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Heterotrophic bacterial communities in the sediment of a nickel-rich River ecosystem

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Abstract

The heterotrophic bacterial assemblage in Nickel-rich river sediment was studied. A total of seventeen (17) isolates were obtained. These were characterized based on their morphological and biochemical properties. Results show that most of the isolates were Gram-negative bacteria (70.6%), nitrate reducers (58.8%) and catalase producers (64.7%). Glucose and sucrose fermenters were also represented. In this study, heterotrophic bacteria had a diversity (H') value of 1.514 and an evenness value of 0.2674. Microbial community diversity, along with environmental factors such as heavy metal content, may greatly affect the quality and nutrient cycle efficiency of the river sediment.

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Introduction

Microorganisms in sediments play key roles in ecologically-important biogeochemical processes (Kaplan *et al.*, 2011) their activities show interdependence between minerals and microbes, and the widespread occurrence of microorganisms in sediments contributes to the immobilization of metals through sorption and precipitation reactions. Depending on the prevailing environmental conditions and activity of indigenous microbial populations, individual cells can facilitate the formation and accumulation of distinct minerals (Riding and Awramik, 2000). Once in the aquatic environment, a number of processes could also lead to the settling of microorganisms and their subsequent resuspension from the bed sediments (Abia, 2017). On the other hand, microbial metabolism can also be dependent on downstream transport of fine particles which include deposition and resuspension events (Drummond *et al.*, 2014).

Heavy metals can be absorbed by suspended solids when discharged into aquatic ecosystems. These can be strongly accumulated in sediments and biomagnified along aquatic food chains (Tang *et al.*, 2014; Yi *et al.*, 2011; Gumgum *et al.*, 1994). Heavy metals affect the number, diversity, and activities of microorganisms in the sediments. The toxicity of these metals on microorganisms depends on a number of factors such as temperature, pH, minerals, organic matter, inorganic anions and cations, and chemical forms of the metal (Kaplan *et al.*, 2011; Friedlovȧ, 2010; Giller *et al.*, 1998; Bååth, 1989).

Microorganisms isolated from metal-rich ecosystems possess mechanisms that regulate metal ion accumulation which include microbial resistance to heavy metals (Filali *et al.*, 2000; Korapati *et al.*, 2010), such as, Nickel (Alboghobeish *et al.*, 2014; Patel *et al.*, 2006).

Recently, the effect of metal toxicity on sediment microorganisms has received special attention because microorganisms are key components for recycling of nutrients (Hu, 2006). This study aims to gather information about the microbial communities in the surface sediments of a Nickel-Rich River at Surigao del Norte, Philippines. Specifically, this study aims to characterize the isolated bacteria and assess the bacterial diversity and physico-chemical parameters of the study area. This undertaking can serve as basis in the assessment of the impact of nickel abundance on sediment bacterial communities and formulation of sustainable ways to process nickel mine effluents.

Materials and methods

Study Area

The sampling area is a Nickel-rich river system (5,045mg kg^{-1}) located in Western Mindanao, at the southern part of the Philippines with coordinates 09°29.859'N and 125°49.456'E.

Sediment Collection

Composite samples of river sediment were collected by taking 200g subsamples at a distance of about 1m apart at three (3) sites. Composite samples were mixed thoroughly in a tightly sealed plastic bag containing subsamples of equal amounts (Gao *et al.*, 2005; Swift and Bignell, 2001). These were then placed in a cooler to keep them field moist and to preserve biological properties while transporting them to the laboratory for microbiological analyses.

Microbiological Analyses

Nutrient agar was used as the culture medium for isolating heterotrophic bacteria. The media and diluents were prepared according to standard procedures. Sediment samples were diluted serially with previously prepared blanks and plated onto agar plates. Dilutions of 10^{-5} to 10^{-7} were prepared for sediment samples. Approximately 0.1ml volume was introduced to each agar plate. Spread Plate Method was used to determine the viable count of heterotrophic microorganisms from the samples. Cultures were then incubated at 23°C for 24 hours. Isolates were morphologically and biochemically characterized using standard methods. Biochemical characterizations included Gram reaction, hydrogen Sulfide formation, indole formation and motility. nitrate reduction, sugar fermentation, citrate utilization, conversion of metabolic intermediates to neutral products, lactose fermentation, urease production and catalase reaction (Bengtsson *et al.*, 2013; Madigan *et al.*, 2012; Jiang *et al.*, 2006, Tamaki *et al.*, 2005; Cavallo *et al.*, 1999; Prescott, 1999).

