Antibiogram and Heavy Metal Tolerance Patterns of *Pseudomonas* Isolates Collected from Imus River Watershed in Cavite, Philippines

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Abstract: This study was conducted to identify the species of *Pseudomonas* present in the Imus River Wateshed and determine the antibiotic resistance and metal tolerance patterns of *Pseudomonas* isolates. Water samples were collected from five stations, namely: Balite Bridge (Station 1), Dasmariñas Bridge (Station 2), Palanan Bridge (Station 3), Imus Toll Bridge (Station 4) and Island Cove Bridge (Station 5). The highest total plate count and *Pseudomonas* count was obtained in Dasmariñas Bridge Station (1.40×10^8 and 9.65×10^7), followed by Palanan Bridge Station (1.92×10^5 and 9.7×10^3), Island Cove Balite Bridge Station (1.64×10^4 and4.4×10^2), Bridge Station (1×10^4 and 3.4×10^2) and Imus Toll Bridge Station (5.2×10^3 and 4.01×10^3). The *Pseudomonas* isolates were identified as *P. aeruginosa* and P. *stutzeri*. Antibiotic susceptibility testing revealed that all isolates were found resistant to ampicillin, amoxicillin, cephalexin and erythromycin, and susceptible to streptomycin, ofloxacin, doxycycline and tetracycline. All *P. stutzeri* showed resistance to clindamycin compared to 77.89% of *P. aeruginosa* isolates. Twenty-one (22.11%) and 74 (77.89%) isolates of *P. aeruginosa* exhibited tetra- and penta-drug resistance, respectively while *P. stutzeri* isolates exhibited penta-drug resistance. Majority of *Pseudomonas* isolates were resistant to 400 ppm concentration of heavy metals with exception of mercury and cobalt. They were found tolerant to 800 ppm concentration of zinc and arsenic. Penta- and hexa-tolerance of isolates were observed at 400 ppm concentration of heavy metals while bi- and tri-tolerance were recorded on 800 ppm concentration of heavy metals.

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Keywords: pollution, bioindicator, public health, environmental health

1. Introduction

Environmental pollution is the addition of any material (solid, liquid, or gas) or even energy (such as heat, sound, or radioactivity) to the environment at a rate that is greater than its capacity for dispersion, dilution, decomposition, recycling, or storage in some harmless form. The three main categories of pollution air, water, and land are typically categorized according to their effects on the environment **(Nathanson.J.2022)**. Water pollution happens when dangerous substances, frequently chemicals or microbes, infiltrate a stream, river, lake, ocean, aquifer, or other body of water, the water quality deteriorates and they make the water poisonous for both biotic and abiotic components in the environment. Water is highly susceptible to pollution. The fact that a water is considered as the universal solvent, more substances can be dissolved by water than by any other liquid on earth. One of the many water pollutants is the harmful toxic heavy metal. Metallic substances with a relative high density compared to water are referred to as heavy metals. Despite the fact that heavy metals are naturally occurring substances that are present throughout the earth, most environmental pollution and human exposure are caused by anthropogenic activities like mining and smelting operations, industrial production and use, as well as domestic and agricultural use of metals and compounds that contain metals **(Tchounwou.P.2018**). As a result of several human activities such as agriculture, combustion, corroded underground pipes, industrial plants, sewage, smelting, and vehicles, heavy metals primarily infiltrate aquatic habitats. Since heavy metals cannot be easily to dissolved in water, they typically bind to suspended particles, which ultimately settle as sediment. Thus, in an aquatic environment, heavy metals can enter the food chain and become available for bioaccumulation **(Algul.F & Beyhan.M.2020)**. Microorganisms are extremely susceptible to heavy metal contamination and are crucial to the ecosystem's energy and material movement. Elements such as Chromium (Cr), Lead (Pb), and Zinc (Zn) showed generally negative relationships with the relative abundance of bacteria, and heavy metals had a major impact on how microbial communities were structured **(Li.C. et al.2020)**.

