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Isolation and Identification of Heavy Metals and Antibiotics Resistant Strains from Antananarivo Dumpsite, Madagascar

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Abstract: Heavy metals contamination is now widespread in the nature. At higher concentration, heavy metals become toxic and disturb the ecosystem including soil microorganisms. To adapt to such constraints, some microorganisms have developed tolerance mechanisms. Indeed, in the environment, the resistance of microorganisms to heavy metal often promotes to antibiotic resistance. This work aims to isolate strains from soil samples collected in Andralanitra landfill, to test their tolerance to heavy metals, to identify tolerant strains and to verify their resistance to antibiotics. According to the dilution method, a total of 48 strains were obtained, 14 were isolated on PDA medium, 10 on Sabouraud agar medium, 10 strains on Mossel agar medium, 7 on AS1 medium, 5 strains on TSA medium and 2 strains with King B medium. Resistance test to heavy metals performed by the wells method showed that out of the 48 isolated strains, 26 were capable to grow in the presence of heavy metals (solution composed of copper, zinc, cadmium, chromium, nickel, lead) at different concentrations. The highest number of tolerant strains was recorded at the concentration of 100mg/L ≤ C ≤ 1000mg/L. Four (4) strains were tolerant to the heavy metals solution at a concentration between 100mg/L and 1500mg/L. The molecular identification of these four most resistant strains by 16S rDNA gene sequencing and ITS gene sequencing allowed to classify them as belonging to the genera *Ochrobactrum pseudogrignonense*, *Arthrobacter nicotianae*, *Penicillium crustosum* and *Penicillium commune*. The antibiotic sensitivity test using disc diffusion method on Mueller-Hinton agar revealed that *Ochrobactrum pseudogrignonense* and *Penicillium commune* were resistant to Trimethoprim, *Arthrobacter nicotianae* showed resistance to Trimethoprim and Ciprofloxacin, *Penicillium crustosum* was resistant to all tested antibiotics.

Keywords: Isolation, Characterization, Resistance, Heavy Metals, Antibiotics, Dumpsite, MADAGASCAR

1. Introduction

In the world, dumpsite is the most used method for solid waste elimination. However, it is identified as one of the major risks for groundwater contamination [1]. Without control, waste accumulation in open air increases the threat on environmental health [2]. In Madagascar, the increasing establishment of large industrial units and the mismanagement of municipal waste generate various pollutants that are dangerous for the ecosystem. Solid wastes are all accumulated and confined in the sole municipal landfill of Andralanitra without any prior treatment.

Among these wastes, heavy metals which are the major toxic compounds produced by various industrial, pose greater problem for the environment if they are not treated properly prior to their disposal [3]. They can become mobile in the contaminated soil according to their speciation and the soil

pH. A fraction of the total mass may therefore become available to living organisms [4]. The contamination of the soil by heavy metals causes the decrease of the biomass and the diversity of telluric microbial population. Moreover, this contamination affects their growth, their morphology, and their biochemical activities.

The microorganisms to tolerate high concentration of metals have adopted several mechanisms which are often specific to one or several types of metals [5, 6]. The heavy metals resistance mechanisms may involve physiological adaptation or genetic structure of the microorganisms [7, 8]. Some of these mechanisms have been identified as responsible of alteration of normal cell physiology leading to development of drug resistance in microorganisms [9].

Many researchers suggested that metal exposure indirectly promotes bacteria resistance to unrelated toxic substances, particularly antibiotics [10]. Several works demonstrated a link between heavy metal resistance and antibiotic resistance [11]. For bacteria, the genes responsible of these two types of resistance are placed on the same plasmid [12, 13].

The purpose of the present study is to isolate microorganisms from dumpsite soil samples, to determine their resistance to different types of heavy metals, to identify tolerant strains, and to verify their resistance to antibiotics.

2. Materials and Methods

2.1. Study Site

Andralanitra landfill with a surface of 18ha is the only existing dumpsite within the urban district of Antananarivo. It is located in the north-eastern about 9 km far from the town (47°34'25.5"E of longitude and 18°54'46.1"S of latitude). It has been used since 1966 as solid wastes dumping site for industries and households of 2 200 000 inhabitants [14] in Antananarivo and its surroundings. About 450 tons of wastes per day are accumulated in this site without any treatment.