Determination of Physico-Chemical Parameters

Different laboratory equipment was used to determine the physical and chemical parameters in the area. Sediment pH and temperature was determined using HM Digital PH-200 waterproof pH meter. Furthermore, the concentrations of nickel (Ni) were determined by atomic absorption spectrophotometry.

Data Analysis

The diversity (Shannon Diversity Index and Evenness) of the cultivable heterotrophic bacterial community was assessed according to phenotypic characteristics using Paleontological Statistics Software.

Results and discussion

Abundance of Isolates

A total of seventeen (17) cultivable bacterial isolates were obtained in the surface sediments of the Nickel-rich river ecosystem. Substantial quantitative differences in total percentage of CFU ml^{-1}of the seventeen (17) isolates were observed. Isolate KL04 had the highest mean CFU ml^{-1} of 6.0 x 10^{6} corresponding to an overall percentage of 39.88. This was followed by isolate KL02, yielding a percentage of 32.30 with a mean CFU ml^{-1} of 4.86 x 10^{6} (Fig. 1).

Phenotypic Characteristics

Isolates showed unique variations in colony morphology. The most common colony shape and margin observed were circular (64.7%; n=11) and entire (35.3%; n=5), respectively. Most isolates have a flat elevation and whitish coloration. Ten isolates (58.8%) have an opaque colony; this may have implications on virulence. Filiform (41.2%; n=7) and diffuse (58.8%; n=10) growth patterns on nutrient agar slants were observed, with the latter as the most common growth pattern exhibited by the isolates. A study by Simpson *et al.* (1987) on the correlation between virulence and colony morphology revealed that most virulent strains exhibit both opaque and translucent colonies.

Bacterial shape varies with factors such as growth rate, nutritional conditions and interaction with other microorganisms (Kirchman, 2008; Young, 2006). In a study by Sjöstedt (2012) about the effect of temperature on aquatic bacterial community, volume of vibrio or rod-shaped bacteria is significantly higher at all temperatures. Rods allow cell to cell signaling and are associated with motility (Constantino *et al.*, 2016) in solid surfaces (Young, 2006), such as sediments. Similarly in this study, rod-shaped bacteria dominated the assemblage. Furthermore, Gram-negative bacteria (n=12) were the most encountered bacteria. The outer surface of bacterial cytoplasmic membranes are exposed to the environment and interact with periplasmic proteins that bind substrates or process large molecules for transport into the cell in Gram-negative bacteria. Bacterial species with unique morphologies usually show Gram negative reactions and these include the aquatic planctomycetes, characterized by cells with a distinct stalk that allows the organisms to attach to a solid substratum and the helically shaped spirochetes (Madigan *et al.*, 2012). In line with this, an article by Moriarty (1982) examined the bacteria in marine sediments from the surface aerobic layer (0-1cm) and deeper anaerobic layer (20-21 cm) and showed that the surface layer is made up of 90% Gram-negative and 10% Gram-positive bacteria. In the anaerobic zone, Gram-negative bacteria comprised 70% and 30% Gram-positive bacteria. Among Gram-negative bacteria, the predominant genus includes Aeromonas; Photobacterium and Pseudomonas were also found (Cavallo *et al.*, 1999).

Biochemical Characteristics

Nitrate reduction

Nitrogen is the most growth limiting element affecting productivity. Several factors affect the ability of microorganisms such as heterotrophic bacteria, in transforming Nitrogen. These include the presence of nitrates, oxygen, organic carbon, sulfide concentration, sediment depth, temperature and anthropogenic inputs (Bhawsar, 2014; Papaspyrou *et al.*, 2014; Burgin AJ, Hamilton, 2007; Megonigal *et al.*, 2003; Herbert, 1999; Potter *et al.*, 1999; Bell *et al.*, 1990).