The Department of Environment and Natural Resources reported that 180 of the country's 421 rivers and other bodies of water are so severely contaminated that they could soon be deemed biologically dead. The DENR investigation revealed that home sewage was the main offender, followed by commercial and industrial wastes **(Bengwayan.M.2019)**. Imus river is among the significant rivers that needs to be studied, Imus River passes through the municipalities of Silang, Dasmariñas City, and Tagaytay City after starting in Tagaytay City **(Braaten.H. et al.2021)**. The river is one of the six significant rivers in the province of Cavite with a length of 38.4 km. These rivers discharge into Manila Bay, a significant source of pollution. Region IV-A (CALABARZON), which includes Cavite, has experienced rapid urbanization and human settlement, resulting in significant changes. The province's rivers now contain more pollution than before **(Rubio.J. et al.2022)**.

2.Significance Of The Study

Some microorganisms tolerate a vast number of chemicals and pollutants when present in an environment. One example of these is the group of *Pseudomonas* spp. They have the advantages over coliforms as a fecal pollution indicator because they are primarily associated with human feces and have better survival characteristics than the coliform groups. If *Pseudomonas* spp. were proven present in the river, awareness about its presence can be used to prevent epidemic outbreak of *Pseudomonas* spp. infection in the community. Thus, hospital and local clinical laboratories can identify diseases or illnesses caused by *Pseudomonas* spp.

and administer proper medications as well as give precautions for information exchange. Along with the above idea, environmental administrators and the local government can design rehabilitation projects for polluted river such as bioremediation using effective microorganisms.

3.Review Of Related Studies

Water is thus so easily contaminated. It is easily dissolved and mixed with toxic compounds from companies, municipalities, and farms, which results in water pollution **(Denchak.M.2018**). Human activity is typically the cause of water contamination. It is the product of human behavior carried out for one's own benefit. These could be categorized under the different human activities that cause pollution. The main factors of pollution are the expansion of the human population, industrialization, and agricultural activities. Overcrowding in urban areas causes water contamination to worsen. The main contaminants in intertwining habitats are household, industrial, and agricultural wastes **(Owa.F.2013)**.

Pseudomonads is one of the most dangerous fish infections, it can cause hemorrhagic septicemia and ulcerative syndrome. Many bacterial infections have an impact on a variety of aquatic species and cause significant economic losses on a global scale. According to estimates, bacterial infections cause up to 50% of farm fish to die before being marketed. The major causes of the economic losses are considered to be low growth, high mortalities, and poor flesh quality. Although Pseudomonas aeruginosa is a normal component of fish microbiota, under stressful circumstances like malnutrition and overcrowding, the bacteria have evolved into highly opportunistic and pathogenic forms that can lead to serious illness like hemorrhagic septicemia, gill necrosis, abdominal distension, splenomegaly, friable liver, and congested kidney **(Algammal.A.2020)**.

In Turkey, the frequency of Pseudomonas species in freshwater fish and water from aquaculture farms was assessed on a monthly basis between 2013 and 2017 and during the first two seasons of 2018. The study's farms were spread throughout six distinct Turkish regions. 90 Pseudomonas strains in total, divided into 20 species, were isolated from water and both sick and healthy fish. The oxidase and catalase activity, hemolysis, tolerance to temperature and sodium chloride, generation of fluorescent pigments, and antibiotic resistance spectra were used to characterize the strains' phenotypic characteristics. This is the first report on the isolation of multiple Pseudomonas species from freshwater salmonid fish, including P. brenneri, P. defensor, P. haemolytica, P. lactis, P. lundensis, P. lurida, P. mandelii, P. meridiana, P. migulae, P. proteolytica, P. simiae, and P. Two of these species, P. haemolytica and P. lactis, have been isolated for the first time from fish farms, a setting very dissimilar to the raw milk that served as the basis of their initial isolation. Seven allegedly brand-new Pseudomonas species were also isolated from water and raised in rainbow trout farms. Many Pseudomonas species were found to have moved from the Aegean and Central Anatolia to the Eastern Anatolia and Black Sea regions between 2013 and 2018 **(Duman.M.2021)**.

