Research Article

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Bacterial Community of Castor Plants Growing on Municipal Solid Waste Dumpsites of Wukari Metropolis

Volume 6 Issue 1 - 2021

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Received: December 19, 2020 | **Published:** January 08, 2021

Abstract

Ricinus communis L. are usually found growing abundantly in wastelands where MSW are disposed in urban and semi-urban settlements. The aim of the research is to ascertain the ecological factors that influence the proliferation of Castor bean plants *Ricinus communis L.* on MSW dumpsites. A study was conducted between the months of December 2018 to April 2019 on fourteen MSW dumpsites from fourteen selected localities in Wukari Metropolis. Three localities were categorized into highly populated (Hospital site, Marmara 1 and Marmara 2) sites; six were moderately populated (GRA, New Site, T-junction, Avyi 1, Mission Quarters 1, Mission Quarters 2) and five (New Market, Kwararafa 1, Timber Shed, Rice Mill, Albaco Maiko) were sparsely populated sites. Results revealed that Hospital Site, Marmara 1and Marmara 2 exhibited the highest biochemical activities due to high population density and waste generation at the study sites. The bacterial community reveals high diversity of gram-positive (Lactobacillus, Bacillus and Staphylococcus aureus) and gram-negative (Pseudomonas and Klebsiella) due to the presence of large volumes of untreated sewage, household garbage, agricultural wastes/poultry farms/abbatoirs, frozen food shops, hospital wastes, fermented sorghum-an alcoholic beverage called "burukutu" and "nono" – fresh cowmilk in the MSW dumpsites. The presence of Klebsiella in the dumpsites reveals their ubiquity of belonging to the coliforms group and potential indicator organism as an index of possible water contamination and from hospital acquired infection including Staphylococcus aureus (food poisoning). The survival, growth and proliferation of *Ricinus communis L.* on the MSW dumpsites is dependent on its symbiotic relationship with the five Orders of bacteria that plays critical roles in organic matter synthesis, degradation, detoxification and novel metabolic abilities thus creates a suitable habitat.

Keywords: Bacterial Isolates, MSW dumpsites, Microhabitat factor, rhizosphere, *Ricinus communis* L

Introduction

The microbial world is the largest unexplored reservoir of biodiversity which exists in diverse ecological niches, including extreme environments. Exploration of microbial diversity holds great promise because of the role of microbes in nutrient cycling, environmental detoxification and novel metabolic abilities in pharmaceuticals and industrial processes.[1,2]

The vast majority of soil microbes are heterotrophic and many have evolved close relationships with plants, which are the major source of terrestrial primary production. Many microbes-plant interactions are commensalism– they do not harm the plant, whereas the microbe gains some advantage.[3] Many other important mutualistic interactions are beneficial to both the microorganism and the plant.

Plants are colonized by large numbers of unicellular microorganisms which may be free-living commensals, epiphytes, symbionts (endophytes), or obligate parasites.[4] The plant holobiont is an ecosystem that includes associated microbiota, and is a valid unit of natural selection. The holobiont is often dependent on its microbiota for crucial functions.[4,5] The association of plants with microbes is phylogenetically ancient, going back to the macroalgae.[6]

The 40–90% of the net photosynthesized carbon that enters the soil as a wide variety of materials including alcohols, ethylene, sugars, amino and organic acids, vitamins, nucleotides, polysaccharides and enzymes creates a unique environment for soil microorganisms called the rhizosphere.[4] The rhizosphere offers an assortment of plant exudates that are more easily degraded than lignin and cellulose.[4] Rhizosphere is home to a variety of bacteria that compete with saprophytic and plant pathogenic fungi also in the rhizosphere.[3] The plant root surface called the rhizoplane also provides an exceptional environment for microorganisms as the gaseous, soluble and particulate materials move from plant to soil. Rhizosphere and rhizoplane play critical roles in organic matter synthesis and degradation.[3] *Pseudomonas* and *Bacillus* are the most abundant rhizobacteria found in soils and near plants in general.

