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Research Article

Bioremediation Potential of Heavy Metal Multi-Tolerant *Pseudomonas Spp.* Isolated From a Municipal Waste Dumpsite at Ile-Ife, Osun State, Nigeria

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ABSTRACT

Municipal wastes are probable source of heavy metals that may be noxious to the environment and the biotic component due to indiscriminate waste disposal method and open waste dump practiced in Africa.

Three *Pseudomonas spp.* isolated from a municipal waste dumpsite were evaluated for their tolerance to five metals (Pb, Cd, As, Ni and Cr). The best isolate that showed multiple resistance to all the five metals was identified as *Pseudomonas aeruginosa* by the 16S rRNA sequence with a Maximum Tolerable Concentrations (MTC) of 2000 mg/l for Pb, 1200 mg/l for Cd, 4500 mg/l for As, 600 mg/l for Ni and 2000 mg/l for Cr. *Pseudomonas aeruginosa* was subjected to mutation by ethidium bromide and acridine orange; thirteen mutant strains were recovered. The *Pseudomonas aeruginosa* MutAb, MutAc and MutAe exhibited a 25% increase in the MTC to lead; *Pseudomonas aeruginosa* MutAa-Ae recorded an improvement of 32% in the MTC to cadmium while *Pseudomonas aeruginosa* MutAa, MutAb, MutAd and MutAe exhibited 25% increase in the MTC to arsenic after mutational enhancement. All *Pseudomonas aeruginosa* strains were resistant to all the antimicrobials except for *Pseudomonas aeruginosa* MutAc which was sensitive to ofloxacin, nitrofurantoin and gentamicin. *Pseudomonas aeruginosa* MutAe exhibited the removal of 98.9% Pb from solution, compared to the *Pseudomonas aeruginosa* wild type with 97.4% at pH 7.

The outcome of the research specifies that the efficiency of native microbes in bio-removal procedure can be boosted by mutation for effective bioremediation of effluents with Pb metal contaminations.

Keywords: *Pseudomonas aeruginosa*; Bioremediation; Waste dumpsite; Enhancement; Heavy metals; Antimicrobial resistance

INTRODUCTION

Waste occurs in diverse forms such as solids and liquid; the solid forms are known as solid waste, which are produced from municipal, commercial and industrial sources [1]. According to Scarlat et al., one hundred and twenty five million tons per annum of wastes were produced in Africa in 2012 with 65% (81 million tons) of the total amount from Countries South of the Sahara, which is estimated to rise to two forty-four million tons by 2025 [2]. Wastes are frequently dumped by the public in indiscriminate places, at several dumping locations on the border of urban areas, at bare lands spread all over the city. It has been reported that haphazard and open disposal of waste can lead to environmental degradation, introduction of harmful constituents such as heavy metals into water and soil ecology, causes illnesses, unpleasant smell and threaten the wellbeing of humans and living organisms [3-5]. Regardless of the improvement in the environmental safety and ecology, the level of waste management remain inadequate [6].

Toxic metals such as arsenic (As), cadmium (Cd), mercury (Hg), and lead (Pb) have no biological role, non-biodegradable and considered a global pollution problem due to their persistence and the lethal effects in the environment and to the populace [7]. They depreciate the quality of aquatic ecosystem, poisonous at minute concentration to biological system, and causes endocrine disruption and carcinogenic [8].

There are extensive range of practices and managements to lessen or recover heavy metals from contaminated environments which include physicochemical treatment, phytoremediation and microbial bioremediation strategies [9]. Bioremediation processes have more benefits compared with conventional treatments because of the cost effectiveness and environmentally friendly. Microbes,

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have the competence to devour organic left-over and transform the left-over to harmless products by producing various metabolites to breakdown the difficult left-over into simple complexes [10]. Some strains of microorganisms isolated from heavy metal polluted environments have exhibited bioremediation potentials, since they have mechanisms that permit them to acclimatize to hostile environments. Some of the methods established by microbes include, metal sorption, extracellular precipitation uptake and accumulation, mineralization and enzymatic oxidation or reduction to a non-toxic form, and efflux of heavy metals from the cell [11,12].