2.2. Soil Sampling

Soil samples were collected from the study area in a sterile plastic container, kept in ambient temperature and transported to the laboratory where they were conserved at -4°C until use.

2.3. Soil Analysis

Soil analysis was performed using Rodier's method [15] for the evaluation of heavy metals. For that, 2g of soil were weighed in a capsule and mixed with 4mL of sodium nitrate (100g/L); the mixture was then dried in the oven at 110°C. Thereafter, the capsule was placed in the muffle furnace at 450°C for 2 hours, and left to cool. The residue was transferred into a beaker with a few milliliters of water and the capsule was rinsed with concentrated HCl and boiling water successively. Five milliliters (5mL) of HNO_3 were then added to the residue; the mixture was boiled for ten minutes and evaporated. The residue was taken up in 20 mL of HCl (2N); the mixture was boiled and filtered. The filtrate was collected in a flask; beaker and filter were rinsed with 10mL of HCl (2N) and twice with boiling water; finally the volume was adjusted. The minerals obtained were analyzed by atomic absorption spectrometry.

2.4. Strains Isolation

In order to obtain a large number and diversity of strains, different types of media were used for the isolation: AS1 (Antibiotic selection 1), King B, Mossel, Sabouraud agar, TSA (Trypto-casein soya agar) and PDA (Potato-Dextrose Agar).

Strains isolation was performed using the dilution method with the previously cited media. One gram of soil sample was suspended in 9mL of sterile distilled water. The mixture was shaken thoroughly and a serial dilution was prepared. One milliliter of the dilutions 10^{-2} and 10^{-3} was taken and spread aseptically into the media plates cited above. Plates were then incubated at 30°C for 48 hours to 4 weeks [16].

2.5. Heavy Metals Resistance Test

The heavy metal resistance was evaluated using the wells method. Petri dishes containing Mueller-Hinton agar media were inoculated with 100µL of tested strains spore suspension (10^6CFU/mL). Wells were then formed with a sterile cork borer and 50µL of heavy metals solution (copper, zinc, cadmium, chromium, nickel, lead) at different concentrations (100, 250, 500, 1000, 1500mg/L) were poured into the wells. The diameter of inhibition zone was measured after incubation at 30°C for 7 days. The strains were considered resistant when the inhibition diameter was less than 7mm and non-resistant when it was higher than 10mm [17, 18].

2.6. Molecular Identification

Resistant isolates were subjected to molecular identification by 16S rDNA gene sequencing and ITS gene sequencing. For that, pure colonies of each isolate were picked, suspended into colony lysis buffer (10mM TrisCl pH 8, 1mM EDTA, 50 mMKCl, 0.1% Tween 20) and boiled for 10min. The solution was then directly used for PCR using the primers pA (AGAGTTTGATCCTGGCTCAG) and pH (AAGGAGGTGATCCAGCCGCA) for 16S rDNA gene and ITS1 (TCCGTAGGTGAACCTGCGC) and ITS4 (TCCTCCGCTTATTGATATGC) for ITS gene. PCR products sequencing was performed by Sanger method using the primers pA and ITS1.

Sequencing products were checked and assembled with CLC Workbench program for the final sizes of 993 pb for the isolate S6, 1048 pb for S9, 953 pb for S13 and 562 pb for the strain S14. The sequences were compared with the genbank database available at http://www.ezbiocloud.net/identify for 16S rDNA gene and at www.mycobank.org for ITS gene

2.7. Antibiotics Sensitivity Test

Sensitivity test to antibiotics of heavy metals resistant isolates was performed using disk diffusion method on

Mueller-Hinton agar media. The antibiotics used were: Cefotaxime 5µg (COX), Cefotaxime 30µg (CTX), Ciprofloxacine 5µg (CIP), Ketoconazole 50µg (KET), Sulphamethoxazole trimethoprim 25µg (SXT), Cefixime 10µg (CFM), Aztreonam 30µg (ATM), Fusidic acid 10µg (FA), Trimethoprim 5µg (TMP), Spectinomycin 100µg (SPT), Nalidixic acid 30µg (NA) and Erythromycin 15µg (ERY). Inhibition zone was noted after 48hours of incubation at 35-37°C. Strains were considered susceptible when the inhibition zone diameter was higher than 12mm [19]. Tests were performed in duplicate and the sterility of medium plate was confirmed by incubating two plates of un-inoculated agar plate with the inoculated plates.