In this study, ten isolates were able to reduce nitrate to nitrite, these isolates include KL3, KL4, KL5, KL8, KL10, KL12, KL13, KL15, KL16, and KL17. Moreover, one (1) isolate, KL3, was able to completely denitrify nitrate to ammonia or molecular nitrogen. Denitrification is a microbially facilitated process involving the stepwise reduction of nitrate to nitrite (NO2-), nitric oxide (NO), nitrous oxide (N_2O), and, eventually, to dinitrogen (N_2). Thus, denitrifying bacteria are necessary part of the nitrogen cycle as it allows nitrogen to be recycled back into the atmosphere. Some organisms only produce nitrate reductase and therefore can accomplish only the first reduction leading to the accumulation of nitrite. Others (e.g. *Paracoccus denitrificans* or *Pseudomonas stutzeri*) reduce nitrate completely. Complete denitrification is an environmentally significant process. In the absence of denitrification, nitrogen compounds may accumulate to toxic levels which are detrimental for living organisms. Denitrification is also important in biological wastewater treatment, where it can be used to reduce the amount of nitrogen released into the environment, thereby reducing eutrophication and massive algal blooms, including those of toxic algae and cyanobacteria (e.g., *Microcystis*), affecting human populations relying on surface waters for municipal, recreational or agricultural needs. The fact that denitrification enzymes are located on or near the outer cell surfaces further increases the vulnerability of the entire denitrification pathway to chemical disruption such as in heavy metal contamination (Sobolev and Begonia, 2008).

Glucose, Sucrose and Lactose fermentation.

Populations of fermenting bacteria are responsible for the anaerobic degradation of organic matter through the hydrolysis of biomolecules and for supplying substrates to other functional groups (Nielsen *et al.*, 2012). Fermentative bacteria can be isolated from urban riverbed sediments due to high organic load (Singh *et al.*, 2010). Six (6) isolates were able to ferment glucose as a carbon source; these include isolates KL02, KL03, KL04, KL05, KL07, and KL16. A study by Kong *et al.* (2008) on fermenting microorganisms in wastewater treatment plants revealed that most monosaccharide-fermenting bacteria were members of the Gram-positive phyla Firmicutes and Actinobacteria, with some related to the genera Streptococcus and Tetrasphaera. In this present study, more than 50% of the heterotrophic bacteria are non-glucose fermenters and most belong to the Gram negative group. Furthermore, of the 6 glucose-fermenting isolates, only four were able to ferment sucrose as a carbon source, these include isolates KL02, KL03, KL05, and KL07. On the other hand, no bacterial isolate was able to ferment lactose, indicating the absence or low concentration of lactose-fermenting bacteria in the surface sediments of the river. It is also indicated in the study on fermentative conversion of sugars by selected bacterial consortium from riverbed sediments by Singh *et al.* (2010) that glucose was the most preferred carbon source compared to other sugars including sucrose.

Lactose is usually fermented by Gram-negative bacilli of the genera *Escherichia, Klebsiella, Enterobacter, Serratia* and *Citrobacter* which may be isolated from a variety of environmental sources (Guentzel, 1996). The majority of *Escherichia coli* and enterococci bacteria in aquatic systems are associated with sediments and these associations influence their survival and transport characteristics (Jamieson *et al.*, 2005). Furthermore, a study by Whitman and Nevers (2003) suggests that exposure of sediments to water affect the abundance of lactose fermenters particularly *E. coli* wherein their concentrations are highest in shoreline sediments rather than submerged samples. Absence of lactose-fermenting isolates does not necessarily indicate the absence of coliform bacteria in the sediment since some may be weak fermenters or may not ferment lactose at all.

Sulfate reduction

Abundance and diversity of sulfate-reducing bacteria (SRBs) vary with several factors including depth and availability of nutrients in sediments (Fichtel *et al.*, 2012; Martins *et al.*, 2011; Tamaki *et al.*, 2005). A study by Jiang *et al.* (2009) revealed that SRBs only comprise 2-20% of the total bacteria in an estuarine river sediment core. Furthermore, the physiological properties of SRBs allow them to play important roles in nutrient cycling which include industrial applications such as in optimizing waste treatment (Ayangbenro *et al.*, 2018; Martins *et al.*, 2011). In this study, the absence of a heterotrophic bacterial isolate able to reduce sulfate to sulfide have implications on the biogeochemistry of the surface sediments where the samples were taken as well as the natural potential for remediation of toxic metal ions in the area.

Indole production

Isolate KL13 was able to cleave indole from tryptophan using the enzyme tryptophanase. Large quantities of indole are naturally produced by a variety of bacteria. This molecule influence bacterial physiology and ecological balance, including spore and biofilm formation (Kim and Park, 2015; Lee and Lee, 2010).