Pseudomonas aeruginosa a Gram negative rod bacterium found in the family Pseudomonadaceae. It can adapt to the presence of some heavy metals and antibiotics [13,14]. *Pseudomonas aeruginosa* is one of the best bacterial strain for mitigating the effect of heavy metals pollution snags and extant in practically all polluted environment [15,16]. It has been reported as an appropriate bio-sorbent for the elimination of cadmium and other heavy metals from polluted waste, aquatic system and soil [15]. *Pseudomonas aeruginosa* has mastered various detoxification mechanisms, such as metal reduction and precipitation as metal salts for the elimination of heavy metals. They also produce a yellowish green fluorescence, known as Pyoverdin which are appropriate biosorbent for the elimination of cadmium and other heavy metals from polluted environment [13,15].

Despite several reports associated to the probing and bio-prospection of bacteria that may perhaps aid in bioremediation of heavy metals, there is paucity of information on the biotechnological enhancement of the native bacterial isolates from waste dump site in Nigeria for heavy metal bioremediation purposes. This study aims at isolation, characterization; identification and evaluation of tolerance mechanisms of native *Pseudomonas spp*. obtained from a municipal waste dump site and their mutant strains.

MATERIALS AND METHODS

Bacterial strains

The *Pseudomonas spp*. was isolated from the soil sample of a municipal waste dump site located within a University Community at Ile-Ife. This site is a located within the co-ordinate No7.53351, 4.52505E.

Probable identity of the multi-tolerant bacteria

Pure isolates of selected *Pseudomonas spp*. were identified using the Bergey's Manual of Determinative Bacteriology [17]. Further identification was done by molecular methods. The amplification of the 16S rRNA gene sequences of *Pseudomonas spp* was carried out with primers 27F and 1525R, the nucleotide sequences gotten were aligned using BioEdit and related with analogous sequences at the National Center for Biotechnology Information (NCBI) with the aids of the BLAST (Basic Local Alignment Search Tool) algorithm [18].

Ethidium bromide and acridine orange stock solutions

150 mg of ethidium bromide and acridine orange were weighed and dispersed in 15 ml of deionized water, this preparation is equivalent to 10 mg/ml of the mutagen. The stock solutions were used to prepare lower concentrations of the ethidium bromide and acridine orange as required [17].

Maximum Tolerable Concentration (MTC)

The agar dilution assay was used according to the modified method of Yahaya et al. The MTC of the *Pseudomonas spp*. were evaluated for cadmium, lead, chromium, arsenic, and nickel. A standardized solution of the bacteria was spot inoculated to sterile Minimal Salt Agar (MSA) augmented with various concentrations of the heavy metals (200 mg/l to 5000 mg/l) after autoclaving. The control plates were set-up as positive and negative controls, all the plates were incubated at 30°C for 48 hours [19].

Generation of mutant strains

A standardized overnight culture of the *Pseudomonas spp*. was used for the experiment; and the methodology of Feruke-Bello et al. and Shakibaie et al [17, 20]. The rate of survival of the *Pseudomonas spp*. Were calculated as:

%survival=Total count (cfu/ml) of mutants × 100

Total count (cfu/ml) of wild type

Antimicrobial resistance pattern of the *Pseudomonas aeruginosa*

The test was performed by disk diffusion assay. Eight antibiotics: ceftazidime (CAZ, 30 µg), cefuroxime (CRX, 30 µg), gentamicin (GEN, 10 µg), cefixime (CXM, 5 µg), ofloxacin (OFL, 5 µg), augmentin (AUG, 30 µg), nitrofurantoin (NIT, 300 µg), and ciprofloxacin (CPR, 5 µg) were used. A suspension of fresh culture of *Pseudomonas aeruginosa* was adjusted to 0.5 McFarland standards, 0.1 ml was spread on Mueller Hinton's agar, and the antibiotic discs were placed on the surface of the plates, incubated at 37°C for 24h. The plates were examined for zone of inhibition which was measured and categorized as resistant, susceptible, or intermediate based on CLSI (2020) standards [21].

The Multiple Antibiotic Resistance (MAR) phenotypes were evaluated mathematically as:

MAR=x /y,

Where, x is the amount of antibiotics to which bacterial isolate displayed resistance and y is the sum of antibiotics which the bacterial isolate was evaluated [22,23]. The MAR index of ≥ 0.2 showed that the bacteria are from elevated risk sources while below 0.2 specified that the bacteria are from small risk sources.