3. Results

3.1. Soil Analysis

The results of the soil analysis are summarized in Table 1.

Table 1. *Soil analysis results.*

Elements	Concentrations (mg/kg)	Reference value (AFNOR)
Lead (Pb)	728.81	100
Nickel (Ni)	20.00	50
Cadmium (Cd)	1.36	2
Iron (Fe)	7.22	-
Manganese (Mn)	670.37	100
Copper (Cu)	260.92	100

3.2. Strains Isolation

A total of forty eight (48) strains were isolated from the samples collected in Andralanitra dumpsite. The strains were coded S1 to S48. Out of the 48 strains, 14 were isolated on PDA medium, 10 on Sabouraud agar medium, 10 strains on Mossel agar medium, 7 on AS1, 5 strains with TSA medium and 2 strains with King B medium (Figure 1).

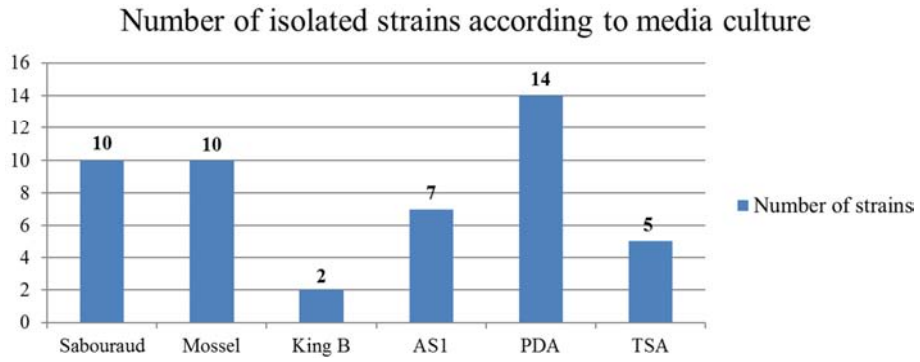

Figure 1. *Number of isolated strains according to media culture.*

3.3. Heavy Metals Resistance Test

The results of the heavy metals resistance test of isolated strains showed that among the 48 strains tested, 26 strains were tolerant to the heavy metals at different concentrations (Figure 2, figure 3). The highest number of tolerant strains was recorded at the concentration of 100mg/L ≤ C ≤ 1000mg/L. Four (4) strains were tolerant to the heavy metals solution at a concentration between 100mg/L and 1500mg/L.

