Heavy Metal Co-Resistance with Antibiotics amongst Plant Growth Promoting Bacteria Isolates from Rhizosphere of Nypa Fruticans

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ABSTRACT

Heavy metal co-resistance with antibiotics has the potential to amplify antimicrobial resistance genes in the environment, and this could have an impact on clinical settings. This study aimed to investigate the presence of heavy metal co-resistance with antibiotics among plant growth promoting bacterial isolates from the rhizosphere of Nypa fruticans. The heavy metals content of the water habouring the Nypa fruticans including copper(0.07mg/L), chromium (2.3 mg/L), lead (4.22 mg/L), iron (12.4 mg/L), cadmium (0.45 mg/L), nickel (3.12 mg/L), arsenic (0.47 mg/L) and zinc (5.37 mg/L) were above acceptable limits of WHO. A total of 10 bacteria including *Bacillus subtilis*, *Bacillus cereus*, *Pseudomonas fluorescens*, *Klebsiella oxytoca*, *Serratia marcescens*, *Achromobacter denitrificans*, *Bulkholderia vietnamiensis* and *Azospirrilum brasilense*, *Azobacter vinelandii* and *Enterobacter aerogenes* were isolated and identified to specie level using 16s RNA gene sequences, which showed above 95 % similarity with reference sequences from GenBank. The isolates showed high resistance to lead, chromium and zinc. Most of the isolates were resistant to streptomycin, ciprofloxacin and augmentin. Also, most of the isolates demonstrated positive growth promoting activities of indole acetic acid production, nitrogen fixation, siderophore production and phosphate solubilization. Pot trials on the effect of heavy metal and antibiotic tolerant bacteria on growth of beans (*Phaseolus vulgaris* l.) in heavy metal polluted soil revealed that beans treated with consortiumof all the isolates recorded high shoot and root length. Hence these isolates can be used for bioremediation of metal polluted site and as well act as plant growth promoting bacterial in agricultural system.

Keywords: Heavy metals, Antibiotics, Resistance, Plant Growth Promoting Bacteria

INTRODUCTION

Heavy metals are naturally occurring elements with a high atomic weight and at least five times the density of water [1]. Their numerous industrial, residential, agricultural, medical, and technical applications have resulted in widespread distribution in the environment, raising worries about their possible impact on human health and the environment. Their toxicity is determined by a variety of factors, including the dose, route of exposure, chemical species, as well as the age, gender, genetics, and nutritional state of those exposed [2].

Heavy metals are needed for microorganisms to function properly in small amounts, but they become toxic at higher concentrations [3, 4]. Certain metals—cobalt, copper, and nickel, for example—act as micronutrients and are employed in redox reactions, as parts of different enzymes, to stabilize molecules through electrostatic interactions, and to control osmotic pressure. However, most metals are nonessential, have no nutrient value, and are potentially toxic to microorganisms. These toxic metals interact with essential cellular components through covalent and ionic bonding. Elevated concentrations of essential and non-essential metals can cause harm to cell membranes, modify the specificity of enzymes, interfere with cellular processes, and deteriorate DNA structure [5]. Because heavy metal pollution can be dangerous at extremely low quantities, it has become a major public health issue. They are non-biodegradable, bioaccumulate in tissues, and biomagnified along the food chain [6, 7].

Lead, chromium, copper, cadmium, mercury, and zinc, are the most significant heavy metal contaminants in the environment that pose a risk to human and animal health and initiate resistance. Heavy metal accumulation in soil and aquatic environment are thought to be major problems threatening the health of people and animals. The growing industrial activities and human population are causing these metals to accumulate in many ecosystems [8, 9]. As a result, indigenous microorganisms have evolved strategies to deal with these heavy metals. Employing the heavy metals as electron acceptor, efflux pump, and complexing them are few of the strategies [10, 11].

Thousands of deaths occur annually as a result of bacteria developing widespread resistance to antibiotics [12]. The most significant issue is the steadily rising number of microorganisms that are resistant to standard antibiotics, including last-resort medications like vancomycin. Resistance genes can spread quickly across the globe, which is a concerning development that calls for international cooperation and has an impact on public health globally [12]. The misuse and or overuse of antibiotics is known to have contributed to the emergence of antimicrobial resistance. To date, there is growing concern over the possibility of antimicrobial resistance transmission via the food chain [13].

In bacterial isolates, co-resistance of heavy metals and antibiotics are linked through similar mechanisms which may be chromosome or plasmid mediated [14]. Because of this synergy, antibiotic resistance genes may be amplified in the environment and potentially spreading to clinical settings [9]. In addition, antibiotic resistant genes are found naturally in low quantity. But research has shown that their prevalence rises when other contaminants including sewage, heavy metals, and crude oil are present [15, 16]. When this occurs, resistant genes are spread between microorganisms through vertical and horizontal route, which can lead to multi-metal resistance and multi-drug resistance, hence, worsening consequences of infectious diseases [9]. Also, these polluted ecosystems may serve as repositories for resistant genes that transition from one environment to another [17].