Organic components like animal wastes, kitchen garbage, and untreated sewage of MSW are decomposed by microbes which are also responsible for getting rid of industrial and household wastes. In tropical countries, the high temperatures and humid conditions accelerate degradation, increase the amount of leachate and directly affect the surrounding ecosystems by penetrating the soil and contaminating groundwater. Bacteria play an important role in the decomposition of organic materials particularly in the early stages of decomposition when moisture levels are high.[3] *Bacillus substilis* and *Pseudomonas fluorescens* are examples of decomposer bacteria.

Wide varieties of refuse dumpsites are increasingly scattered all over Nigeria arising from population explosion resulting increased waste generation. Most of these MSW dumpsites have been utilized as bio-fertilizers by poor farmers as vegetable farmlands in both rural and urban areas, and encourages the proliferation of waste-loving plants like *Amaranthus spinosa, Ricinus communis* L. etc. These municipal solid wastes (MSW) dumpsites consist mostly of undesirable materials mainly of household wastes also called household garbage.[7,8] In Wukari, the MSW include wastes of the agricultural feedlots/abbatoirs/ slaughter houses/poultry houses, untreated sewage and animal wastes, restaurants, commercial centers, crafts, trades, hotels, schools, public services, and hospitals and other recreational areas that present physicochemical features or equivalent to the toxicity of household garbage. Most researches have been largely focused on heavy metal levels and mineral composition of MSW dumpsites, toxicity, effects on soil, vegetation, food crops,[9] solid waste composting, effects on soil properties and vegetables grown on refuse dumpsites with respect to their fertile nature and contaminants. In areas with a suitable climate, *Ricinus communis* L. establishes itself easily where it can become an invasive plant and can often be found on waste land.[10] However, there is scanty literature on the preference and proliferation of these plants like *Amaranthus spinosa, Ricinus communis* L. on MSW dumpsites and the bacterial community of MSW in a rural/semi-rural setting. *Ricinus communis* L. is an important drought-resistant shrub belonging to the family Euphorbiaceae.[11] The castor bean plant can reach a height of 2-3 meters (6.6-9.8ft) in a year and grows into a tree in the subtropical zone attaining heights of between 11-13metres. It is available at low cost and is known to tolerate varying weather conditions.[12,13] In the Eastern part of Nigeria and Wukari, the seeds of *Ricinus communis* L. are used to prepare a fermented food condiment[14] and income generation.

Currently, researches on *Ricinus communis* L. have been more focused on the chemical components of its oil and potential as an energy crop. Castor bean plants are found mostly around MSW dumpsites near small slow-moving water bodies in Wukari. Therefore a monitoring survey of the preference of the growth of castor bean plants on MSW dumpsites than other microhabitats is necessary. The first objective of this research is to ascertain the existence of the soil bacterial community of the MSW that encourages the prolific growth of *Ricinus communis* L. Bacterial are a major player in decomposition at the early stage, while fungi tend to dominate at the later stages of decomposition.

Materials and methods

Description and characteristics of the study area and sampling sites

Wukari local government area is in the southern part of Taraba State. It is bounded to the north-west by Benue State, to the south east by Takum local government area, and north east by Bali, Karim Lamido and Gassol local government areas of Taraba State. Wukari local government has a total area of about1835km^2. The Donga River flows through the area and the Benue River forms a boundary with Nasarawa State to the North West. The area is a typical rural settlement and the main occupation of Wukari people is farming and fishing. It also has several numbers of streams that attracts activities such as bathing, swimming, laundry and other activities.