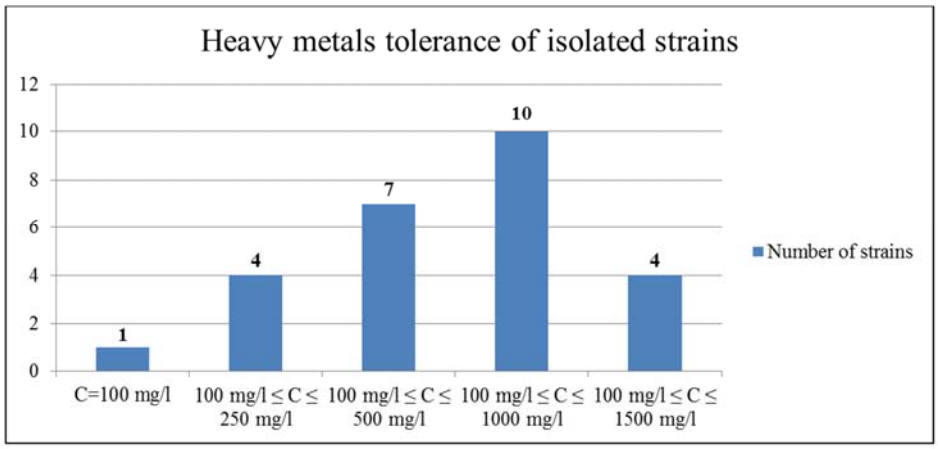

Figure 2. *Heavy metals tolerance of isolated strains.*

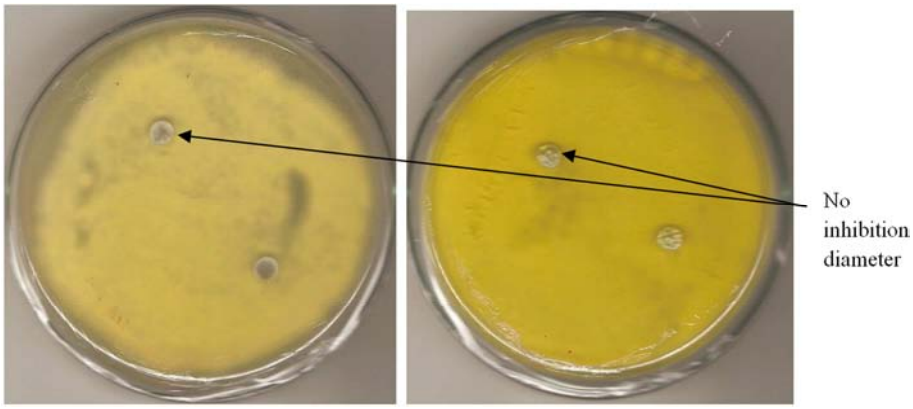

Figure 3. *Resistance of strain S13 to heavy metals at concentrations of 1000 and 1500mg/L.*

3.4. Molecular Identification

Molecular characterization using 16S rRNA gene sequencing method of the two resistant isolates showed that the isolate S6 belongs to *Ochrobactrum pseudogrignonense* with 99.07% of similarity (Figure 4) and the isolate S9 to *Arthrobacter nicotianae* with 99.90% of similarity (Figure 5). The identification of the two other strains by ITS gene sequencing showed that S13 and S14 matched with high similarity with *Penicillium crustosum* (Figure 6) and *Penicillium commune* (Figure 7).

TGCAGTCGAGCGCCCCGCAGGGGAGCGGCAGACGGGTGAGTAACGCGTGGGAAT
CTACCTTTTGCTACGGAATAACTCAGGGAAACTTGTGCTAATACCGTATGTGCCCT
TTTGGGGAAAGATTTATCGGCAAGAGATGAGCCCGCGTTGGATTAGCTAGTTGGT
GGGGTAAAGGCCTACCAAGGCGACGATCCATAGCTGGTCTGAGAGGATGATCAGC
CACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATA
TTGGACAATGGGCGCAAGCCTGATCCAGCCATGCCGCGTGAGTGATGACGGTCTT
AGGATTGTAAAGCTCTTTCACCGGTGAAGATAATGACGGTAACCGGAGAAGAAGC
CCCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGGGCTAGCGTTGTTC
GGATTTACTGGGCGTAAAGCGCACGTAGGCGGACTTTTAAGTCAGGGGTGAAATC
CCAGAGCTCAACTCTGGAACTGCCTTTGATACTGGAAGTCTTGAGTATGGTAGAGG
TGAGTGGAATTCCGAGTGTAGAGGTGAAATTCGTAGATATTCGGAGGAACACCAG
TGGCGAAGGCGGCTCACTGGACCATTACTGACGCTGAGGTGCGAAAGCGTGGGGA
GCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAATGTTAGCCG
TCGGGGTGTTTACACTTCGGTGGCGCAGCTAACGCATTAAACATTCCGCCTGGGGA
GTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTG
GAGCATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCAGCCCTTGACATACC
GGTCGCGGACACAGAGATGTGTCTTTCAGTTCGGCTGGACCGGATACAGGTGCTG
CATGGCTGTCGTCAGCTCGTGTCGTGAGATG

Figure 4. *Sequence of the 16S RNA gene of the isolate S6 (Ochrobactrum pseudogrignonense).*

ATGAAGCCCAGCTTGCTGGGTGGATTAGTGGCGAACGGGTGAGTAACACGTGAGTAA
CCTGCCCCCGACTCTGGGATAAGCCCGGGAAACTGGGTCTAATACCGGATATGACCT
CGCACCGCATGGTGCGGGGTGGAAAGATTTATCGGTGGGGGATGGACTCGCGGCCTA
TCAGCTTGTTGGTGAGGTAATGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGA
GGGTGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCA
GTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGAT
GACGGCCTTCGGGTTGTAAACCTCTTTCAGTAGGGAAGAAGCGAAAGTGACGGTACC
TGCAGAAGAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGC
AAGCGTTATCCGGATTTATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTCGCGTCTGCC
GTGAAAGTCCGAGGCTCAACCTCGGATCTGCGGTGGGTACGGGCAGACTAGAGTGAT
GTAGGGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAA
CACCGATGGCGAAGGCAGGTCTCTGGGCATTTACTGACGCTGAGGAGCGAAAGCATG
GGGAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGTTGGGCACTAGG
TGTGGGGGACATTCCACGTTTTCCGCGCCGTAGCTAACGCATTAAGTGCCCCGCCTGG
GGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGG
CGGAGCATGCGGATTAATTTCGATGCAACGCGAAGACCTTACCAAGGCTTGACATGT
GCCAGACCGCTCCAGAGATGGGGTTTCCCTTCGGGCTGGTTCACAG