Plant growth-promoting bacteria (PGPB) represent a huge and heterogenous group of bacteria that can be found as free-living in bulk soil or in rhizosphere, interacting in a mutualistic relationship with a huge variety of plant species [18]. PGPB can act as biofertilizers; promote plant growth due to their ability to solubilize some elements (mainly P, K and Zn), nitrogen fixation and production of siderophores (small molecules able to improve iron uptake capacity) [19]. In addition, PGPB are frequently used in bioremediation techniques to eliminate or render inert soil contaminants, including pesticides, herbicides, solvents, organic compounds, and heavy metals [20]. Lastly, these microbes can be used to assist plants in overcoming biotic and abiotic stressors [21, 22]. Hence, the aim of this study was to determine the heavy metal co-resistance with antibiotics amongst plant growth promoting bacteria isolates from rhizosphere of Nypa fruticans.

RESULTS

Physicochemical Properties of Water Habouring the Rhizosphere of Nypa fruticans

Physicochemical properties of the water bearing the Nypa fruticans is presented in Table 1. The river water at the time of collection had an average slight acidic pH of 5.79 and temperature of 26^{o}C. Dissolved oxygen (12.9 mg/L), Biological Oxygen Demand (33.02 mg/L), and Chemical Oxygen Demand (22.3 mg/L) were higher than the acceptable limit set by the World Health Organization (WHO). Other parameters like conductivity (267 (µS/cm), alkalinity (13.6 mg/L), nitrate (2.8 mg/L), phosphate (8.1 mg/L), sulphate (20.6 mg/L), chloride (25.1 mg/L), Total Dissolved Solids (294.3 mg/L), Total Soluble Solids (77.5 mg/L), potassium (2.8 mg/L), magnesium (10.4 mg/L), calcium (11.3 mg/L), and manganese (0.05 mg/L), were in compliance with WHO.

Heavy Metal Content of Water habouring the Nypa fruticans

The result on heavy metal content of the water habouring the Nypa fruticans is shown in Table 2. All the heavy metals tested, including copper (0.07 mg/L), chromium (2.3 mg/L), lead (4.22 mg/L), iron (12.4 mg/L), cadmium (0.45 mg/L), nickel (3.12 mg/L), arsenic (0.47 mg/L) and zinc (5.37 mg/L) were above the recommended limit set by the WHO.

Table 1. Physicochemical properties of water habouring the rhizosphere soil of Nypa fruticans

Parameters	**Nypa fruticans**	**Acceptable Limit by WHO**
pH	5.79± 0.00	6-9
Temperature	26.0± 0.03	40
Conductivity (µS/cm)	267± 0.00	400
Alkalinity (mg/L)	13.57± 0.00	250
Nitrate (mg/L)	2.82± 0.00	10
Phosphate (mg/L)	8.10± 0.00	5
Sulphate	20.60±0.80	50
Dissolved Oxygen (mg/L)	12.91±0.30	10
Biological Oxygen Demand (mg/L)	33.02±0.25	10
Chemical Oxygen Demand (mg/L)	22.31±0.00	50
Chloride (mg/L)	25.11± 0.00	250
Total Dissolved Solids (mg/L)	294.33±0.00	500
Total Soluble Solids (mg/L)	77.50±0.21	500
Potassium (mg/L)	2.88± 0.11	3
Magnesium (mg/L)	10.42± 0.10	50
Calcium (mg/L)	11.34± 0.00	75
Manganese (mg/L)	0.05± 0.00	0.5

Values are means of duplicates: ± Standard error of the mean, WHO=World Health Organization

Table 2. Heavy metal content of the water habouring Nypa fruticans

Parameters	**Mean±SD**	**Acceptable Limit by WHO**
Copper (mg/L)	0.07± 0.00	0.05
Chromium (mg/L)	2.30±0.01	0.1
Lead (mg/L)	4.22±0.00	0.01
Iron (mg/L)	12.4± 1.1	0.3
Cadmium (mg/L)	0.45±0.60	0.01
Nickel (mg/L)	3.12±0.01	0.2
Arsenic (mg/L)	0.47±0.00	0.03

Zinc (mg/L)	5.37± 0.00	3.00

Values are means of duplicates: ± Standard error of the mean, WHO=World Health Organization

Isolation and Characterization of Bacterial from Rhizosphere of Nypa fruticans

The results of the morphological and biochemical tests are shown in Table 3. The preliminary results revealed that a total of 10 bacteria including two *Bacillus, Klebsiella, Pseudomonas, Azobacter, Serretia, Enterobacter, Achromobacter, Burkholderia* and *Azospirilum* species. *Bacillus* specieswas characterized as rod shaped motile Gram-positive bacteria which showed catalase (+), Voges Proskauer (+), methyl red (+), citrate (+), with H_2S (-). *Burkholderia* was characterized as rod shaped motile Gram-negative bacteria, catalase (-), Voges Proskauer (+), methyl red (+), citrate (+), and H_2S (-). *Klebsiella* species were Gram-negative non-motile rod-shaped bacteria with catalase (+), Voges Proskauer (-), methyl red (-), citrate (-), and H_2S (-). *Serratia* species appeared as Gram-negative motile rod with catalase (+), Voges Proskauer (+), methyl red (-), citrate (+), and H_2S (-). *Pseudomonas* species appeared as Gram-negative motile rod-shaped bacteria with catalase (+), Voges Proskauer (+), methyl red (-), citrate (+), and H2S (-). *Azobacter* species appeared as Gram-positive motile rod-shaped bacteria with oxidase (+), catalase (+), Voges Proskauer (+), methyl red (+), citrate (+), gas (+) and H2S (-). *Serretia* species appeared as Gram-negative motile rod-shaped bacteria with oxidase (-), catalase (+), glucose (+), lactose (-), indole (-), Voges Proskauer (+), methyl red (-), citrate (+), gas (-) and H2S (-). *Enterobacter* species appeared as Gram negative motile rod with oxidase (-), catalase (-), glucose (+), lactose (+), Voges Proskauer (+), methyl red (-), citrate (+), gas production (+), gas (+) and H_2S (-). *Achromobacter* species appeared as Gram negative motile rod with oxidase (+), catalase (+), glucose (-), lactose (+), Voges Proskauer (-), methyl red (+), citrate (-), gas production (+), gas (+) and H_2S (-). *Bulkholderia*-species appeared as Gram negative motile rod with oxidase (+), catalase (+), glucose (+), lactose (+), Voges Proskauer (-), methyl red (+), citrate (+), gas production (+), and H_2S (+).