The ability of a metal to have harmful effects on microorganisms is known as heavy metal toxicity, and it depends on the bioavailability of the metal and the amount or the dose received. Several mechanisms contribute to heavy metal toxicity, including disruption of lethal enzymatic processes, acting as redox catalysts in the generation of reactive oxygen species (ROS), destroying ion control, and directly influencing DNA and protein synthesis. The presence of heavy metals can change the physiological and biochemical characteristics of microorganisms. Both chromium (Cr) and cadmium (Cd) can cause oxidative damage and

denaturation in microorganisms as well as reduce their ability to bioremediate an environment **(Igiri.B. et al.2018).**

Pseudomonas strains are often carriers of plasmids containing genes that confer the capacity for antibiotic resistance **(Todar.K.2004)**. If the notion that R factors originated in antibiotic producing organisms were correct, it is possible that *Pseudomonas* strains were among the first groups of organisms to have received these factors from streptomycetes as a consequence of their association in the same ecological niches. *P. aeruginosa* is resistant to many antibiotics but some antimicrobial agents like ceftazidime, ticarcillin, piperacillin, imipenem, meropenem, gentamicin, tobramycin **(Todar.K.2004; Volk.W. & Wheeler.M.1988)**, amikacin, ciprofloxacin and aztreonam **(Todar.K.2004)**, colistin and carbenicillin **(Volk.W. & Wheeler.M.1988)** can counteract it.

4.Objectives Of The Study

- identify the species of *Pseudomonas* present in Imus River Watershed
- determine the antibiotic resistance and metal tolerance patterns of *Pseudomonas* spp. isolated from Imus River Watershed

5.Hypotheses Of The Study

- There is no species of *Pseudomonas* which is tolerant to antibiotic and heavy metal.

6. Study Area

The study area is the Imus River watershed covers four towns of Cavite that include Silang, Dasmarinas, Imus, and Bacoor. It stretches from Balite II, Silang and ends at Island Cove-Binakayan, Kawit. The sites of sampling were Balite Bridge in Silang, DLSU-Dasmariñas Station in Dasmarinas, Imus Toll Bridge and Palanas Bridge both located in Imus, and the Island Cove Bridge in Kawit. They are the busiest places for transportation and commercialization of different establishments appropriate for the study of the Imus River Watershed.

6.1.Statistical Techniques Used in the Present Study

Water Sampling

There were three sampling sites per sampling station in Imus River. Each sampling site had a five meter interval. With the use of a boat, samples were collected from the upstream region starting from Silang down to Kawit. Collection of samples was done in a position equating to the draw off point. Bottles were held on to the base and plunged below the surface of the water. The bottle was turned upward slowly with the mouth directed against the current (when there was no current, the bottle was moved away slowly by hand). The containers were filled with water leaving ample space of at least 2.5cm from the mouth. The containers were closed immediately. The samples were placed in a container with ice right after sampling.

Preparation of Culture Media

Culture media were prepared according to standard procedures. The media used were Plate Count Agar (PCA) and Cetrimide Agar (CA). They were sterilized using an autoclave at 115 lbs / in^2 for 15-20 minutes.

Total Count and Viable Count of *Pseudomonas* spp.

Water samples were serially diluted to 10^{-5} to 10^{-7}. One tenth milliliter of water sample was spread onto the CA and PCA plate with the use of an L-rod. After 24-48 hours of incubation at 37^0 C, colonies were counted and viable counts (cfu/ml) were computed using the formula:

cfu / ml = (average number of colonies x DF)/volume plate

The percentage of *Pseudomonas* spp. present was computed using the formula:

% of *Pseudomonas* spp. = cfu *Pseudomonas* / total cfu x 100

Characterization of *Pseudomonas* spp.

Pseudomonas spp. isolates were picked out from the plates containing the highest dilutions. They were purified by streaking on agar plates until only one type of colonies was observed.

Physiological tests were used to identify *Pseudomonas* spp. These included fermentation of carbohydrates (glucose, maltose, lactose, sucrose, and mannitol), Methyl Red test and Voges-Proskaeur test, Indole Production, Hydrogen Sulfide Production, Lysine Decarboxylase, Phenylalanine Deamination test, Citrate Utilization, and Urea Hydrolysis.

Identification and Classification of *Pseudomonas* spp.

Identification was based on characteristics compared to the list in the Bergey's Manual on Determinative Bacteriology (9^{th} edition). Figure 2 shows the schematic diagram for *Pseudomonas* spp. identification.