Figure 5. *Sequence of the 16S RNA gene of the isolate S9 (Arthrobacter nicotianae).*

CTGGGTCCACCTCCCACCCGTGTTTATTTTACCTTGTTGCTTCGGCGGGCCCGCCTT
AACTGGCCGCCGGGGGGCTTACGCCCCCGGGCCCGCGCCCGCCGAAGACACCCTC
GAACTCTGTCTGAAGATTGTAGTCTGAGTGAAAATATAAATTATTTAAAACTTTCA
ACAACGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATACGT
AATGTGAATTGCAAATTCAGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCC
TGGTATTCCGGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCACGGCTT
GTGTGTTGGGCCCCGTCCTCCGATCCCGGGGGGACGGGCCCGAAAGGCAGCGGCGG
CACCGCGTCCGGTCCTCGAGCGTATGGGGCTTTGTCACCCGCTCTGTAGGCCCGGC
CGGCGCTTGCCGATCAACCCAAATTTTTATCCAGGTTGACCTCGGATCAGGTAGGG
ATACCCGCTGAACTTAAGCATATCAATAAGCGAGGAA

Figure 6. *Sequence of the ITS gene of the isolate S13 (Penicillium crustosum).*

CCCCGTGTTTATTTTACCTTGTTGCTTCGGCGGGCCCGCCTTAACTGGCCGCCGGGGGG
CTTACGCCCCCGGGCCCGCGCCCGCCGAAGACACCCTCGAACTCTGTCTGAAGATTGA
AGTCTGAGTGAAAATATAAATTATTTAAAACTTTCAACAACGGATCTCTTGGTTCCGGC
ATCGATGAAGAACGCAGCGAAATGCGATACGTAATGTGAATTGCAAATTCAGTGAAT
CATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCG
AGCGTCATTGCTGCCCTCAAGCCCGGCTTGTGTGTTGGGCCCCGTCCCCCGATCTCCGG
GGGACGGGCCCGAAAGGCAGCGGCGGCACCGCGTCCGGTCCTCGAGCGTATGGGGCT
TTGTCACCCGCTCTGTAGGCCCGGCCGGCGCTTGCCGATCAACCCAAATTTTTATCCAG
GTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAGCGGAGG
AA

Figure 7. *Sequence of the ITS gene of the isolate S14 (Penicillium commune).*

3.5. Antibiotics Sensitivity Test

Susceptibility test of the four heavy metals resistant isolates against some antibiotics revealed that *Ochrobactrum pseudogrignonense* and *Penicillium commune* are sensitive to all antibiotics except Trimethoprim. *Arthrobacter nicotianae* showed resistance to two antibiotics, Trimethoprim and Ciprofloxacin. *Penicillium crustosum* is resistant to all selected antibiotics (Table 2, figure 8, figure 9).