Azospirillum –species appeared as Gram negative motile rod with oxidase (+), catalase (+), glucose (+), lactose (+), indole (-), Voges Proskauer (-), methyl red (+), citrate (+), gas production (-), and H_2S (-) (Table 3).

Table 3. Bacterial Isolates from rhizosphere of Nypa fruticans

ID	Gram Reaction	Shape	Motility	Oxidase	Catalase	Glucose	Lactose	Indole	Methyl Red	Voges Proskauer	Citrate	Triple Sugar Iron	H_2S	Gas	Probable Organism
P1	+	Rod	+	-	+	+	+	-	-	+	+	-	-	-	*Bacillus* sp.
P2	+	Rod	+	-	+	+	+	-	-	+	+	-	-	-	*Bacillus* sp.
P3	-	Rod	-	-	+	+	+	-	-	+	-	Acid butt /Acid slant	-	+	*Klebsiella* sp.

P4	-	Rod	+	+	+	+	-	-	-	-	+	Alkali butt/ Alkali slant	-	-	*Pseudomonas* sp.
P5	+	Rod	+	+	+	+	+	+	+	+	+	Acid butt /Acid slant	+	+	*Azotobacter* sp.
P6	-	Rod	+	-	+	+	-	-	-	+	+	Acid butt/ Alkali slant	-	-	*Serratia* sp.
P7	-	Rod	+	-	-	+	+	-	-	+	+	Acid butt /Acid slant	-	+	*Enterobacter* sp.
P8	-	Rod	+	+	+	-	+	-	+	-	-	Alkali butt/ Alkali slant	-	+	*Achromobacter* sp
P9	-	Rod	+	-	+	+	+	+	+	-	+	-	+	+	*Burkholderia* sp.
P10	-	Rod	+	+	+	+	+	-	+	-	+	-	-	-	*Azospirillum* sp.

Polymerase Chain Reaction (PCR) Amplification of the 16S rRNA of the bacterial Isolates

The results for PCR amplification of the 16S rRNA of the bacterial isolates is shown in Figure 1. The result revealed that isolates represented Lanes 1 to 10 were positive with expected amplicon sizes of 1492 bp.

Identity of Sequences of the Bacterial Isolates

Basic Logical Alignment Search Tool (BLAST) results of the 16S rRNA gene sequences and identity of the bacterial isolates in National Center for Biotechnology Information (NCBI) provided indications of possible relationships and similarities with reference sequences from GenBank. These relationships and similarities are presented in Table 4. *Bacillus cereus* and *Enterobacter aerogenes* showed 100 % identity similar to that of reference sequences from GenBank. Other identified isolates showed above 98 % similarity with reference sequence.

Resistance of the Isolates to Heavy Metals

Resistance of the bacterial isolates to heavy metals is illustrated in Figure 2. The result showed that *Bacillus subtilis, Bacillus cereus, Pseudomonas fluorescens, Serratia marcescens, Achromobacter denitrificans, Bulkholderia vietnamiensis* and *Azospirrilum brasilense* showed high resistance to lead, chromium and zinc. In contrast, *Klebsiella oxytoca*, *Azobacter vinelandii* and *Enterobacter aerogenes* were susceptible to lead, chromium and zinc. However, lead was more toxic to the isolates followed by zinc and chromium.