The removal efficiency of cadmium by *Pseudomonas aeruginosa* and its mutants at different pH

The Pb removal experiment was conducted in conical flasks (250 ml) containing 50 ml MSB appended with 500 mg/l Lead at pH range of 6, 7, and 8 in an aerobic settings. A *Pseudomonas aeruginosa* suspension in logarithmic phase was inoculated into the solution. The solutions were agitated on orbital shaker at 170 rpm for 3-5 days. The Pb concentration in the solution after the incubation period was measured via Atomic Absorption Spectrophotometer (AAS). The %removal of lead was considered by the difference between the initial and final lead concentrations multiplied by 100 [17].

Statistical analysis

Graphpad Prism 8.4.3 was used to evaluate the statistical analysis.

RESULTS AND DISCUSSION

Identification of the heavy metal resistant *Pseudomonas spp.*

The *Pseudomonas spp.* isolated from the municipal waste dumpsite at Ile-Ife were oxidase positive, produce pigments and fluorescence under UV light lamp. They were able to utilize various carbon sources which showed range of metabolic forms that may boost their degradability (Tables 1 and 2). Anjanapriya and Lalitha, reported the isolation of a heavy metal resistant *Pseudomonas spp.* from municipal solid waste dumpsites in Madurai, India [23]. Chetam et al. also reported the presence of *Pseudomonas spp.* from the waste dumpsite in Eagle Island [24]. The occurrence might be as a result of extensive array of physiological abilities and extracellular enzymes [17]. Which permit the organism to propagate in various environments and endure tough environmental situations in addition to polluted environments that wield selective force on the spread of particular clusters of soil bacteria. The molecular analysis of 16S rRNA gene amplification indicated that the best heavy metal resistant bacteria which showed multiple resistance to the various metals was *Pseudomonas aeruginosa* (GBB 215) (99% homology) when matched with related sequences in the non-redundant nucleotide databank at the NCBI with the aid of their world-wide website (BLAST).

Determination of Maximum Tolerable Concentration (MTC) of the isolated bacteria to different heavy metals in mg/l

The Maximum Tolerable Concentrations (MTC) of the Three *Pseudomonas spp.* were indicated in Table 3. All the isolates showed multiple resistance to all the heavy metals (Pb, As, Cd Cr and Ni), with regards to the toxicity, nickel and cadmium were the most toxic and As was the least toxic. The toxicity order was Ni>Cd>Pb>Cr>As which might be an outcome of the usage of related mechanisms for their toxicity as reported by Malik et al. [25-27].

According to Piotrowska-Seget, et al. heavy metals may impede the development of microbes at minute concentrations and limited microbes can endure them [28]. Several investigators recounted the isolation of microbes with multiple resistance to numerous metal ions [29,30]. The results obtained in this study indicated that *Pseudomonas spp.* (GBB 214) had MTC of 2000 mg/l, 1200 mg/l, 4500 mg/l, 1200 mg/l and 400 mg/l for Pb, Cd, As, Cr and Ni respectively. *Pseudomonas aeruginosa* (GBB 215) had MTC of 2000 mg/l, 1200 mg/l, 4500 mg/l, 2000 mg/l and 600 mg/l for Pb, Cd, As, Cr and Ni respectively while *Pseudomonas* C *spp.* (GBB 237) had MTC of 1000 mg/l, 100 mg/l, 3000 mg/l, 100 mg/l and 1600 mg/l for Pb, Cd, As, Cr and Ni respectively. It was observed that isolate GBB 215 (*Pseudomonas aeruginosa*) had the highest tolerance to the five heavy metals with MTC of 2000 mg/l for Pb, 1200 mg/l for Cd, 4500 mg/l for As, 600 mg/l for Ni and 2000 mg/l for Cr. Several researchers have reported that *Pseudomonas aeruginosa* adjust to polluted environment as well tolerate heavy metals owing to its biosorption ability [31-34]. Saha and Santra, also reported the isolation of bacteria with MTC of 3000 mg/l-4000 mg/l of Pb which was higher that the MTC observed for Pb; while MTC of 10-30 mg/l for Cd and 250-350 mg/l for As was reported; which was lower than the MTC recorded in this study [35].

The multiple and high resistance to heavy metals observed might be a reflection of the prevailing environmental condition of the soil at the waste dumpsite. Bacteria exposed to elevated amount of heavy metals develop numerous physiological and genetic mechanisms required for their adaptation and existence under such circumstances [36].