A pre-survey of 21 locations was carried out during the dry season between December 2017 to March/April 2018 to identify areas where castor plant grew on MSW dumpsites, was conducted within three wards namely Avyi, Hospital Ward and Puje in Wukari Metropolis. Twelve locations across the three wards were identified and selected as representatives of the metropolis. They include Kwararafa 1 (Abo prisoners), Mission Quarters 1, Mission Quarters 2, New Market (Concord), rice mills, New site and Timber shed from Puje ward; Marmara 1 (Abattior), Marmara 2 (Veterinary farm site), T-junction, Maiko/Albaco and Hospital site in Hospital ward (African church) and GRA (Bariki), Avyi 1 in Avyi ward. The field study of characteristics of the major MSW dumpsites near streams in each of the sampling locations was obtained and tabulated as shown in Table 1. Soil samples were collected near the roots of *Ricinus communis* L. growing on MSW dumpsites for further laboratory analyses.

Table 1 Characteristics of the fourteen study localities

Sampling Locations	Characteristics
Marmara 1(Abattoir)	Consist of small stream housing ancient crocodile, small water bodies, trees like Neem tree, Mango tree and grasses, also refuse dumps, goat slaughtering area, block molding site and school. Highly populated
Marmara 2 (Veterinary farm site)	Located around residential area, township roadside, refuse dumps and grasses. Highly populated
Avyi 1	The moderately populated area with refuse dumps, abandoned building and grasses.
Mission Quarters 1&2	The moderately populated area with stream, residential site with Mango tree, Neem tree and grasses.
T-Junction	The moderately populated area with refuse dumps, school and residential area with grasses.
Hospital site	Highly populated area, residential area, roadside and refuse dumps.
New site	The moderately populated area with refuse dumps, mechanic village and incomplete buildings.
New market (New concord)	The sparsely populated area with refuse dumps, predominantly tall plant with large yellowish or greenish colour leaves.
Kwararafa 1 (Abo prisioners)	The sparsely populated locality with small water body where ancient prisoners take their bath, refuse dumps, filling station, carpentery and also Neem tree.
Timber shed	The sparsely populated area with refuse dumps.
Rice mill	The sparsely populated area with refuse dumps, water body, school and trees like Mango tree, Neem tree and cassava tree.
Maiko albaco	Also Sparsely populated area with refuse dumps, water body, plant with a yellowish greenish colour and short brown leaf.
GRA(Bariki)	The moderately populated area with the refuse dump, along Air strip house hold site and hotel.

Soil samples were collected at a depth of 25cm using a soil auger for physic-chemical, bacterial and biochemical characteristics of soils on which the castor plants grew. The standard or quantitative plate count method was used for the enumeration and isolation of soil bacteria using the corresponding soil samples where castor plants grew according to Harley and Prescott,[15,16] and were restricted to bacteria only. It is the classical and most widely used isolation method used for the quantitative and qualitative studies of soil microorganisms.

The characterization of the bacterial isolates was determined by the following biochemical tests which include Gram staining;[17] Catalase test, oxidase test;[18] coagulase test: This test was used to distinguish *staphylococcus aureus* (coagulate positive) from *staphylococcus epidermidis* and *staphylococcus saprophyticus* (coagulate negative);[18] sugar fermentation test.[18]

Physicochemical parameters such as, particle-size distribution, textural class, organic matter and organic carbon content and soil pH were determined according to Bouyoucos;[19] Walkley and Black;[20] Nelson and Sommers[21] and Bates[22] respectively.

Results and discussion

Sample distribution and cultural characteristics of bacterial isolates

The cultural characteristics of the bacterial isolates are shown in Table 2. *Lactobacillus* (32.14%) and *Pseudomonas species* (25%) were the most frequently occurring bacterial isolates observed at about 92.86% of the 14 locations used in this study, while *Staphylococcus aureus* (14.19%) and *Bacillus Species* (10.17%) were the least occurring isolates (Table 2). *Klebsiella species* had a percentage occurrence of 17.86%.