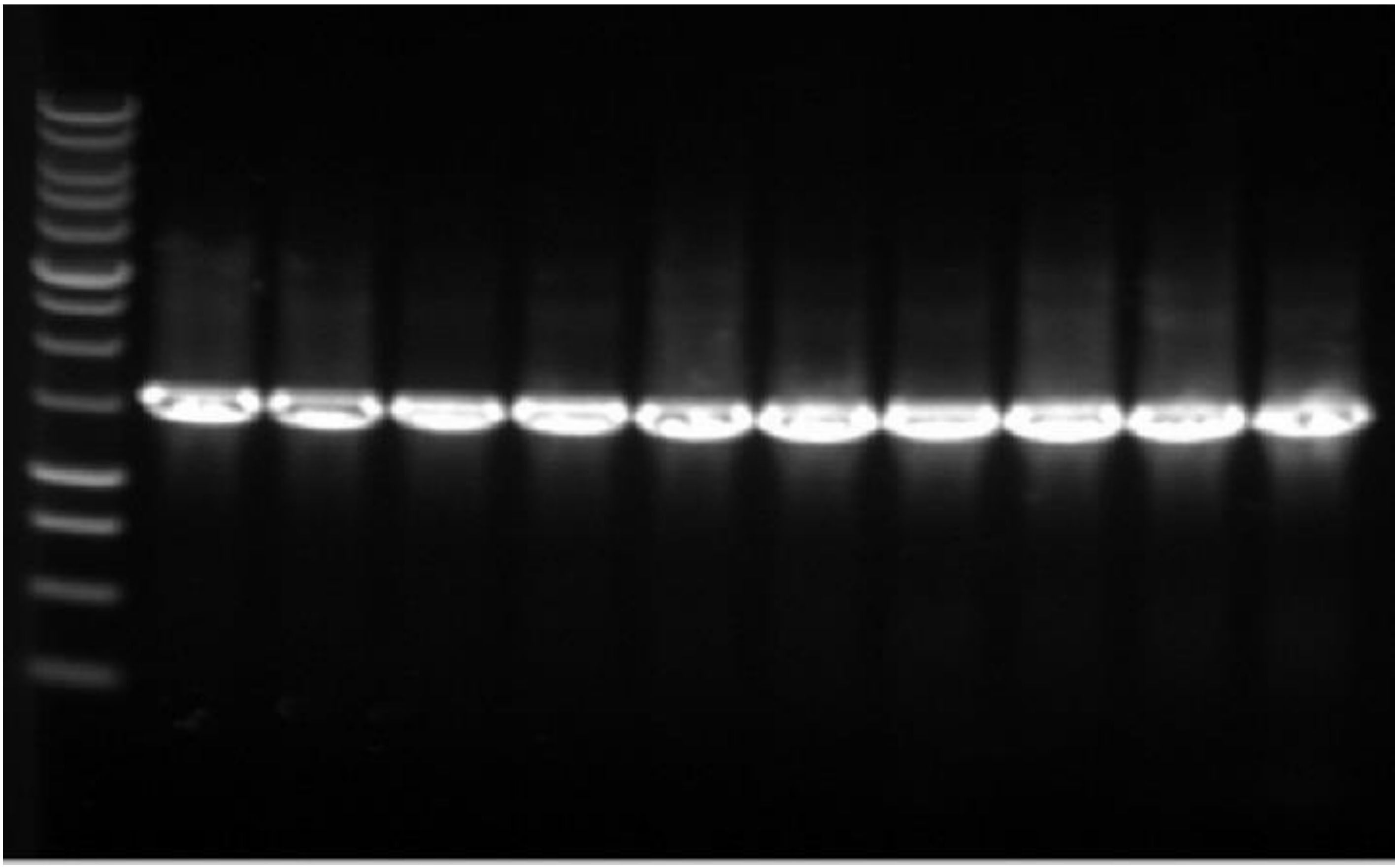

Figure 1 PCR amplification of 1492 bp 16S rRNA gene of 10 bacterial isolates from rhizosphere of Nypa fruticans

Keys: M= DNA ladder, 1kb (Marker), Lanes 1-10 = Isolates P1-P10

Table 4. 16S rRNA gene sequence analysis and identity

Isolate code	**Sequence lengt**	**Max score**	**Query cover (%)**	**E-value**	**Percentage identity (%)**	**Closest Bacterial Identity**
P1	837	1530	100	0.0	99.65	*Bacillus subtilis*
P2	951	1757	100	0.0	100	*Bacillus cereus*
P3	1184	2176	100	0.0	99.75	*Klebsiella oxytoca*
P4	1032	1877	100	0.0	99.90	*Pseudomonas fluorescens*
P5	1030	1858	100	0.0	98.64	*Azotobacter Vinelandii*
P6	815	1495	100	0.0	99.75	*Serratia marcescens*
P7	720	1330	100	0.0	100	*Enterobacter aerogenes*
P8	964	1772	100	0.0	99.79	*Achromobacter denitrificans*
P9	916	1685	100	0.0	99.89	*Burkholderia vietnamiensis.*
P10	1070	1973	100	0.0	99.91	*Azospirillum brasilense*