Ability to utilize citrate as sole carbon source

Citrate utilization is a defining characteristic that could be used to distinguish between coliforms and may involve several transport systems (Brocker *et al.*, 2009; Bott, 1997). Furthermore, the ability of certain species of enterobacteria in utilizing citrate vary according to the concentration of oxygen. In aerobic conditions, several species of enterobacteria are able to utilize citrate. On the other hand, during anaerobic conditions, some species are capable of growth on citrate such as *Klebsiella pneumoniae* and *Salmonella typhimurium*, but not *Escherichia coli* (Bott, 1997). In this study, one isolate, specifically, KL1, was able to utilize citrate as sole carbon and energy source.

Utilization of glucose to acidic and non-acidic products

Bacteria able to utilize glucose to a mixture of acids (acetic, lactic and formic acid), carbon dioxide, and some ethanol are considered fermenters. This pathway yields large amounts of acids, causing strong and sometimes even lethal acidification of the environment (Vivijs *et al.*, 2014). The neutral to slightly alkaline pH of the sampling site is indicative of the absence of heterotrophic isolates which are able to perform mixed-acid fermentation.

Urease production

Isolate KL05, was able to produce urease, an enzyme that hydrolyzes urea to carbon dioxide and ammonia. Urea is a waste product of many living organisms, and is the major organic component of human urine. Urease activity in sediment is generally attributed to heterotrophic microorganisms, although it has been demonstrated that some chemoautotrophic ammonium - oxidizing bacteria are capable of growth on urea as sole source of carbon, nitrogen, and energy (Marsh *et al.*, 2005). It must be noted that urease is a nickel-dependent metalloenzyme (Boer *et al.*, 2014). Furthermore, environmental urease activity is often measured as an indicator of the health of microbial communities. Microbial ureases are important enzymes in environmental transformations of certain nitrogenous compounds (Mobley and Hausinger, 1989).

Catalase production

Eleven isolates (64.71%) were able to produce catalase. Strong catalase reaction was observed in isolates KL01, KL06, KL13, and KL17 and a weak catalase reaction in isolates KL04, KL07, KL08, KL09, KL10, KL14 and KL15. In order to survive, organisms rely on defense mechanisms that allow them to repair or escape the oxidative damage of hydrogen peroxide. Some bacteria produce the enzyme catalase which facilitates cellular detoxification. Catalase neutralizes the bactericidal effects of hydrogen peroxide (Wheelis, 2008) via the breakdown of hydrogen peroxide into oxygen and water thus protecting them and its concentration in bacteria has been correlated with pathogenicity (Mahon *et al.*, 2011). Catalase is known to be absent in obligate anaerobes and is produced by bacteria that respire using oxygen. Therefore, catalase production is the protective mechanism used mostly by the bacteria living within the area against toxic forms of oxygen. Isolates exhibited variations in their biochemical properties. Table 1 shows some of the biochemical properties of isolates with high abundance (CFU ml^{-1}). Isolate KLO4, with highest mean CFU ml^{-1} of 6.0 x 10^{6}, was able to reduce nitrate to nitrite via nitrate reductase enzyme, ferment glucose and produce catalase to protect from the harmful effects of H_2O_2. In contrast, KL04 was not able to reduce sulfate to sulfide, produce indole and urease, ferment sucrose and lactose, utilize citrate as sole carbon source and utilize glucose to acidic and non-acidic products.

Also, isolate KL04 is a motile gram-negative; presumptive identification of KL04 as *Pseudomonas*, prevalent in sediments such as soils, based on biochemical properties. While isolate KL02, with second highest mean CFU ml^{-1} of 4.86 x 10^{6}, was able to ferment both glucose and sucrose but lacks the enzyme catalase and unable to reduce nitrate to nitrite. In addition, KL02 is a motile gram-negative; with its capability to ferment both glucose and sucrose, presumptive identification of KL02 as member of family Enterobacteriaceae. Fig. 1 shows the percentage of the seventeen (17) bacterial isolates that manifest the listed biochemical properties.

Table 1. Some biochemical properties of isolates with high CFU ml^{-1}.