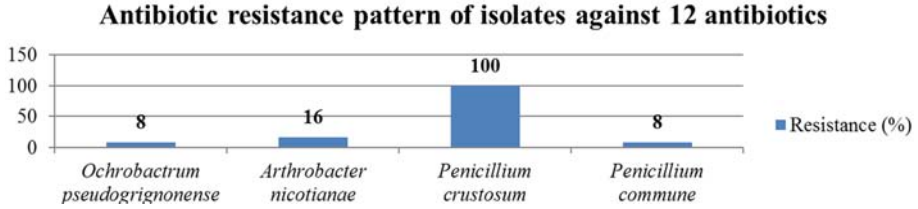

Figure 8. *Antibiotic resistance pattern of isolates against 12 antibiotics.*

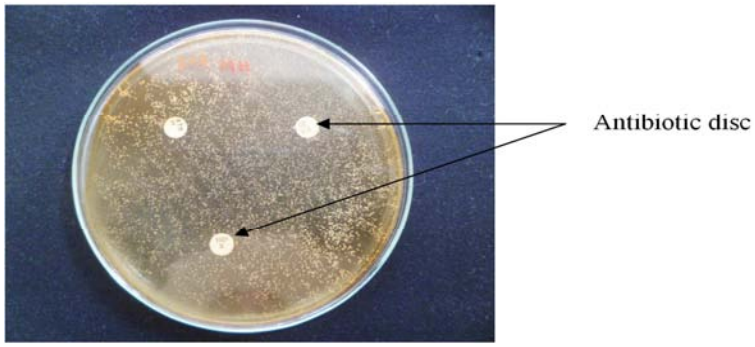

Figure 9. *Resistance of Penicillium crustosum against 3 antibiotics.*

Table 2. *Strains resistance to antibiotics.*

Antibiotics / Strains	Cefotaxime 5µg	Cefotaxime 30µg	Ciprofloxacine 5µg	Ketoconazole 50µg	Sulphamethoxazole trimethoprim 25µg	Cefixime 10µg
Ochrobactrum pseudogrignonense	-	-	-	-	-	-
Arthrobacter nicotianae	-	-	R	-	-	-
Penicillium crustosum	R	R	R	R	R	R
Penicillium commune	-	-	-	-	-	-

Table 2. *Continue.*

Antibiotics / Strains	Aztreonam 30µg	Fusidic acid 10µg	Trimethoprim 5µg	Spectinomycin 100µg	Nalidixic acid 30µg	Erythromycin 15µg
Ochrobactrum pseudogrignonense	-	-	R	-	-	-
Arthrobacter nicotianae	-	-	R	-	-	-
Penicillium crustosum	R	R	R	R	R	R
Penicillium commune	-	-	R	-	-	-

R=Resistance, - = Sensitive.

4. Discussion

In this study, soil samples were collected in Andralanitra dumpsite and processed for strains isolation. All isolated strains were tested for heavy metals resistance, the most resistant isolates were then identified and tested for antibiotics resistance.

The soil samples analysis showed the presence of lead, nickel, cadmium, iron, manganese and copper. The lowest contaminant concentrations were recorded with cadmium and iron while the highest concentrations were noted with lead and manganese. Compared to AFNOR standards for heavy metals in soils, concentrations of lead and manganese were respectively 7 times and 6 times higher, indicating that the study site is highly polluted by these both elements.

For the isolation, a total of forty eight (48) strains were isolated from the samples using dilution method on different culture media. Previous studies showed that certain genera of microorganisms such as *Citrobacter sp, Thiobacillus ferrooxidans, Bacillus cereus, Bacillus subtilis, Pseudomonas aeruginosa, Micrococcus luteus, Rhizopus arrhizus, Aspergillus flavus, Saccharomyces cerevisiae, Acinetobacter baumannii* isolated from contaminated soil with heavy metals were demonstrated to be able to reduce soil pollution [20, 21, 22]. Hence, the choice to use several culture media to allow the isolation of a wide variety of strains. The greatest number of isolated strains was obtained on PDA media. This could be explained by its composition, containing dextrose as a carbohydrate source which serves as a growth stimulant and potato infusion that provides a nutrient base for luxuriant growth of most fungi [23].

Heavy metals resistance test revealed that four strains were resistant to the highest concentration (1500mg/L) of heavy metals. It was noted that the number of tolerant strains decreased as the concentration of heavy metals increased indicating, the toxic effects of metals on microorganisms [24]. Heavy metals tolerant strains play an important role in bioremediation process of contaminated soils [25]. Therefore, their isolation from polluted area constitutes the aim of many researches. Twelve (12) strains capable to grow on soil contaminated by mercury, lead, argent, zinc and copper at variable concentrations (1,0-5,0mM) were isolated from industrial and agricultural areas in Mauritius [26]. Ansari et *al.,* [27] isolated from industrial soil, six strains resistant to cadmium, lead and arsenic. Our results are in accordance with Adebisi et *al.,* [28] who isolated 26 strains from soil samples collected in Awotan-Nigéria dumpsite. Those strains were tolerant to heavy metals as lead, nickel, copper and arsenic. In bioremediation, the use of resistant strains offers many perspectives because the ability of telluric microorganisms to interact with plants allows the combination of bioremediation and phytoremediation process [29, 30].