Improper disposal of MSW produces both gaseous and liquid seepages that contaminate drinking water sources as well as vegetables are grown on such dumpsites in most rural or semi-rural and some larger regions. The proliferation of *Ricinus communis* L. on the selected MSW dumpsites in the 14 localities reflects the symbiotic relationships of their roots with rhizobacteria which acts as plant growth promoters and biocontrol agent. *Pseudomonades* are resistant to chemical remediation agents at contamination sites.

Table 2 Cultural characteristics and frequency of occurrence of soil bacterial isolates

Colony appearance	Soil bacterial isolates	Frequency of occurrence(%)
SGMSM	Staphylococcus aureus	4(14.29)
SWCSM	Bacillus species	3(10.71)
SMSM	Pseudomonas species	7(25)
	Klebsiella species	5(17.86)
SWSM	Lactobacillus species	9(32.14)
Total Bacterial Isolates		28

SGMSM, small golden milk spherical & mucoid; SMSM, small milkish spherical & mucoid; SWSM, smooth whitish spherical & mucoid; SWCSM, small white cream spherical & mucoid

Pseudomonas and Bacillus are decomposer bacteria associated with the mycorrhizal fungi that form a mycorrhizosphere.[3] Mycorrhizosphere is formed due to the flow of carbon from the plant into the mycorrhizal hyphal network and then into the surrounding soil as the external hyphal network radiates into the soil.[3] These bacteria are called mycorrhization helper bacteria because they may play a role in the development of mycorrhizal relationships involving ectomycorrhizal fungi. Pseudomonas and Bacillus are the most abundant rhizobacteria found in soils and near plants in general. Pseudomonas is a plant growth promoter present in agricultural soils and well adpted to grow in the rhizosphere. They possess many traits to acts as biocontrol agent and promote plant growth ability. They also contribute to bioremediation. Rhizoremediation is the detoxification of soil contaminants by microbes that are stimulated by the release of nutrients, oxygen and other factors by plant roots.[3]

Bacillus occurs naturally in soil, rhizosphere and roots of crop plants and in marine sediments. In order to understand the relative contribution of different components of the microbiota to the plant holobiont, it should be noted that abundance alone may not truly reflect the relative importance of the species/strain in question. Numerically less abundant species could be key players within the microbiota, assuming the role of "keystone" species.[23] Plants colonized by *Bacillus* spp. take up more water, which is an important mechanism for plant protection against drought-induced damage.[24]

Lactobacillus is found on plant surfaces and in dairy products, meat, water, sewage, beer, fruits and many other materials. They are normal flora of the human body, in the mouth, intestinal tracts and vagina. They are rarely pathogenic. Lactobacillus is the largest genus in the order Lactobacillales. Many lactobacilli operate using homofermentative metabolism (they produce only lactic acid from sugars), and some species use heterofermentative metabolism (they can produce either alcohol or lactic acid from sugars).[25] Klebsiella is another species that belong to the coliforms groups that are used as indicator organisms as an index of possible water contamination by human pathogens derived from contamination of water with human and other animal faecal wastes. Also Staphylococci are normally associated with skin, skin glands and mucous membranes of warm-blooded animals. Staphylococcus aureus is a major cause of food poisoning.

Morphological characteristics of the bacterial groups isolated from the 14 sampling localities

The morphological characteristics and correspondence colony appearances of the bacteria isolated from the 14 locations are shown in Figure 1. The morphological characteristics of two groups of pairing of *Lactobacillus* and *Pseudomonas species* and *Pseudomonas* and *Klebsiella species* shared the same colony appearances of small milky spherical and mucoid (SMSM) at sampling locations 12, 13 and 14 (Figure 1). Similarly *Klebsiella* and *Pseudomonas species* shared the same colony appearance of SMSM (Table 2).

The occurrence of Pseudomonas and Lactobacillus pair reveals the presence of improperly disposed untreated sewage, commercial frozen food shops and abbatoirs at the localities and acts as decomposer rhizobacteria and non-pathogenic bacteria respectively.