Antibiotic Resistance Testing

The isolates were grown on NA. They were replica plated on PCA supplemented with 100 ppm of ampicillin, amoxicillin, cephalexin, erythromycin, streptomycin, ofloxacin, doxycycline, tetracycline, and clindamycin. NA without any antibiotics served as the control. The plates were incubated for 24-48 hours at 30^0 C (Ilagan *et al*. 2004).

The percentage of *Pseudomonas* spp. resistant to different concentrations of antibiotics was computed using the formula:

% of *Pseudomonas* spp. = frequency/total number of isolates x 100

Metal Tolerance Testing

The *Pseudomonas* isolates were grown on Nutrient Agar (NA) plates. They were replica plated on PCA containing 0 (control), 100, 150, 200, 250, 400, 800 and 1600 ppm of each metal. The metals tested include copper, manganese, zinc, arsenic, mercury, iron, cobalt, and lead using iron sulphate, copper sulphate, lead acetate, zinc sulphate, mercuric chloride, manganese sulphate, arsenic oxide and cobalt chloride, respectively. The plates were incubated for 24-48 hours at 30^0 C (Ilagan *et al*. 2004).

The percentage of *Pseudomonas* spp. tolerant to different concentrations of metals was computed using the formula:

% of *Pseudomonas* spp. = frequency/total number of isolates x 100

Preservation and Maintenance of Cultures

All isolates were subcultured and preserved in 2 ml distilled water stock.

6.2.Data Analysis and Interpretation

Identity of *Pseudomonas* Isolates

The 95 (94.06%) out of 101 isolates were *Pseudomonas aeruginosa* while six (5.94%) were identified as *Pseudomonas stutzeri*. In terms of morphological characteristics, all isolates are gram-negative and irregular in appearance, with undulate margin, raised elevation, smooth surface, and viscous consistency. They varied in colony color and pigmentation, some were green to yellow, blue to green and white. *Pseudomonas aeruginosa* is a free-living bacterium, commonly found in soil and water. *P. aeruginosa* is tolerant to a wide variety of physical conditions which include temperature and moisture. It is resistant to high concentrations of salts and dyes, weak antiseptics, and many commonly used antibiotics.

It was possible to isolate *P. aeruginosa* from unpolluted water. This claims that *P. aeruginosa* is a penetrating species that can be found in sewage, water, and soil and can enter water following rainfall. In contrast, they found that Psuedomonas numbers in the Nile River were greater in the summer and lower in the winter. It was reported that Psuedomonas counts were higher during the rainy season in the Ganga river in India. Moreover, greater Psuedomonas counts have been observed in the summertime in the St. Clair River in Sarnia. The current study, in which greater numbers of Psuedomonas aeruginosa were seen in summer, followed by monsoon, and least in winter, provides strong support for the

aforementioned finding. Due to increased nutrient concentration, organic matter, and a decrease in water volume, higher counts are seen in the summer **(Bhasin.S et al.2020).**

According to the bacterial count, the Karoon River had a high microbial load during the spring and summer months. The Enterobacteriaceae family (which includes Escherichia coli, Salmonella, Shigella, Klebsiella, and Enterobacter) and Pseudomonas aeruginosa accounted for the majority of the species identified from these samples. In every station, P. aeruginosa and E. coli were the most prevalent contaminants. coli and Klebsiella sp. was seen in the summer, and winter, fall, and autumn seasons, respectively, had the lowest levels of contamination with these bacterial species. *Shigella* sp., *Salmonella* sp, and *Enterobacter* sp. was highest in the spring and lowest in the fall. As in summer season, the environmental temperature is more conducive for growth of these bacteria and the maximum bacterial number was reported in summer season **(Besharati.S. et al.2018)**.

Table 1.0. Identity of *Pseudomonas* Isolates

Identity	**Number**	**Percentage**
Pseudomonas aeruginosa	95	94.06
Pseudomonas stutzeri	6	5.94

Resistance of *Pseudomonas* Isolates to Different Antibiotics

All isolates were tested for their resistance to nine different antibiotics, namely: Ampicillin, Amoxicillin, Cephalexin, Erythromycin, Streptomycin, Ofloxacin, Doxycycline, Tetracycline and Clindamycin. All isolates (101) were resistant to ampicillin, amoxicillin, cephalexin and erythromycin. On the other hand, 77.89% (74) of *P. aeruginosa* were resistant to clindamycin and all (6) isolates of *P. stutzeri* were resistant to clindamycin. All isolates (101) were susceptible to streptomycin, ofloxacin, doxycycline and tetracycline.