Isolates	Nitrate Reducer	Catalase Producer	Glucose Fermenter	Sucrose Fermenter
KL02	-	-	+	+
KL04	+	+	+	-
KL09	-	+	-	-
KL10	+	+	-	-

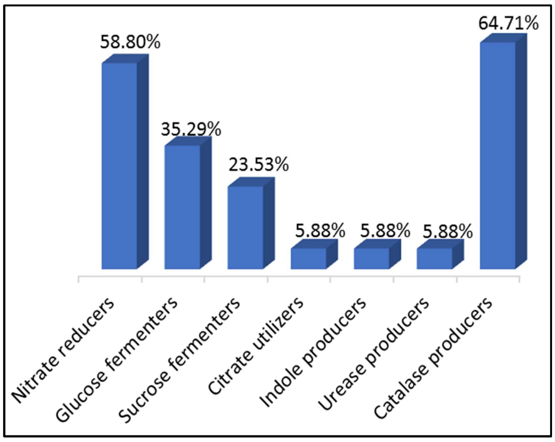

Fig. 1. Percentage of biochemical properties exhibited by the heterotrophic bacterial isolates.

There were some biochemical properties that isolates did not manifest. No sulfate- reducing and lactose- cultivable heterotrophic bacteria were found. Furthermore, no bacteria were able to perform the mixed acid and 2,3-butanediol fermentation pathways. In accordance with the data gathered by Ramamoorthy *et al.* (2009) wherein sulfur-reducing bacteria were lowest where the sediment heavy metal content is at its highest. Results could indicate that heavy metals were high thereby rendering the inability to perform microbial processes since heavy metals are known to be toxic to microorganisms due to their capacity to deactivate enzymes. Moreover, a study done by Wyszkowska *et al.* (2005) revealed that soil contamination with nickel decreased the activity of dehydrogenases, urease and acid and alkaline phosphatase.

Physico-Chemical Parameters

The pH of the sediment samples ranged from 7.78 - 8.18 and were observed to be moderately alkaline with a mean pH value of 7.99 ± 0.201 and the temperature ranged from 29.8 - 31.33 °C. These are within the normal limits in freshwater bodies for boating, fishing and irrigation (Class C) and navigable waters (Class D) provided by the Department of Environment and Natural Resources of the Philippines Administrative Order No. 2016- 08, with a pH range of 6-9 and temperature range of 29-31 as part of its water quality guideline for primary parameters.

Bacterial Diversity

Evenness index shows whether there is similarity or variation in the pattern of distribution of isolates. The higher the value of evenness index, the more uniform is the distribution of isolates. Evenness value corresponds to diversity index's value. If the isolates are evenly distributed then the H' value would be high. Therefore, the area is slightly diverse with an H' value of 1.514 and E value of 0.2674.

Low evenness value indicates that one isolate was abundant in the area, corresponding to isolate KL04. The diversity of microorganisms in ecosystems is immense but critical in determining sediment quality because they are involved in so many important sediment processes. This microbial pool maintains sediment homeostasis.

The larger the microbial diversity and functional redundancy, the quicker the ecosystem can return to stable initial conditions after exposure to stress or disturbance (Sharma *et al.*, 2010). Species diversity can give rise to ecosystem stability through the ability of the species or functional groups it contains to respond differently and in a compensatory fashion to perturbations in the sediment environment (Sturz and Christie, 2003).

Conclusion

Heterotrophic bacteria thrive in the surface sediments of a nickel-rich river ecosystem. A total of seventeen heterotrophic bacterial (17) isolates were obtained in the sediment sample and isolate KL04 had the highest mean CFU ml^{-1} of 6.0 x 10^6, corresponding to an overall percentage of 39.88%. This motile gram-negative isolate was able to reduce nitrate to nitrite but not sulfate, ferment glucose and produce catalase to protect from the harmful effects of H_2O_2. Based on its biochemical properties, it is presumed to belong the *Pseudomonas* group. Next to isolate KL04 was KL02 with an overall percentage of 32.30% corresponding to a mean of 4.86 x 10^6 CFU ml^{-1}. The presumptive identification of KL02 is of the bacterial family Enterobacteriaceae.

Nitrate reducers which play a major role in the nitrogen cycle comprise 58.8% (n=10) of the isolates. Furthermore, most of the isolates were also catalase producers (61.74%). In this study, a diverse microbial community was found with low evenness value. The diversity and physiological properties of these indigenous bacterial communities are important in understanding microbial processes that may improve sediment quality in disturbed ecosystems.

References

Abia ALK, James C, Umobwa-Jaswa E, Momba NMB. 2017. Microbial Remobilisation on Riverbed Sediment Disturbance in Experimental Flumes and a Human-Impacted River: Implication for Water Resource Management and Public Health in Developing Sub-Saharan African Countries. International Journal of Environmental Research and Public Health **14(3),** 306.