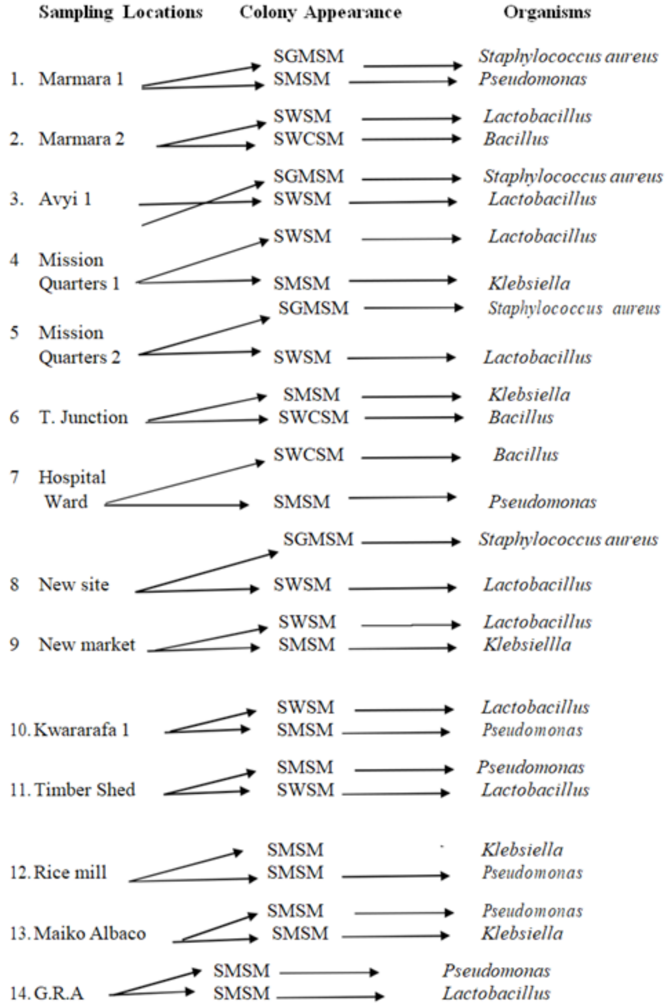

Figure 1 Morphological and cultural characteristics of bacteria isolated from fourteen (14) localities in wukari metropolis.

The presence of Klebsiella in the soil samples shows the presence of hospital wastes as a component of the MSW dumpsites. The ability of *K. pneumoniae* to colonize the hospital environment, including carpeting, sinks, flowers, and various surfaces, as well as the skin of patients and hospital staff, has been identified as a major factor in the spread of hospital-acquired infections.[26,27]

Klebsiella species are found everywhere in nature. This is thought to be due to distinct sublineages developing specific niche adaptations, with associated biochemical adaptations that make them better suited to a particular environment. They can be found in water, soil, plants, insects and other animals including humans.[26,28] Klebsiella is a common opportunistic pathogen for humans and other animals, as well as being resident or transient flora (particularly in the gastrointestinal tract). Other habitats include sewage, drinking water, soils, surface waters, industrial effluents, and vegetation. They are also called associative nitrogen fixers or diazotrophs.[28,29] The bacteria attach strongly to root hairs and less strongly to the surface of the zone of elongation and the root cap mucilage.[30]

They have no specific growth requirements and grow well on standard laboratory media, but grow best between 35 and 37˚C and at pH 7.2..[31] The genus Klebsiella is seemingly ubiquitous in terms of its habitat associations. Until recently, almost all these Klebsiella have been identified as one species, ie, K. pneumoniae. However, phenotypic and genotypic studies have shown that "K. pneumoniae" actually consists of at least four species,[26] all with distinct characteristics and habitats. General habitat associations of Klebsiella species are as follows: K. pneumoniae--humans, animals, sewage, and polluted waters and soils; K. oxytoca--frequent association with most habitats; K. terrigena--unpolluted surface waters and soils, drinking water, and vegetation; K. planticola--sewage, polluted surface waters, soils, and vegetation; and K. ozaenae/K. rhinoscleromatis--infrequently detected (primarily with humans).[26]