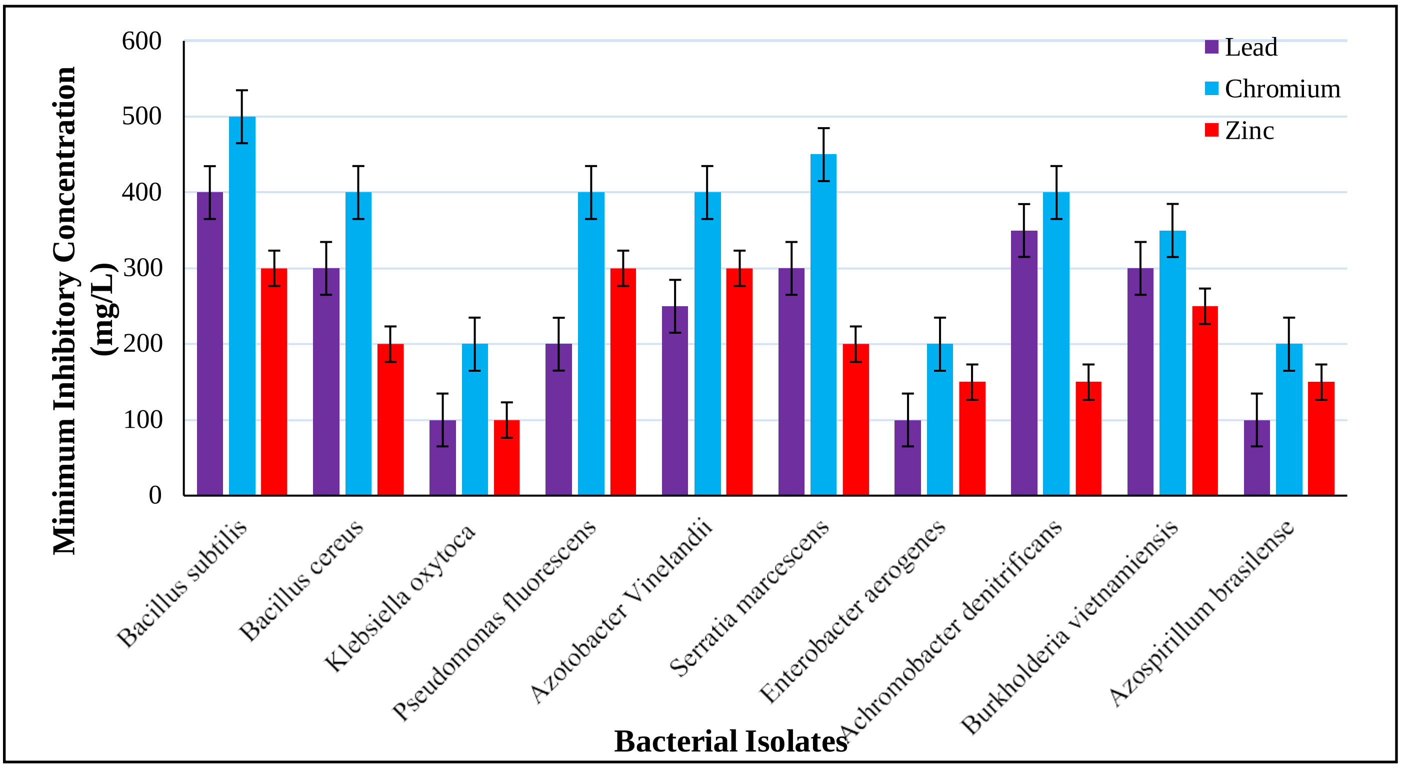

Figure 2. Resistance of the bacterial isolates to heavy metals

Antibiotic Resistance and Susceptibility Profile of the Bacterial Isolates

Antibiotic and susceptibility profile of the bacterial isolates is shown in Table 5. Antibiotic resistance patterns of the isolates revealed that all the 10 bacterial isolates were resistant to streptomycin. However, 3, 2, 5, 4, 3, 9, 6, 2, and 8 out of the 10 bacterial isolates were resistant to cephalexin, cefepime, augmentin, pefloxacin, ceftazidime, gentamyicin, ciprofloxacin, thioredoxin and ofloxacin respectively. *Bacillus subtilis, Bacillus cereus, Pseudomonas fluorescens*, *Serratia marcescens*, *Achromobacter denitrificans,* and *Bulkholderia vietnamiensis* were more resistant to all the test antibiotics than other isolates. The most active antibiotics against the isolates were cephalexin, cefepime, ceftazidime and thioredoxin while streptomycin, augmentin, pefloxacin, gentamyicin, ciprofloxacin, and ofloxacin were less active to the isolates.

Plant growth Promoting Characteristics of the Resistant Bacteria

Plant growth promoting activities of the bacterial isolates resistant to heavy metals and antibiotics are shown in Table 6. All the bacterial isolates were positive to siderophore production, nitrogen fixation, and phosphate solubilization. *Bacillussubtilis*, *Bacillus cereus*, and *Serratia marcescens* showed negative to indole acetic acid production. However, *Pseudomonas fluorescence*, *Azotobacter Vinelandii*, *Achromobacter denitrificans*, and *Burkholderia vietnamiensis* were all positive to indole acetic acid production, siderophore production, nitrogen fixation, and phosphate solubilization.

Table 5. Resistance of the bacterial isolates to antibiotics

Isolate Code	**Bacterial Isolates**	**Antibiotics**									
		S	**CEP**	**CEF**	**AU**	**PEF**	**CTZ**	**CN**	**CPX**	**TRX**	**OFX**
P1	*Bacillus subtilis*	R	R	R	R	R	R	R	R	R	R
P2	*Bacillus cereus*	R	S	S	R	S	S	R	R	S	R
P3	*Klebsiella oxytoca*	R	S	S	S	S	S	S	S	S	R

P4	*Pseudomonas fluorescens*	R	R	R	R	R	S	R	R	S	R
P5	*Azotobacter Vinelandii*	R	S	S	S	S	S	R	S	S	S
P6	*Serratia marcescens*	R	S	S	R	S	S	R	R	R	R
P7	*Enterobacter aerogenes*	R	S	S	S	R	S	R	S	S	S
P8	*Achromobacter denitrificans*	R	R	S	R	S	R	R	R	S	R
P9	*Burkholderia vietnamiensis.*	R	S	S	R	R	R	R	R	S	R
P10	*Azospirillum brasilense*	R	S	S	S	S	S	R	R	S	R

Keys= S= sensitive, R=Resistance, S=Streptomycin, CEP=Cephalexin, CEF=Cefepime, AU=Augmentin, PEF=Pefloxacin, CTZ=Ceftazidime, CN=Gentamycin, CPX=Ciprofloxacin, TRX= Thioredoxin, OFX=Ofloxacin