Generation of mutant

The best two bacterium that is *Pseudomonas* A and *Pseudomonas aeruginosa* were selected for further studies based on their elevated and multiple resistances to the five heavy metals. The sub-inhibitory concentration of 3.0 mg/l for ethidium bromide and 0.3 mg/l for acridine orange for *Pseudomonas* A and *Pseudomonas aeruginosa* respectively (Table 3). The colony forming unit (cfu/ml) detected for different strains of the heavy metal resistant *Pseudomonas aeruginosa* are reported in Table 3. Acridine orange was identified as a superior mutagen than ethidium bromide for *Pseudomonas aeruginosa* [17]. Shakibaie et al. reported that acridine orange and acriflavine had extreme consequence on the bioremoval of copper and zinc [20]. *Pseudomonas aeruginosa* possess a survivor rate of 7.87 in ethidium bromide and 19.77 in acridine orange. A total of thirteen *Pseudomonas aeruginosa* mutant strains, eight from ethidium bromide and five from acridine orange were isolated from the MSA augmented with diverse concentrations of the lead salt. The generated strains of *Pseudomonas aeruginosa* were allotted codes based on the particular mutagen, ethidium bromide were (MutEa–MutEh) while acridine orange (MutAa-MutAe). The pigments, morphological and biochemical features of the mutant strains were not affected by the mutagen.

Evaluation of Maximum Tolerable Concentration (MTC) of the *Pseudomonas aeruginosa* mutant strains to different heavy metals in (mg/L)

The capability of microorganisms to endure in polluted environments has been ascribed to the structure of their cell walls which can network and bind well with heavy metals [37], in addition to a variety of genetic mechanisms that assist them to withstand the effects of the toxic metals [38].

The MTC for the heavy metal resistant bacterial isolate *Pseudomonas aeruginosa* Wt were 4500 mg/l, 2000 mg/l and 1200 mg/L for arsenic, lead and cadmium respectively. Feruke-Bello et al. reported a similar result in a multiple resistant heavy metal resistant *Klebsiella variicola* isolated from a discharged effluent in Nigeria [17]. The same MTC as that of the wild type were recorded for *Pseudomonas aeruginosa* MutEa–MutEh for lead while *Pseudomonas aeruginosa* MutAb, MutAc and MutAe recorded 25% (2500 mg/L) increase in their MTC to lead. In addition, a 25% increase (2000 mg/L) in MTC to cadmium was observed in *Pseudomonas aeruginosa* MutEa, *Pseudomonas aeruginosa* MutEd, *Pseudomonas aeruginosa* MutEe, *Pseudomonas aeruginosa* MutEf, *Pseudomonas aeruginosa* MutEg and *Pseudomonas aeruginosa* MutEh whereas 32% (2200 mg/L) increase was observed in *Pseudomonas aeruginosa* MutAa- *Pseudomonas aeruginosa* MutAe (Table 4). However, *Pseudomonas aeruginosa* MutEb and MutEc maintained the same MTC for cadmium as the wild type. In the presence of arsenic, the MTC observed for *Pseudomonas aeruginosa* MutEa-*Pseudomonas aeruginosa* MutEh showed an increase of 6%, while the MTC for *Pseudomonas aeruginosa* MutAa, *Pseudomonas aeruginosa* MutAb, *Pseudomonas aeruginosa* MutAd and *Pseudomonas aeruginosa* MutAe showed 25% increase.

Table 1: Morphological features of the heavy metal resistant microbes isolated from soil of O.A.U waste dump site.

Bacterial code	Shape	Size	Pigmentation	Edge	Surface	Opacity	Elevation	Gram's reaction
GBB 214	Irregular	Medium	Cream	Undulate	Rough and dull	Opaque	Flat	- Rods
GBB 215	Circular	Small	Cream	Entire	Smooth and glistering	Opaque	Low convex	- Rods
GBB 237	Circular	Medium	Cream	Entire	Smooth and glistering	Opaque	Low convex	- Short Rods

Table 2: The biochemical features of the heavy metal resistant microbes isolated from soil of O.A.U waste dump site.