Antibiotics were first isolated from mold and bacterial products that exhibited a selective toxicity to many infectious agents but showed little or no toxicity toward mammalian cells. Antibiotics given to different animals and excreted to either soil or water after they were administered in animal husbandry, fish farming and other fields are called environmental antibiotics **(Mavrodi.D. et al.2006)**. Although they help cure many diseases, their presence in the environment may alter the ecology of watersheds and may produce hazardous strains of antibiotic-resistant bacteria **(Kummerer.K.2004; Mavrodi.D. et al.2006)**. This case pose an alarm to the public health. According to **Reynolds.D & Kollef.M (2021)**, *Pseudomonas aeruginosa*, which causes pneumonia, surgical site infections, urinary tract infections, and bacteremia, is a frequent source of nosocomial infections. *P. aeruginosa* is thought to be present in 7.1%–7.3% of all illnesses connected to healthcare. Over the past ten years, prevalence has increased. It is the cause of an even larger proportion of healthcare-associated infections in patients in intensive care units (ICUs). It caused 23% of all ICU-acquired infections, according to a large multinational observational point-prevalence study of infections in ICU patients, with a respiratory source being the most frequent location of P. aeruginosa infection.

Table 1.1. Percentage of *Pseudomonas* spp. resistant to different antibiotics

Antibiotic Classification	**Specific Antibiotic**	**Percentage**	
		P. aeruginosa **n= 95**	***P. stutzeri*** **n= 6**
Beta-Lactams	Ampicillin	100	100
	Amoxicillin	100	100
Cephalosporin	Cephalexin	100	100
Macrolide	Erythromycin	100	100
Aminoglyside	Streptomycin	0	0
Quinolone	Ofloxacin	0	0
Tetracycline	Doxycycline	0	0
	Tetracycline	0	0
Others	Clindamycin	77.89	100

Antibiotic Resistance Patterns of *Pseudomonas* Isolates

Two types of resistance patterns were exhibited by *Pseudomonas* spp. These are tetra-resistance and penta-resistance.Tetra-resistance was exhibited by 21 (22.11 %) *P. aeruginosa* isolates. Penta-resistance was exhibited by 74 (77.89%) *P. aeruginosa* isolates and 6 (100%) *P. stutzeri* isolates. According to **Chegini.Z. et al. (2020)**, one of the most significant bacterial infections is the Multi-Drug Resistant (MDR) *Pseudomonas aeruginosa* causes illness with a high death rate due to resistance to many drugs. This bacteria drives significant tissue damage with various determinants of virulence, and its biofilm development produces persistent and antibiotic-resistant infections. Thus, due to the non-applicability of antibiotics for the elimination of *P. aeruginosa* biofilm, alternative ways have been studied by researchers, and phage therapy is one of these novel therapeutic solutions. By degrading the extracellular matrix, enhancing antibiotic penetration into the inner layer of the biofilm, and preventing the quorum-sensing activity, bacteriophages can be employed to eliminate *P. aeruginosa* biofilm.

In the study of **Lee.N. *et al.* (2004)**, clinical bacteremia caused by *P. aeruginosa*, *P. putida* and *P. stutzeri* (group of metallo beta-lactamase producers) was regarded as a therapeutic challenge disease since *Pseudomonas* spp. are multidrug-resistant. *Pseudomonas* isolates from rivers exhibited multiple antibiotic resistance. Seven isolates exhibited resistance to eight antibiotics, 20 isolates to seven, 21 isolates to six, 26 isolates to five, 36 isolates to four, 23 isolates to three and seven isolates to two antibiotics. The antimicrobial agents used were β-lactams, macrolide, aminoglocosides, quinolones, tetracyclines and clindamycin. They concluded that multiple antibiotic resistance of *Pseudomonas* could have been acquired by gene transfer **(Ilagan.Y. et al.2005)**.

Table 2.0. Antibiotic resistance patterns of *Pseudomonas* spp.