Table 6. Plant growth promoting characteristics of the resistant bacteria

Isolate Code	**Bacteria**	**Indole Acetic Acid production**	**Siderophore production**	**Nitrogen fixation**	**Phosphate solubilization**
P1	*Bacillussubtilis*	–	+	+	+
P2	*Bacillus cereus*	–	+	+	+
P4	*Pseudomonas fluorescence*	+	+	+	+
P5	*Azotobacter Vinelandii*	+	+	+	+
P6	*Serratia marcescens*	–	+	+	+
P8	*Achromobacter denitrificans*	+	+	+	+
P9	*Burkholderia vietnamiensis.*	+	+	+	+

Effect of Heavy Metal and Antibiotic Tolerant Bacteria on Growth of Beans (Phaseolus vulgaris L.) in Heavy Metal Contaminated Soil

Growth promoting activities of the metal and antibiotic tolerant isolates on growth of beans in the presence of heavy metal polluted soil is shown in Table 7. Beans treated with *Bacillus subtilis* and *Pseudomonas fluorescens* had the highest and lowest shoot length of 14.2 cm and 8.1 cm respectively in the presence of Pb polluted soil. Beans treated with consortium of all the bacterial isolates had the highest root length of 3.7 cm grown in lead polluted soil while the lowest was beans treated with *Pseudomonas fluorescens* with root length of 1.3 cm. Beans treated with *Bacillus cereus*and *Serratia marcescens* had the highest and lowest shoot length of 7.1 cm and 4.1 cm respectively in the presence of chromium polluted soil. Consortium of all the isolates had the highest root length of 3.2cm in chromium contaminated soil while the lowest root length (1.3 cm) was the control without inoculation and metals. For zinc polluted soil, beans treated with *Pseudomonas fluorescens* and *Burkholderia vietnamiensis*had the highest and lowest shoot lengths of 6 cm and 3 cm respectively while control and had the highest (3.9 cm) and lowest (1.5 cm) root length respectively.

Nitrate, Phosphate, and Sulphate Contents of Soil Before and After Growth of Beans

The nitrate, phosphate, and sulphate content before and after growth of beans in the presence of heavy metals are presented in Figure 4.2. Nitrate, phosphate, and sulphate content before growth were 8.65, 2.6, and 2.1 mg/L respectively. After growth of beans in lead polluted, the nitrate, phosphate, and sulphate contents of the soil were 6.88, 1.27 and 1.36 mg/L respectively. Whereas after growth in chromium polluted soil, phosphate, and sulphate contents were 7.6, 0.17, and 0.69 mg/L respectively. However, after growth of beans in the presence of zinc, the nitrate, phosphate, and sulphate contents were 6.45, 0.37, and 0.57 mg/L respectively (Figure 4.2).

Table 4.7: Effect of heavy metal and antibiotic tolerant PGPB on different growth parameters of bean (Phaseolus vulgaris L.) in heavy metal contaminated soil

Treatment (Bacteria+ heavy metal)	**Pb**		**Cr**		**Zn**	
	Shoot length (cm)	**Root length (cm)**	**Shoot length (cm)**	**Root length (cm)**	**Shoot length (cm)**	**Root length (cm)**
Control (no metal, no inoculation)	10.8±0.0	1.45±0.2	4.2±0.0	1.3±0.0	5.7±0.1	3.9 ±0.0
Bacillussubtilis	14.2±0.4	3.5±0.7	6.3±0.5	2.7±0.0	4.2±0.8	3.0±0.9
Bacillus cereus	9.8±0.0	2.9±0.5	7.1±0.0	2.4±0.0	3.3±0.0	2.6±.0.0
Pseudomonas fluorescens	8.1±0.0	1.29±0.0	4.3±0.6	2.9±0.0	6.0±0.0	1.7±0.0
Azotobacter Vinelandii	9.3±0.2	1.7±0.0	5.6±0.0	2.0±0.8	4.0±0.0	2.9±0.0
Serratia marcescens	8.8±0.0	2.7±0.3	4.1±0.3	2.4±0.1	3.7±0.0	1.9±0.7
Achromobacter denitrificans	8.2±0.0	1.5±0.0	5.7±0.7	1.8±0.0	4.6±0.0	1.7±0.0
Burkholderia vietnamiensis.	7.9±0.2	3.1±0.6	6.1±0.3	2.3±0.2	3.8±0.7	1.5±0.0
Consortium	12.1±0.0	3.7±0.0	5.8±0.0	3.2±0.0	5.5±0.5	3.3±1.1