Alboghobeish H, Tahmourespour A, Doudi M. 2014. The study of Nickel Resistant Bacteria (NiRB) isolated from wastewaters polluted with different industrial sources. Journal of Environmental Health Science and Engineering 12, 44.

Ayangbenro AS, Olanrewaju OS, Babalola OO. 2018. Sulfate-Reducing Bacteria as an Effective Tool for Sustainable Acid Mine Bioremediation. Frontiers in Microbiology 9, 1986.

Bååth E. 1989. Effects of heavy metals in soil on microbial processes and populations (A Review). Water, Air, and Soil Pollution **47(3-4),** 335-379.

Bell LC, Richardson DJ, Ferguson SJ. 1990. Periplasmic and membrane-bound respiratory nitrate reductases in *Thiosphaera pantotropha* - the periplasmic enzyme catalyzes the 1st step in aerobic denitrification. FEBS Letters **265,** 85-87.

Bengtsson A, Eriksson L, Pedersen K. 2013. Methods development for analysis of microbial abundance and distribution of fractures in natural granitic rock aquifers. Microbial Analytics Sweden AB.

Bhawsar S. 2014. Importance of Denitrification. Biotech Articles, Agriculture.

Boer JL, Mulrooney SB, Hausinger RP. 2014. Nickel-dependent metalloenzymes. Archives of Biochemistry and Biophysics **5(544),** 142-52.

Bott M. 1997. Anaerobic citrate metabolism and its regulation in enterobacteria. Archives of Microbiology **167,** 78-88.

Brocker M, Schaffer S, Mack C, Bott M. 2009. Citrate Utilization by *Corynebacterium glutamicum* Is Controlled by the CitAB Two-Component System through Positive Regulation of the Citrate Transport Genes *citH* and *tct CBA*. Journal of Bacteriology **191(12),** 3869-3880.

Burgin AJ, Hamilton SK. 2007. Have we overemphasized the role of denitrification in aquatic ecosystems? A review of nitrate removal pathways. Frontiers in Ecology and the Environment **5,** 89-96.

Cavallo RA, Rizzi C, Vozza T, Stabili L. 1999. Viable heterotrophic bacteria in water and sediment in 'Mar Piccolo' of Taranto (Ionian Sea, Italy). Journal of Applied Microbiology **86,** 906-916.

Constantino MA, Jabbarzadeh M, Fu HC, Bansil R. 2016. Helical and rod-shaped bacteria swim in helical trajectories with little additional propulsion from helical shape. Science Advances **2(11),** e1601661-e1601661.

Department of Environment and Natural Resources of the Philippines. Administrative Order No. 2016- 08. Subject: Water Quality Guidelines and General Effluent Standards of 2016.

Drummond JD, Davies-Colley RJ, Stott R, Sukias JP, Nagels JW, Sharp A, Packman A. 2014. Retention and remobilization dynamics of fine particles and microorganisms in pastoral streams. *Water Research* **66,** 459-472.

Fichtel K, Mathes F, Könneke M, Cypionka H, Engelen B. 2012. Isolation of sulfate-reducing bacteria from sediments above the deep-subseafloor aquifer. Frontiers in Microbiology **20(3),** 65.

Filali BK, Taoufik J, Zeroual Y, Dzairi FZ, Talbi M, Blaghen M. 2000. Waste water bacterial isolates resistant to heavy metals and antibiotics. Current Microbiology **41(3),**151-6.

Friedlova M. 2010. The Influence of Heavy Metals on Soil Biological and Chemical Properties. Soil and Water Research **5(1),** 21-27.

Gao X, Olapade OA, Leff LG. 2005. Comparison of benthic bacterial community composition in nine streams. Aquatic Microbial Ecology **40,** 51-60.

Giller K, Witter E, Mcgrath S. 1998. Toxicity of heavy metals to microorganisms and microbial processes in agricultural soils. Soil Biology and Biochemistry **30(1-11),** 1389-1414.

Guentzel MN. 1996. *Escherichia, Klebsiella, Enterobacter, Serratia, Citrobacter,* and *Proteus*. In: Baron S (Ed.), Medical Microbiology, 4th Edition, Chapter 27, University of Texas Medical Branch at Galveston, Galveston.

