Tsang, S. M. Shaheen, S. S. Chen, Y. S. Ok, Y. Hashimoto, D. Hou, N. S. Bolan, and J. Rinklebe, Soil amendments for immobilization of potentially toxic elements in contaminated soils: A critical review, *Environment International*, 134 (2020) 1-29.

[2] M. L. El Hachimi, M. Fekhaoui, A. E. Abidi and A. Rhoujatti, Contamination des sols par les métaux lourds à partir de mines abandonnées : le cas des mines Aouli-Mibladen-Zeïda au Maroc, *Cah Agriculture*, 23 (2014) 213-219.

[3] M. A. Ashraf, M. J. Maah and I. Yusoff, Chapter - Soil Contamination, Risk Assessment and Remediation, Book - Environmental Risk Assessment of Soil Contamination, (2014), 54 p.

[4] T. Weldeslassie, H. Naz, B. Singh, and M. Oves, Chemical Contaminants for Soil, Air and Aquatic Ecosystem, Modern Age Environmental Problems and their Remediation, (2018), 22 p.

[5] Y. Li, E. Padoan, and F. Ajmone-Marsan, Soil particle size fraction and potentially toxic elements bioaccessibility: A review. *Ecotoxicol. Environ. Saf.*, 209 (2021) 1-9.

[6] K. Chitra, and G. B. Kamala, Evaluation of soil quality and heavy metal contaminants in agricultural soils, *J. Univ. Shanghai Sci. Technol.*, 23(10) (2021) 267-274.

[7] L. Chen, X. Zhu, J. Beiyuan, W. Hu, Z. Zhang, C. Duan, Q. Cui, H. He, X. Huang, and L. Fang Phytoremediation of potentially toxic elements (PTEs) contaminated soils using alfalfa (Medicago sativa L.): A comprehensive review, *Chemosphere*, 292 (2022) 1-18.

[8] F. Bilias, T. Nikoli, D. Kalderis and D. Gasparatos, Towards a soil remediation strategy using biochar: effects on soil chemical properties and bioavailability of potentially toxic elements, *Toxics*, 9 (2021) 1-35.
[9] S. Kuppusamy, T. Palanisami, M. Megharaj, K. Venkateswarlu, and R. Naidu, Ex-situ remediation technologies for environmental pollutants: a critical perspective. in: *Rev. Environ. Contam. Toxicol.*, 236 (2016) 117–192.
[10] X. Bohand, C. Monpeurt, S. Bohand and A. Cazoulat, Toxic waste dumped in abidjan (Ivory Coast) and health consequences, *Médecine Tropicale*, 67 (6) (2007) 620-624.
[11] F.M. Adebiyi, V. Thoss and A.S. Akinola, Comparative Studies of the Elements that are Associated with Petroleum Hydrocarbon Formation in Nigerian Crude oil and Bitumen Using ICP-OES, *J. Sustain. Energy Eng.*, 2(1) (2014) 10-18.
[12] S.F. Taghizadeh, R. Rezaee, H. Badibostan, G. Karimi, Aflatoxin B1 in walnuts: a probabilistic cancer risk assessment for Iranians, *Toxicol. Environ. Chem.*, 102 (2020) 1-16.
[13] J. L. Kirk, L. A. Beaudette, M. Hart, P. Moutoglis, J. N. Klironomos, H. Lee and J. T. Trevors; Methods of studying soil microbial diversity, *J. Microbiol. Methods*, 58(2) (2004) 169-188.
[14] Normes gouvernementales de Côte d'Ivoire pour le sol (2009)
[15] K. E. Ashelford, M. J. Day, and J. C. Fry, Elevated Abundance of Bacteriophage Infecting Bacteria in Soil, *Appl. Environ. Microbiol.*, (2003) 285–289.
[16] S. Ashok, V. Akila, P. M. Ayyasamy, and S. Rajakumar, Bioremediation of Ethylbenzene by Soil Column Study and Bioreactor Study for Polluted Soil and Water Samples Using Optimized Bacterial Consortium, Integrated Waste Management in India, Springer International Publishing, (2016) 155-168.
[17] P. Baldrian, Forest microbiome: diversity, complexity and dynamics, *FEMS Microbiology Reviews*, 41 (2) (2017) 109-130.
[18] N. A. Soleimani, The Biodegradation of Crude Oil by Bacillus subtilis Isolated from Contaminated Soil in Hot Weather Areas, *Int. J. Environ. Res.*, 8(2) (2014) 509-514.
[19] S. Wasi, S. Tabrez, and M. Ahmad, Use of Pseudomonas spp. for the bioremediation of environmental pollutants: a review. *Environ. Monit. Assess.*, 185 (2013) 8147–8155.
[20] D. Cluzeau, M. Guernion, R. Chaussod, F. Martin-Laurent, C. Villenave, J. Cortet, N. Ruiz-Camacho, C. Pernin, T. Mateille, L. Philippot, A. Bellido, L. Rougé, D. Arrouays, A. Bispo, G. Pérès. Integration of biodiversity in soil quality monitoring: Baselines for microbial and soil fauna parameters for different land-use types, *Eur. J. Soil Biol.*. 49 (2012) 63-72.

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