Biochemical characteristics of the bacterial isolates

The morphological and biochemical characteristics of the Bacterial Isolates at the fourteen localities are presented in Table 3. The positive results of the oxidase and catalase tests which are typical characteristics of Pseudomonas were evident at Avyi 1, Mission Quarters 1, New site, New Market, Kwararafa 1, Timber Shed and GRA and also exhibited cell arrangement of rod in cocci (Table 3). The genus *Pseudomonas* is behaviorally very versatile, with free-living as well as parasitic forms capable of colonizing a wide variety of host organisms and ecological niches within hosts.[4] All species and strains of *Pseudomonas* have historically been classified as strict aerobes. *Pseudomonas* species also typically give a positive result to the oxidase test, the absence of gas formation from glucose, glucose is oxidized in oxidation/fermentation test using Hugh and Leifson O/F test.[32,33]

Table 3 Morphological and biochemical characteristics of soil samples at the fourteen localities at the study area

Sampling distribution	Gram staining	Cell arrangement	Coagulase	Sucrose	Lactose	Oxidase	Glucose
Marmara 1	+	Cocci in chain	+	+	+	-	+
Marmara 2	+	Rod in chain	-	+	+	-	+
Mission Quarter 1	-	Rod in cocci	-	-	-	+	-
Mission quarters 2	+	Rod in chain	-	+	+	-	+
T. junction	-	Rod in pair	+	+	+	-	+
Hospital Site	+	Rod in chain	-	+	+	+	+
New site	-	Rod in cocci	-	-	-	+	-
New Market	-	Rod in cocci	-	-	-	+	-

Citation: Ibrahim JM, Anongo MC. Bacterial Community of Castor Plants Growing on Municipal Solid Waste Dumpsites of Wukari Metropolis. *MOJ Eco Environ Sci.* 2021;6(1):1‒6. DOI: 10.15406/mojes.2021.06.00206

Table Continued...

Sampling distribution	Gram staining	Cell arrangement	Coagulase	Sucrose	Lactose	Oxidase	Glucose
Kwararafa1	-	Rod in cocci	-	-	-	+	-
Timber shed	-	Rod in cocci	-	-	-	+	-
Rice Mill	-	Rod in pair	+	+	+	-	+
Maiko/Albaco	-	Rod in pair	+	+	+	-	+
G RA	-	Rod in cocci	-	-	-	+	-

Oxidase, glucose, sucrose and lactose were the commonest metabolic activity while gram staining and coagulase were the least metabolic activity. Hospital site and Marmara 1 exhibited the highest biochemical characteristics while New Site, New Market, Kwararafa 1, Timber Shed and GRA had the least biochemical activity (Table 3).

Selected physico-chemical characteristics of the soil samples from the 14 localities

Physico-chemical characteristics of the fourteen localities selected for this study are shown in Table 4. Soil pH among the 14 localities ranged from slightly acidic (6.2) at T-junction to a near neutral (7.25) chemical environment at Maiko/Albaco and a textural class ranging from sandy loam to loamy sand soils with the exception of Maiko/ Albaco with sandy soil texture. The optimum pH range for most plants is between 5.5 and 7.0; however, many plants have adapted to thrive at pH values outside their range. The pH values could have influenced the textural class of the soil in this research. Though, the % sand at the 14 localities is far higher than the % silt and % clay, the loamy sand natures of the soils are usually derived from the mixture of silt, clay and high organic matter and organic carbon contents. Therefore, the slightly acidic to neutral pH, loamy sand soils, high organic matter and organic carbon contents might have influenced the high diversity of rhizobacteria at the 14 localities. The presence of Pseudomonas, Bacillus and Klebsiella in the toxic MSW dumpsites of Wukari Metropolis stimulates rhizoremediation, decontamination and degradation of the wastes further protect the Castor plants and promote their prolific growth on the dumpsites.