Gumgum B, Unlu E, Tez Z, Gulsun Z. 1994. Heavy-metal pollution in water, sediment and fish from the Tigris River in Turkey. Chemosphere 111-116.

Herbert A. 1999. Nitrogen cycling in coastal marine ecosystems. FEMS Microbiology Reviews **23,** 563-590.

Hu Q, Qi H, Zeng J, Zhang H. 2006. Bacterial diversity in soils around a lead and zinc mine. Journal of Environmental Sciences 74-79.

Jamieson R, Joy DM, Lee H, Kostaschuk R, Gordon R. 2005. Transport and deposition of sediment-associated Escherichia coli in natural streams. Water Research **39(12),** 2665-2675.

Jiang H, Dong H, Zhang G, Yu B, Chapman LR, Fields MW. 2006. Microbial Diversity in Water and Sediment of Lake Chaka, an Athalassohaline Lake in Northwestern China. Applied and Environmental Microbiology **72(6),** 3832-3845.

Jiang L, Zheng Y, Peng X, Zhou H, Zhang C, Xiao X, Wang F. 2009. Vertical distribution and diversity of sulfate-reducing prokaryotes in the Pearl River estuarine sediments, Southern China. FEMS Microbiology Ecology **70(2),** 249-262.

Kaplan H, Ratering S, Hanauer T, Henningsen P, Schnell S. 2011. Impact of a trace metal contamination from mining activity on soil microbial communities and respiration activity of irrigated Kastanozems in Georgia. Geophysical Research Abstracts **13,** EGU 2011-10774.

Kim J, Park W. 2015. Indole: a signaling molecule or a mere metabolic byproduct that alters bacterial physiology at a high concentration? Journal of Microbiology **53(7),** 421-428.

Kirchman DL. 2008. Introduction and overview. In: Kirchman DL (ed.) Microbial ecology of the ocean, 2nd ed. Wiley-Blackwell, Hoboken, NJ 1-26.

Kong Y, Xia Y, Nielsen PH. 2008. Activity and identity of fermenting microorganisms in full-scale biological nutrient removing wastewater treatment plants. Environmental Microbiology **10(8),** 2008-19.

Korapati N, Rao PS, Venu VA. 2010. Isolation and Identification of Bacterial Strains and Study of their Resistance to Heavy Metals and Antibiotics. Journal of Microbial and Biochemical Technology **2(3).**

Lee JH, Lee J. 2010. Indole as an intercellular signal in microbial communities. FEMS Microbiology Reviews **34**(4), 426-44.

Madigan MT, Martinko JM, Stahl DA and Clark DP. 2012. Brock Biology of Microorganisms. 13th edition. Pearson Education, Inc., publishing as Benjamin Cummings, San Francisco, CA **39.**

Mahon C, Lehman D, Manuselis G. 2011. Textbook of diagnostic microbiology, 4th ed. W. B Saunders Co., Philadelphia, PA.

Marsh KL, Sims GK, Mulvaney RL. 2005. Availability of urea to autotrophic ammonia-oxidizing bacteria as related to the fate of 14C- and 15N-labeled urea added to soil. Biology and Fertility of Soils **42,** 137-145.

Martins M, Santos ES, Faleiro ML, Chaves S, Tenreiro R, Barros RJ, Barreiros A, Costa MC. 2011. Performance and bacterial community shifts during bioremediation of acid mine drainage from two Portuguese mines. International Biodeterioration & Biodegradation **65,** 972-981.

Megonigal JP, Hines ME, Visscher PT. 2003. Anaerobic metabolism: Linkages to trace gases and aerobic processes. Biogeochemistry. Elsevier Pergamon 317-424.

Mobley H, Hausinger R. 1989. Microbial urease, significance, regulation, and molecular characterization. Microbiological Reviews **53,** 85-108.

Moriarty D. 1982. Ultrastructure of bacteria and the proportion of Gram-negative bacteria in marine sediments. Microbial Ecology **8(1),** 1-14.

Nielsen JL, Nguyen H, Meyer RL, Nielsen PH. 2012. Identification of glucose-fermenting bacteria in a full-scale enhanced biological phosphorus removal plant by stable isotope probing. Microbiology **158,** 1818-1825.

Patel JS, Patel PC, Kalia K. 2006. Isolation and Characterization of Nickel Uptake by Nickel Resistant Bacterial Isolate (NiRBI). Biomedical and Environmental Sciences **19,** 297-301.