Isolate	*GBB 214*	*GBB 215*	*GBB 237*
Gram stain	- Rods	- Rod	- Short rod
Spore stain	ND	ND	ND
Motility test	+	+	+
Starch hydrolysis	-	-	-
Catalase test	+	+	+
Indole test	-	-	-
H_2S production	+	+	+
Citrate utilization	+	+	+
Nitrate reduction	+	+	+
Methyl red test	+	-	-
Voges–Proskauer test	+	-	-
O/F test	O	O	O
Oxidase test	+	+	+
Glucose	A	A	A
Sucrose	-	-	-
Lactose	-	-	-
Maltose	-	-	-
Mannitol	A	A	A
Probable identity of Isolate	*PseudomonasA sp.*	*PseudomonasB sp.*	*PseudomonasC sp.*

Table 3: The percentage survival of the wild and mutated strains of *Pseudomonas aeruginosa* (GBB 215) in the presence and absence of chemical mutagen.

	PseudomonasA spp.		*Pseudomonas aeruginosa*
Native isolate (removal of EtBr)	5.40×10^6	Native isolate (removal of acridine orange)	1.33×10^7
Mutant strains (addition of EtBr)	4.20×10^5	Mutant strains (addition of acridine orange)	2.63×10^6
% Survival	7.87		19.77

Table 4: MTC of the metal resistant wild and mutant strains of *Pseudomonas aeruginosa*.

	Mg/l				
Bacterial isolates	**Pb**	**Cd**	**As**	**Ni**	**Cr**
Pseudomonas aeruginosa Wt	2000	1200	4500	-	-
Pseudomonas aeruginosa MutEa	2000	2000	4700	-	-
Pseudomonas aeruginosa MutEb	2000	1500	4700	-	-
Pseudomonas aeruginosa MutEc	2000	1500	4700	-	-
Pseudomonas aeruginosa MutEd	2000	2000	4700	-	-
Pseudomonas aeruginosa MutEe	2000	2000	4700	-	-
Pseudomonas aeruginosa MutEf	2000	2000	4700	-	-
Pseudomonas aeruginosa MutEg	2000	2000	4700	-	-
Pseudomonas aeruginosa MutEh	2000	2000	4700	-	-
Pseudomonas aeruginosa MutAa	2000	2200	4700	-	-
Pseudomonas aeruginosa MutAb	2500	2200	4700	-	-
Pseudomonas aeruginosa MutAc	2500	2200	4700	-	-
Pseudomonas aeruginosa MutAd	2000	2200	5000	-	-
Pseudomonas aeruginosa MutAe	2500	2200	5000	-	-

Some researchers Shakibaie et al.; Feruke-Bello et al. also reported an increase in the MTC of cadmium, lead and arsenic once exposed to ethidium bromide and acridine orange by mutational boost procedures. This improvement may perhaps be as a result of the accumulation or obliteration of definite genes in the genome of the bacteria, as the chemical mutagens are alkylating agents stimulating frameshift mutation [39].The MTC of chromium and nickel in the mutant strains were not detected after exposure to the mutagens which might be ascribed to the fact that they are plasmid encoded while lead, cadmium and arsenic resistant gene are chromosomal. Various methods existed for plasmid curing, including chemical and physical agents for the eradication of plasmid; hence ethidium bromide and acridine orange were used for the same purpose in this study.

Antibiotic sensitivity patterns of *Pseudomonas aeruginosa* and the mutant strains

Globally, micro-organisms resistance to antimicrobial continues to be a top menace to public health.

Municipal waste dumpsites remain a likely source of antimicrobial resistance genes due to the haphazard and incessant dumping of solid wastes in the environment [40-43].

The results for the antibiotic sensitivity profile of *Pseudomonas aeruginosa* and their mutant strains were presented in Table 5 according to the CLSI (2020). The *Pseudomonas aeruginosa* Wt (wild type) and its mutant strains showed resistance to almost all the antimicrobials used in this study except for *Pseudomonas aeruginosa* MutAc. The susceptibility pattern showed that 14 (100%) of *Pseudomonas aeruginosa* were resistant to ceftazidime, cefuroxime, cefixime and augmentin. However, 13 (92.9%) of the *Pseudomonas aeruginosa* and its mutant strains were resistant to gentamicin, ofloxacin and nitrofurantoin while 13 (93%) were resistant to ciprofloxacin. Ajuzie et al. also isolated a strain of *Pseudomonas aeruginosa* that was resistant to ciprofloxacin, ofloxacin, augmentin and gentamycin from municipal waste dumpsite [44].