Resistance Patterns	Isolate Identity	No.	%	Response to Different Antibiotic Concentration (100ppm)								
				Amp	Amo	Cer	Ery	Str	Cef	Dox	Tet	Cl
Tetra-R	*P. aeruginosa*	21	22.11	+	+	+	+	-	-	-	-	-
Penta-R	*P. aeruginosa*	74	77.89	+	+	+	+	-	-	-	-	+
	P. stutzeri	6	100	+	+	+	+	-	-	-	-	+

Ap – Ampicillin; Of – Ofloxacin; Am – Amoxicillin; Do – Doxycycline;

Ce – Cephalexin; Te – Tetracycline; Er – Erythromycin;

Cl – Clindamycin; Sr – Streptomycin

Tolerance of *Pseudomonas* Isolates to Different Metals

Pseudomonas spp. isolates were tested for tolerance to different heavy metals at concentrations ranging from 100 ppm to 1,600 ppm similar to the concentration used by **Agustin.J (2005)**. Majority of *Pseudomonas* isolates were resistant to different metals at 100 to 400 ppm which also shows as the concentration of metal increases the percentage of isolates decreases. Although this is not true to all metals. According to the study of **Ojewumi.M. et al. (2018)**, *Pseudomonas aeruginosa* impacts on the bioremediation of soil that has been polluted by different quantities, 5% w/w and 8% w/w, of raw (for imitating oil spills from well-heads) and processed (for simulating oil spills from flow lines/storage tanks) crude oil. The Nigerian Escravos Light blend of crude oil fouling soil samples was used to get absorbance measurements using UV/VIS spectrophotometry equipment. This 30-day measurement design also models light and heavy onshore oil spillage scenarios.

All isolates were resistant to copper at 100 ppm to 250 ppm concentrations. The percentage of isolates decreased abruptly at 400 pp until none to 800 ppm and 1,600 ppm. All *Pseudomonas* isolates were resistant to manganese at 100 ppm to 400 ppm concentrations but all isolates were susceptible to 800 and 1,600 ppm. All isolates were tolerant to zinc most probably because zinc is an essential element for microbial metabolism. All *Pseudomonas* isolates were tolerant to arsenic. Very few (1.98%) isolates were tolerant to mercury only 1.98% tolerated 100 ppm concentration while 0.99% tolerated 150 ppm. All isolates were sensitive to at least 200 ppm. All isolates were tolerant to as high as 400 ppm concentration of iron. All isolates were sensitive to 800 ppm and 1,600 ppm concentrations of iron. The percentage of isolates tolerant to 100 ppm, 150 ppm and 200 ppm concentrations of cobalt was 8.91% while 0.99% of isolates was tolerant to 250 ppm cobalt concentration. All isolates were sensitive to 400 ppm and higher concentrations. All isolates were tolerant to 400 ppm concentration of lead and below while all were susceptible to 800 ppm and 1,600 ppm concentration.

Table 2.1. Percentage of *Pseudomonas* isolates tolerant to different heavy metals.

Concentration (ppm)	**Percentage of Isolates Tolerant to Different Metal Concentrations**							
	Cu	**Mn**	**Zn**	**As**	**Hg**	**Fe**	**Co**	**Pb**
100	100	100	100	100	1.98	100	8.91	100
150	100	100	100	100	0.99	100	8.91	100
200	100	100	100	100	0	100	8.91	100
250	100	100	100	100	0	100	0.99	100
400	5.94	100	100	100	0	100	0	100
800	0	0	100	100	0	0	0	0
1600	0	0	100	100	0	0	0	0