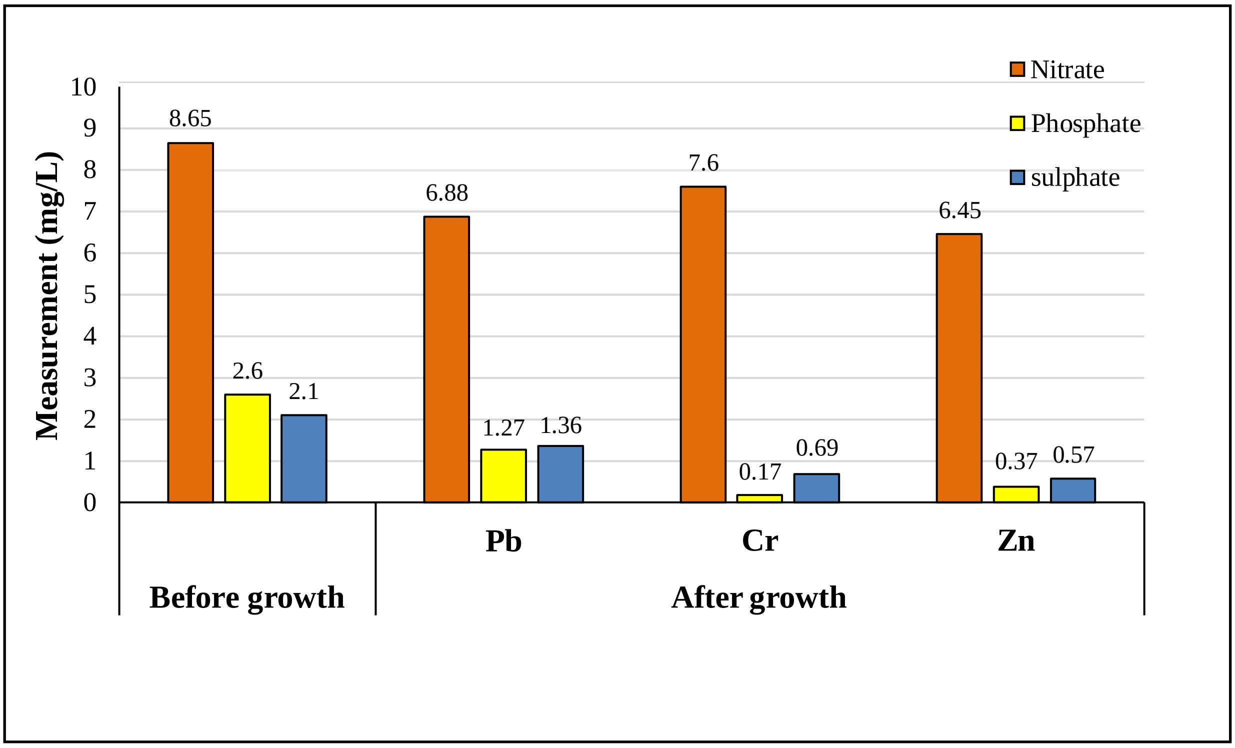

Figure 2. Nitrate, phosphate, and sulphate content of soil before and after growth of beans

DISCUSSION

Physicochemical Characteristics of the Water Habouring the Rhizosphere of Nypa fruticans

The water habouring the Nypa fruticans was highly polluted and unsafe since most of the parameters analyzed were above the acceptable limits of WHO. The river water had a mean slightly acidic pH value of 5.59. The pH of water decides most of the biochemical processes taking place in a particular water body and time [23]. The low pH may be due the presence of soluble organic and inorganic compounds in the water [24]. Similar low pH has been recorded in previous studies [23, 25, 26]. According to Ogbeide and Edene [25], low pH values render water unfit for human consumption. However, According to Inam et al. [27], the pH values reported are favourable for optimal aquatic activities in the studied river. The mean temperature (26 ^{O}C) in this study was found to be in compliance with the standard set by WHO. Temperature is an important factor that affect flora, fauna, chemical, biological and microbial activities in water bodies [28].

Dissolved oxygen (12.9 mg/L), Biological Oxygen Demand (33.02 mg/L), and Chemical Oxygen Demand (22.3 mg/L) were higher than the acceptable limit. These high values may be due to the activities of oil companies within the study area which might have elevated these parameters of the river. This may also affect the chemistry and microbiological activities of the aquatic ecosystem [29].

Other parameters like conductivity, alkalinity, nitrate, phosphate, sulphate, chloride, Total Dissolved Solids, Total Soluble Solids, potassium, magnesium, calcium, and manganese, were in compliance with WHO. Low concentration of nitrate and phosphate and sulphate is indicative of low nutrient in the river which may have been absorbed by aquatic plants alongside heavy metals [30].

Heavy metal content of the samples

All the heavy metals tested, including copper (0.07 mg/L), chromium (2.3 mg/L), lead (4.22 mg/L), iron (12.4 mg/L), cadmium (0.45 mg/L), nickel (3.12 mg/L), arsenic (0.47 mg/L) and zinc (5.37 mg/L) were above the recommended limit set by the WHO. Copper (Cu) is one of the essential elements for human and aquatic life but toxic at high concentrations [31]. It is widely spread in the environment since the metal emanates from both natural and anthropogenic sources [32]. The level of Chromium in this study may have an adverse effect on human and aquatic organisms. Thus, the level of Cr associated with oil exploration and other anthropogenic activities within the study area may not have an immediate negative impact on the environment [23].

Lead is found in air, soil and water due to its widespread applications. It is a well-known element due to its toxicity even at minimal concentration [33, 34]. The Pb emanates from both natural and anthropogenic sources; the major anthropogenic sources include urban, agricultural and industrial wastes. It is also a component of mining and crude exploration processes [35]. The outcome of this study indicates that oil-related and other human activities in the study area might have accelerated the Pb content of aquatic ecosystems. Long-term exposure to the high level of Pb reported in the studied river can cause several health problems including death in both the children and adult populations [36, 37, 38]. It can also impact negatively on the aquatic life of the studied ecosystem [39].

The main sources of Cadmium in aquatic environment are aerial deposition, combustion of fossil fuels, industrial wastes and Cd-related fertilizers in runoffs from adjoining farms [40]. The level of Cd obtained in this study could be detrimental to human and aquatic organisms exposed to the studied river for a long time [41]. Consequently, anthropogenic activities including crude oil exploration carried out within the study area may have contributed a significant level of Cd to the environment [42].