Potter LC, Millington P, Griffiths L, Thomas GH, Cole JA. 1999. Competition between *Escherichia coli*strains expressing either a periplasmic or a membrane-bound nitrate reductase: does Nap confer a selective advantage during nitrate-limited growth? Biochemical Journal **344,** 77-84.

Prescott H. 1999. Laboratory Exercises in Microbiology, 4th Edition. The McGraw-Hill Companies, Inc., New York 685.

Ramamoorthy S, Piotrowski J, Langner H, Holben W, Morra MJ, Rosenzweig RF. 2009. Ecology of Sulfate- Reducing Bacteria in an Iron-Dominated, Mining-Impacted Freshwater Sediment. Journal of Environmental Quality **38,** 1-10.

Riding RE, Awramik SM. 2001. Microbial Sediments. International Microbiology **4(3).**

Sharma S, Ramesh A, Sharma M, Joshi O, Govaerts B, Steenwerth K, Karlen D. 2010. Microbial Community Structure and Diversity as Indicators for Evaluating Soil Quality. E. Lichtfouse (ed.), Biodiversity, Biofuels, Agroforestry and Conservation Agriculture, 317 Sustainable Agriculture Reviews **5.**

Simpson L, White V, Zane S, Oliver J. 1987. Correlation between virulence and colony morphology in *Vibrio vulnificus*. Infection and Immunity **55,** 269-272.

Singh S , Sudhakaran AK, Sarma PM, Subudhi S, Mandal AK , Gandham G, Lal B. Dark fermentative biohydrogen production by mesophilic bacterial consortia isolated from riverbed sediments. International Journal of Hydrogen Energy **35,** 10645-10652.

Sjöstedt J, Hagström A, Zweifel UL. 2012. Variation in cell volume and community composition of bacteria in response to temperature. Aquatic Microbial Ecolology **66,** 237-246.

Sobolev D, Begonia M. 2008. Effects of Heavy Metal Contamination upon Soil Microbes: Lead-induced Changes in General and Denitrifying Microbial Communities as Evidenced by Molecular Markers. International Journal of Environmental Research and Public Health. PMCID: PMC3700007, 450-456.

Sturz A, Christie B. 2003. Beneficial microbial allelopathies in the root zone: the management of soil quality and plant disease with rhizobacteria. Soil and Tillage Research **72,** 107-123.

Swift L, Bignell D. 2001. Standard methods for assessment of soil biodiversity and land use practice. ASB Lecture Note 6B. International Centre for Research in Agroforestry.

Tamaki H, Sekiguchi Y, Hanada S, Nakamura K, Nomura N, Matsumura M, Kamagata Y. 2005. Comparative Analysis of Bacterial Diversity in Freshwater Sediment of a Shallow Eutrophic Lake by Molecular and Improved Cultivation-Based Techniques. Applied and Environmental Microbiology **71(4),** 2162-2169.

Tang W, Shan B, Zhang H, Zhang W, Zhao Y, Ding Y, Rong N, Zhu X. 2014. Heavy Metal Contamination in the Surface Sediments of Representative Limnetic Ecosystems in Eastern China. Scientific Reports, Article no. 7152.

Vivijs B, Moons P, Aertsen A, Michiels C. 2014. Acetoin Synthesis Acquisition Favors *Escherichia coli* Growth at Low pH. Applied and Environmental Microbiology **80(19),** 6054-6061.

Wheelis M. 2008. Principles of modern microbiology. Jones and Bartlett Publishers, Inc., Sudbury MA.

Whitman RL, Nevers M. 2003. Foreshore sand as a source of *Escherichia coli* in nearshore water of a Lake Michigan beach. Applied and Environmental Microbiology **69(9),** 5555-5562.

Wyszkowska J, Kucharski J, Boros E. 2005. Effect of nickel contamination on soil enzymatic activities. University of Warmia and Mazury in Olsztyn, Poland. Plant, Soil and Environment **51,** 523-531.

Yi Y, Yang Z, Zhang S. 2011. Ecological risk assessment of heavy metals in sediment and human health risk assessment of heavy metals in fishes in the middle and lower reaches of the Yangtze River basin. Environmental Pollution 2575-2585.

Young KD. 2006. The Selective Value of Bacterial Shape. Microbiology and Molecular Biology Reviews **70(3),** 660-703.