Metal Tolerance Patterns of *Pseudomonas* Isolates

There was observed metal tolerance pattern of *Pseudomonas* at 400 ppm (Table 2.2). Penta-tolerance was exhibited by 89 (93.68%) *P. aeruginosa* isolates and 6 (100%) *P.stutzeri* isolates. Penta-tolerance was observed on combination of manganese, zinc, arsenic, iron and lead. On the other hand, six isolates of *P. aeruginosa* exhibited hexa-tolerance.Table 2.3 shows the tolerance pattern of *Pseudomonas* at 800 ppm. Bi-tolerance (zinc and arsenic) was exhibited by 74 (77.89%) isolates of *P. aeruginosa* and 6 (100%) isolates of *P. stutzeri.* Tri-tolerance was exhibited by 21 (22.11%) isolates of *P. aeruginosa* (zinc, arsenic and manganese). According to the study of **Raja.C & Selvam.G (2009)**, *Pseudomonas* spp. showed resistance to a variety of toxic substances, heavy metals and antibiotics and can use various compounds as carbon sources. According to **Sah.S et al. (2021)**, Siderophores are low molecular weight, water-soluble, organic ligands having affinity towards iron-binding compounds or iron carriers. Iron (Fe) is the fourth most common element on earth. It is naturally found as Fe (II), but in aerobic conditions, Fe (II) oxidization takes place, converting it to Fe (III). Because this type of iron is insoluble, living things cannot use it for metabolism. Siderophores produced predominantly by gram-negative bacteria, such as genera of *Enterobacter* sp. and *Pseudomonas* sp., exhibit substantial molecular and physiological implications for biocontrol of rhizospheric phytopathogens. Higher production of heterologous siderophores in the rhizospheric Pseudomonas sp. via Fe (III) chelation suggests the inhibition of other microbes with low iron affinity in the rhizosphere. Moreover, the binding of Ferric ion in phytopathogens reduces the competition in the rhizosphere

Table 2.2. Tolerance patterns of *Pseudomonas* spp. at 400 ppm metal concentration

Tolerance Pattern	**Isolate Identity**	**N**	**%**	**Tolerance to Different Heavy Metal Concentration (400ppm)**							
				Cu	**Mn**	**Zn**	**As**	**Hg**	**Pb**	**Co**	**Pb**
Penta-T	*P.aeruginosa*	89	93.68	-	+	+	+	-	+	-	+
	P.stutzeri	6	100	-	+	+	+	-	+	-	+
Hexa-T	*P.aeruginosa*	6	6.32	+	+	+	+	-	+	-	+

**Total number of P. aeruginosa = 95; P. stutzeri = 6*

Table 2.3. Tolerance patterns of *Pseudomonas* spp. at 800 ppm metal concentration

Tolerance Pattern	Isolate Identity	N	%	Tolerance to Different Heavy Metal Concentration (400ppm)							
				Cu	Mn	Zn	As	Hg	Pb	Co	Pb
Penta-T	*P.aeruginosa*	74	77.89	-	-	+	+	-	-	-	-
	P.stutzeri	6	100	-	-	+	+	-	-	-	-
Hexa-T	*P.aeruginosa*	21	22.11	-	-	+	+	-	-	-	-

**Total number of P. aeruginosa = 95; P. stutzeri = 6*

7.Recommendations

Since the study was done in summer, related study should be done during rainy season to provide a thorough year round investigation on *Pseudomonas* spp. in the Imus River Watershed. Further study can be done on the genetic analysis of *Pseudomonas* spp. particularly on antibiotic resistance and metal tolerance.

8.Conclusion

There were two species of *Pseudomonas* found in the river, the *P. aeruginosa* and *P. stutzeri*. The increasing antibiotic resistance and metal tolerance of *Pseudomonas* spp. Indicates that they can live in a polluted water with high toxic metals. The presence and abundance of *Pseudomonas* spp. serve as bioindicator of the degree of pollution.

References (APA)

Agustin, J. 2006. Metal tolerance and antibiotic resistance patterns of bacteria isolated from selected rivers of Cavite. College of Arts and Sciences. Cavite State University. (unpublished work)

Algammal, A.M., Mabrok, M., Sivaramasamy, E. (2020). Emerging MDR-*Pseudomonas aeruginosa* in fish commonly harbor *opr*L and *tox*A virulence genes and *bla*TEM, *bla*CTX-M, and *tet*A antibiotic-resistance genes. *Sci Rep* 10, 15961. https://doi.org/10.1038/s41598-020-72264-4

Algül, F., & Beyhan, M. (2020). Concentrations and sources of heavy metals in shallow sediments in Lake Bafa, Turkey. *Scientific reports*, *10*(1), 1-12.