In addition, 1 (7.1%) of the *Pseudomonas aeruginosa* which is *Pseudomonas aeruginosa* MutAc was sensitive to gentamicin, ofloxacin, nitrofurantoin and intermediate for ciprofloxacin (Table 5). Five classes of antibiotics (cephems, aminoglycoside, β-lactam, fluoroquinolones and nitrofurans) were used in this study. The *Pseudomonas aeruginosa* Wt and all the other mutants had MAR index of 1.0 except for *Pseudomonas aeruginosa* MutAc and *Pseudomonas aeruginosa* MutAd that had MAR index of 0.5 and 0.9 respectively.

A research by Hrenovic et al. established that the waste dumpsite possibly harbors the biggest and assorted resistome together with bacteria that possess intrinsic and acquired ABR [45]. Mwaikono et al. shown that municipal waste dumpsites consist of huge range and multifaceted groups of bacteria. The contact of the bacteria with heavy metals led to the choice of bacterial strain which can resist antibiotics. This occurs since genes coding for metals resistance are situated together with antimicrobial resistance genes [46]. Under situations of metal pressure, metal and antimicrobial resistance in microbes probably aids in adjusting more rapidly via range of resistant dynamics than through modification and usual selection [47].

Table 5: The antibiotic susceptibility pattern of pseudomonas aeruginosa and their mutant strains.

	Antibiotics	Ceftazidime (CAZ)	Cefuroxime (CRX)	Gentamicin (GEN)	Cefixime (CXM)	Ofloxacin (OFL)	Augmentin (AUG)	Nitrofurantoin (NIT)	Ciprofloxacin (CPR)
		30 µg	30 µg	10 µg	5 µg	5 µg	30 µg	300 µig	5µg
Pseudomonas aeruginosa strains	Wt	0^R	0^R	4^R	0^R	4^R	0^R	3^R	14^R
	MutEa	0^R	0^R	4^R	0^R	4^R	0^R	0^R	14^R
	MutEb	0^R	0^R	4^R	0^R	5^R	0^R	0^R	15^R
	MutEc	0^R	0^R	4^R	0^R	5^R	0^R	0^R	15^R
	MutEd	0^R	0^R	3^R	0^R	4^R	0^R	0^R	15^R
	MutEe	0^R	0^R	4^R	0^R	5^R	0^R	0^R	14^R
	MutEf	0^R	0^R	4^R	0^R	5^R	0^R	0^R	14^R
	MutEg	0^R	0^R	5^R	0^R	5^R	0^R	0^R	15^R
	MutEh	0^R	0^R	3^R	0^R	4^R	0^R	0^R	14^R
	MutAa	0^R	0^R	4^R	0^R	6^R	0^R	4^R	15^R
	MutAb	0^R	0^R	5^R	0^R	4^R	0^R	4^R	15^R
	MutAc	0^R	0^R	19^S	0^R	20^S	0^R	21^S	21I
	MutAd	0^R	0^R	4^R	0^R	0^R	0^R	0^R	17^R
	MutAe	0^R	0^R	4^R	0^R	0^R	0^R	0^R	13^R
Break Points	S	≥ 18	≥ 18	≥ 15	≥ 19	≥ 18	≥ 16	≥ 17	≥ 21
	I	15-17	15-17	13-14	16-18	14-17	13-15	15-16	16-20
	R	≤ 14	≤ 14	≤ 12	≤ 15	≤ 13	≤ 12	≤ 14	≤ 15

Bioremoval efficiency of Pb by *Pseudomonas aeruginosa* strains

The practice of using native/wild type microorganisms in bioremediation might minimize their prospect due to antagonism and raised heavy metal concentrations. Bioremediation procedure may perhaps be enhanced by diverse methods, based on the nature of the contaminated environment [48,49]. One of such methods is the use of mutant strains of the bacteria as demonstrated by Feruke-Bello et al.; amendment of environmental variables also permit microbial development and hasten bioremediation methods [26,50].

In this study, the *P. aeruginosa* Wt and four of its mutants (*P. aeruginosa* MutAb, *P. aeruginosa* MutAc, *P. aeruginosa* MutAd, *P. aeruginosa* MutAe that showed higher degree of resistance to multiple heavy metal resistance were selected for further studies. The results revealed that the mutant strains *P. aeruginosa* MutAc and MutAe removed 96.3% and 97.1% of Pb respectively compared to 94.6% observed in the *P. aeruginosa* Wt at pH 6 (Figure 1). The percentage removal of Pb by *P. aeruginosa* MutAc and MutAe increased to 98.4% and 98.9% at pH 7 while *P. aeruginosa* MutAc removed 98.25% of Pb at pH 8. *P. aeruginosa* Wt removed 97.9% of lead from solution at pH 7 which was higher than 85% Pb removal by *Pseudomonas spp*. B50D in solution reported by Giovanella et al [51]. The result was also higher than 33.67% bioremediation of Pb by *Pseudomonas aeruginosa* as reported by Oziegbe et al [52]. It was noted that all the strains had maximum bioremediation potential at pH 7 compared with the percentage removal recorded at pH 6 and pH 8 which corroborated the report by Jin et al. that pH has a substantial outcome on the solubility of heavy metal ions and charge on the exterior of the cell which may disturbs the bio-removal procedures [53].