The high levels of Ni reported can cause health problems such as cancer, allergy and improper functioning of the lungs [43]. It has also been reported that a high level of Ni has detrimental effects on aquatic organisms [44]. The reported high level of Ni might have contributed to the studied ecosystem through the anthropogenic activities in the area [23]. The level of Zinc obtained in this work could be essential for both the human and aquatic lives. This is also an indication that human activities within the studied system may not have contributed a significant quantity of Zn to the environment [45]. Arsenic is one of the non-essential heavy metals found in the environment. Its concentration in ingestible items suggests contamination [46].

Isolation and Identification of Bacterial Isolates

The bacterial isolates were subjected to Gram reactions, biochemical characterizations, PCR and sequencing to identify the organisms to specie level. The ten major bacteria isolated from the rhizosphere of Nypa fruticans were *Bacillus subtilis*, *Bacillus cereus*, *Pseudomonas fluorescens*, *Serratia marcescens*, *Achromobacter denitrificans*, *Bulkholderia vietnamiensis*, *Azospirrilum brasilenseKlebsiella oxytoca*, *Azobacter vinelandii*, and *Enterobacter aerogenes*. Tang *et al*. [47], Fernando and Cruz [48], and Cruz and Cadiente [49], in their research work isolated similar organisms from rhizosphere and root of Nypa fruticans.

Heavy metal Tolerance

The bacteria in this study showed high resistance to heavy metals. These metal-resistant PGPB could enhance plant metal tolerance by improving detoxification rates of plants, enzymes secreted by plant roots, and soil pH modification [50, 51]. Previous studies show that similar bacteria isolates have significant heavy metal tolerance [52, 53]; which agrees the results of the current study. The high levels of resistance found among the bacterial isolates are probably attributed to past or present metal contamination in the growing environment [54, 55]. According to Pagnucco et al [53], metal tolerance in bacteria is an essential trait and may help in developing an effective process for the treatment of heavy metal contaminated environment.

Antibiotic Resistance by the Bacterial Isolates

Most of the bacterial isolates were resistant to antibiotics. Antimicrobial resistance (AMR) is one of the most serious global public health threats in this century [56]. Bacterial resistance to antibiotics is a major global health concern, caused by the misuse and overuse of antimicrobial agents [57], which has led to microorganisms (including bacteria, fungi, viruses, and parasites) becoming resistant to the effects of these medications. This imprudent use of antimicrobials in both the human and animal sector has resulted in the selection of pathogens resistant to multiple drugs. According to Mahdi et al. [56], the origin of acquired antibiotic resistance by PGPB could be the exposure of soils to anthropogenic activity or the production of antibiotics by antibiotics-producing bacteria inhabiting the soil.

Plant Growth Promoting Bacteria

In this study, the isolates were evaluated in terms of plant growth promotion (PGP) activities (Indole Acetic Acid, siderophore production, nitrogen fixation and phosphate solubilization). It was observed that most of the isolates have three or more PGB activities. Several studies showed that rhizosphere bacteria stimulate plant growth and development under stress conditions. These changes in growth and development of beans in metal polluted site in the presence of resistant rhizobacterial isolates might be due to involvement of single or multiple possible mechanisms of actions i.e. solubilization of insoluble phosphate [58, 59].

Siderophores are iron-chelating compounds secreted by microorganisms to absorb iron from their surroundings. Antagonistic microorganisms that produce siderophores inhibit the growth of plant pathogens and increase plant growth by recruiting iron to the root area [60].

phosphate solubilization by soil microorganisms is an important factor for plants as they provide a major nutrient required for plant growth. Many bacteria play an important role in phosphate solubilization and bioavailability by producing organic acids. Several reports have indicated that different bacterial species, particularly rhizosphere colonizing bacteria, have the ability to liberate organic phosphates or to solubilize insoluble inorganic phosphate compounds such as tricalcium phosphate, dicalcium phosphate, hydroxyapatite, and rock phosphate [61, 62]. These bacteria make available the soluble phosphates to the plants, and in return gain root borne carbon compounds, mainly sugars and organic acids, necessary for bacterial growth [64]. Phosphate Solubilizing Bacteria (PSB) may also be useful in the phytoremediation of heavy metal impacted soil or for bioleaching of rare Earth elements for mined ores [65, 66].

Apart from their ability to promote plant growth, PGPRs have also been recognized to improve the health of plants by enhancing their defense system by the mechanism of ISR [67]. As an important rhizosphere growth-promoting bacteria, isolates from this study can simulate the synthesis of plant hormones to directly regulate plant growth and development. It promotes plant growth by increasing the nitrogen and minerals in the soil that can be utilized by plants. This agrees with the findings of Wu et al. [68], who tested similar bacteria isolates (*Pseudomonas fluorescens*) to nitrogen fixation.

CONCLUSION

Heavy metal pollution and antibiotic resistance bacteria have been found in all kinds of environment including those that are pristine and has the potential to cross into clinical settings with huge public health significance. The study evaluated the co-resistance of heavy metals with antibiotics amongst isolates from rhizosphere of Nypa fruticans plant. Isolates that showed multiple drug resistance were also able to tolerate higher concentrations of heavy metals utilized in the study. These findings suggest that pollutants like heavy metals could have their tolerance genes co-evolving with antibiotic resistant gene in a synergistic manner. Furthermore, our MDR isolates showed heavy metal resistance that was dependent on the concentration of the heavy metals.

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