The molecular identification of the four resistant strains allowed to classify them as belonging to the genera *Ochrobactrum pseudogrignonense*, *Arthrobacter nicotianae*, *Penicillium crustosum* and *Penicillium commune*. These results corroborate those found by Yang et *al.,* [31] who demonstrated that *Ochrobactrum pseudogrignonense* strain can tolerate a high concentration of heavy metals. Research works conducted by Liao et *al.,* [32] showed that other *Ochrobactrum* species are able to reduce chromium and other species to absorb different types of metals (lead, cadmium, zinc, copper, cobalt and nickel) [33, 34]. Some *Arthrobacter* species have been reported to have the ability to reduce chromium [35]. Another study conducted by Goutam *et al.,* [36] demonstrated the effectiveness of *Arthrobacter phenanthrenivorans* in the bioremediation of contaminated water by heavy metals (lead, arsenic, cadmium).

Regarding fungi isolates, ubiquitous fungi can adapt to many types of substrates and they are frequently used in the bioremediation of contaminated soils and waters. Indeed, their ability to absorb heavy metals and to degrade many compounds has been shown [37]. Xu et *al.,* [38] demonstrated the role of *Penicillium chrysogenum* in the detoxification and bioremediation of cadmium. Other species such as *Penicilium oxalicum* and *Penicillium citrinium* have been reported for their ability to absorb lead [39] and *P. aurantiogriseum* for biosorption of cadmium and mercury [40]. The ability of fungi to absorb heavy metals is often related to the composition of their cell walls which are rich in polysaccharides and glycoproteins (glucans, chitin, mannan and phosphomannan), these polymers provide abundant sources of ligands that can bind to metal [41].

Antibiotic susceptibility test of the four most tolerant strains showed that *Ochrobactrum pseudogrignonense* and *Penicillium commune* are resistant to one antibiotic, *Arthrobacter nicotianae* showed resistance to two antibiotics and Penicillium crustosum was resistant to all selected antibiotics. These results are in accordance with those of Adebisi et *al.,* [28] who showed that all strains isolated from soil samples collected in a landfill were resistant to at least one of the tested antibiotics. The antibiotic resistance capacity of bacteria may be related to the production of enzymes that inactivate or modify antibiotics or bacteria cell

membrane [42]. These properties are acquired when bacteria undergo genetic changes occurred during mutation or acquisition of new genetic material [43]. According to Roger et *al.,* [44] antibiotic resistance in some bacteria is due to the transfer of resistance gene from one bacterium to another.

Penicillium species are known for their widespread occurrence and their ability to produce mycotoxins and other secondary metabolites [45]. The resistance of *Penicillium crustosum* to antibiotics may be explained by the production of volatile and non-volatile metabolites as penitrems, viridicatins, terrestric acid and roquefortine C [46, 47]. Penitrems and roquefortine C are mycotoxins produced by Penicillium species [48] and are classified as toxic compounds [49]. Viridicatin is a fungal metabolite and terrestic acid is a phytotoxic metabolite of various fungi.

Tolerance to heavy metals and antibiotics is the result of microorganisms' exposure to contaminated area by metals [50]. In terms of survival, the acquisition of these two types of resistance by the bacterium is favorable for its growth in an environment exposed to many constraints.

5. Conclusion

This study demonstrated the isolation of four resistant strains to heavy metals from soil samples collected in Andralanitra landfill. The strains tolerate a concentration of heavy metals of 1500 mg/L and they were identified as belonging to the genera *Ochrobactrum pseudogrignonense*, *Arthrobacter nicotianae*, *Penicillium crustosum* and *Penicillium commune*. The antibiotic resistance test revealed that *Ochrobactrum pseudogrignonense* and *Penicillium commune* were resistant to Trimethoprim, *Arthrobacter nicotianae* to Trimethoprim and Ciprofloxacin, and *Penicillium crustosum* was resistant to the 12 tested antibiotics. The accumulation of pollutants in the study area induced to the emergence of multi-resistant bacteria to heavy metals and antibiotics. As the strain S13 (*Penicillium crustosum*) showed the highest resistance to heavy metals and antibiotics, it could be considered as a remarkable agent for bioremediation of soils contaminated by heavy metals in our perspectives.

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