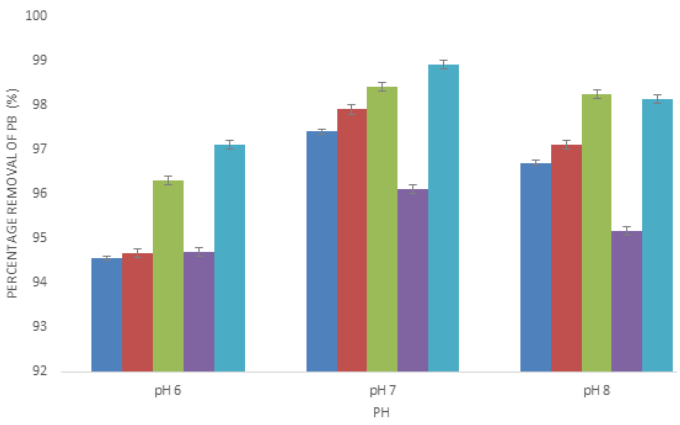

Figure 1: Bioremoval efficiency Pb by *Pseudomonas aeruginosa* strains. **Note:** (■) *P. aeruginosa* Wt; (■) *P. aeruginosa* MutAb; (■) *P. aeruginosa* MutAc; (■) *P. aeruginosa* MutAd; (■) *P. aeruginosa* MutAe.

Pseudomonas spp. is reflected as one of the microbial indicator for assessing pollution in environs [41]. *Pseudomonas aeruginosa* has been reported to possess the capacity to repel and amass metal ions like HgCl, $CuCl_2$ and $CdCl_2$ [54]. Other researchers also reported that lyophilized *Pseudomonas* has competence for cadmium (II) and lead (II) ions uptake from solution through biosorption [55]. The mechanisms used by *Pseudomonas aeruginosa* in reaction to heavy metals pressure can be encrypted within the chromosomal genes, but the resistance has been frequently situated on plasmid [56].

CONCLUSION

Bioremediation still remains one of the utmost capable inventions for handling industrial or municipal waste comprising of heavy metals, chemical spills and hazardous wastes. The isolated *Pseudomonas aeruginosa* Wt showed multiple resistances to cadmium, lead, chromium, arsenic and nickel while the mutant strains *Pseudomonas aeruginosa* MutEa-MutEh and *Pseudomonas aeruginosa* MutAa-MutAe showed multiple resistances to arsenic, lead and cadmium. The resistance to chromium and nickel in the mutant strains were lost after contact with chemical mutagen (ethidium bromide and acridine orange) in a process called plasmid curing; this showed that the resistance to chromium and nickel were plasmid borne while the others are chromosomal. Although the *Pseudomonas aeruginosa* Wt isolated from the municipal waste dumpsite has good bioremediation potential in solution; the mutant's strains generated from acridine orange; *Pseudomonas aeruginosa* MutAc and *Pseudomonas aeruginosa* MutAe showed a better bio-removal capability of Pb from solution, hence these strains could be a probable candidate in bioremediation and bioaccumulation of Pb from solutions.

The *Pseudomonas aeruginosa* strains were resistant to almost all the antibiotics in this study and the MAR index was above 0.2 which indicated that the resistance was point based pollution and from extreme antimicrobial presence in the waste dumpsite. Despite the good performance of *Pseudomonas aeruginosa* Wt and its mutant strains in bio-removal of Pb from solution, the probability of their usage for bioremediation may be slim due to the multiple resistances of the these strains to the commonly used antimicrobials. Antimicrobial resistance is a major menace to public health, and a main concern to global health and their presence in the environment can lead to transfer of resistant genes through the ecological structures into the food chain. The genetic alteration of native flora of a polluted environment could lead to the discovery of strains of bacteria which are good candidate for bioremediation technologies.

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