Table 4 Selected physico-chemical parameters of soil samples collected at the fourteen localities in Wukari

Sampling distribution	Soil pH	% clay	% Silt	% Sand	Textural class	% O.C	% O.M	K/Meq/100g	Na\Meq/100g
Marmara1	6.925	8.76	4.28	86.96	Loamy sand	1.04	1.79	0.32	1.28
Marmara2	7.025	8.76	8.28	82.96	Loamy sand	1.58	2.73	0.46	1.84
Avyi 1	6.775	9.7	1.6	88.7	Loamy sand	1.72	2.97	`2.40	4
Mission quarter	6.675	12.76	34.28	52.96	Sandy loam	1.86	3.22	0.57	2.28
Mission quarters	7	8.7	9.6	81.7	Loamy sand	0.81	1.4	4.5	8
T- junction	6.2	7.7	5.6	86.7	Loamy sand	1.11	1.91	0.75	2
Hospital ward	6.975	12.7	4.6	82.7	Loamy sand	0.81	1.4	1.65	4
New site	7.175	11.7	7.6	80.7	Loamy sand	3.27	5.64	6.3	10
New Market	7.05	8.7	3.6	87.7	Loamy sand	1.16	2	4.5	8
Kwararafa1	6.875	8.76	2.28	88.96	Loamy sand	1.62	2.8	0.52	2.66
Timber shed	7.075	11.76	17.28	70.96	Sandy loam	1.41	2.44	0.52	2.66
Rice Mill	6.875	12.7	24.6	62.7	Sandy loam	2.35	4.05	3	6
Maiko/Albaco	7.25	8.7	1.1	90.2	Sand	1.46	2.52	0.75	4
G RA	6.7	15.76	23.28	60.96	Sandy loam	1.72	2.97	0.54	2.16

Sandy loam soils had a low value of % sand as compared to loamy sand ranging from 52.96 at Mission Quarters to 70.96 at Timber Shed (Table 4). Sandy loam soils are often very acidic encouraging the growth of Lactobacillus at Mission Quarters 1 and 2, Timber shed and GRA as shown in Figure 1 and Table 4. While Staphylococcus strives on both the loamy sand and sandy loam soils indicating a wide of tolerance.[34,35]

Conclusion

This research revealed that Marmara 1, 2 and Hospital ward which are the highly populated sites had higher diversity of bacterial community among the MSW dumpsites. The presence of five Orders belonging to three Classes of the Bacterial Isolates reveals a high species diversity of the bacterial community or rhizobacteria influencing the high proliferation of *Ricinus communis* L. and the existence of a symbiotic relationship between the bacterial community and *Ricinus communis* L. in the MSW dumpsites of Wukari Metropolis. The symbiotic relationship is the basis of survival, preference and proliferation of *Ricinus communis* L. on the MSW dumpsites.

The Class Bacilli was the Commonest among all the sites reflecting a suitable microhabitat for proliferation of the bacterial community and *Ricinus communis* L. The Class Bacilli was the most abundant with higher populations of Bacillus, Lactobacillus and Staphylococcus aureus. The Class Bacilli were the Gram-positive while Klebsiella and Pseudomonas are gram-negative.

Pseudomonas's ability as a plant growth promoter, biocontrol agent and bioremediator prevented the toxic wastes at the MSW dumpsites from affecting the growth and survival of *Ricinus communis* L. The presence of the bacterial community and suitable abiotic factors of the MSW dumpsites are the habitat factors suitable for *Ricinus communis* L. survival and growth.

Acknowledgments

None.

Funding

None.

Conflicts of interest

The authors declare there are no conflicts of interest.

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