Bengwayan, M. (2019). Many Philippine Rivers are Dying. https://landportal.org/node/81429

Bhasin, S., Shukla, N. A., & Shrivastava, S. (2020). Bacterial diversity of river Kshipra with relation to human health. *Environment Conservation Journal*, *21*(1 & 2), 63–74. https://doi.org/10.36953/ecj.2020.211207

Braaten, H. F. V., Karlsson, M., Nordbø, A. J., Hurley, R., O'Neill, C., Jaya, R. K., ... & Olsen, M. (2021). Plastic pollution in Indonesia and the Philippines: current status and upcoming knowledge needs. *NIVA-rapport*.

Denchak, M. (2018). Water pollution: Everything you need to know. *Nat. Resour. Def. Counc. NY*.

Duman, M., Mulet, M., Altun, S., Saticioglu, I. B., Ozdemir, B., Ajmi, N., Lalucat, J., & García-Valdés, E. (2021). The diversity of Pseudomonas species isolated from fish farms in Turkey. *Aquaculture*, *535*, 736369. https://doi.org/10.1016/j.aquaculture.2021.736369

Igiri, B. E., Okoduwa, S. I., Idoko, G. O., Akabuogu, E. P., Adeyi, A. O., & Ejiogu, I. K. (2018). Toxicity and bioremediation of heavy metals contaminated ecosystem from tannery wastewater: a review. *Journal of toxicology*, *2018*.

Ilagan, Y., Ilagan, M., Sale, M., and Velasco, C. (2005). Metal tolerance and antibiotic resistance pattern of a population of *Pseudomonas* spp. isolated from rivers. CvSU Research Journal. 19(1):1-6.

Kummerer, K. (2004). Resistance in the environment. Journal of Antimicrobial Chemotherapy. 54 (2):311-320.

Lee, N., Yan, J., Lee, H., Liu, K., Huang, S., and Ko, W. (2004). Clinical experiences of bacteremia caused by metallo beta-lactamse –producing gram negative organisms. J Microbiol Immunol Infect. 37:343-349.

Li, C., Quan, Q., Gan, Y., Dong, J., Fang, J., Wang, L., & Liu, J. (2020). Effects of heavy metals on microbial communities in sediments and establishment of bioindicators based on microbial taxa and function for environmental monitoring and management. *Science of the Total Environment*, *749*, 141555.

Mayrodi, D., Blankenfeldt, W., and Thomashow, L. (2006). Phenazine compounds in fluorescent *Pseudomonas* spp. biosynthesis and regulation. Microbiol Mol Biol. 56(4):662-676.

Nathanson, J. A. (2022, November 30). *pollution*. *Encyclopedia Britannica*. https://www.britannica.com/science/pollution-environment

Ojewumi, M. E., Okeniyi, J. O., Ikotun, J. O., Okeniyi, E. T., Ejemen, V. A., & Popoola, A. P. I. (2018). Bioremediation: Data on *Pseudomonas aeruginosa* effects *on the bioremediation of crude oil polluted soil*. *Data in brief*, *19*, 101–113.*19*, 101–113. https://doi.org/10.1016/j.dib.2018.04.102

Owa, F. D. (2013). Water pollution: sources, effects, control and management. *Mediterranean journal of social sciences*, *4*(8), 65-65.

Raja, C., and Selvam, G. (2009). Plasmid profile and curing analysis of *Pseudomonas aeruginosa* as metal resistant Int. J. Environ. Sci. Tech. 6(2):259-266.

Reynolds, D., & Kollef, M. (2021). The Epidemiology and Pathogenesis and Treatment of Pseudomonas aeruginosa Infections: An Update. *Drugs*, *81*(18), 2117–2131. https://doi.org/10.1007/s40265-021-01635-6

Rubio, J. S., Mercurio, A. L., Ching, J. A., Guyamin, M. C., Zamora, G. C., & Bala, J. P. (2022) SURVEY ON PLASTIC LITTERS ALONG IMUS RIVER, CAVITE, PHILIPPINES.

Tchounwou, P. B. (2018). Heavy metal toxicity and the environment, in Molecular, clinical and environmental toxicology. *Journal of Analytical & Bioanalytical Techniques*.

Todar, K. (2004). *Pseudomonas* and Related Bacteria. University of Wisconsin-Madison. Department of Bacteriology.

Volk WA, and Wheeler MF. (1988). Basic microbiology. 6th Ed. Harper & Row Publishers, Inc. p. 196-199; p. 587-588.