Isolation and Characterization of *Rhizobacteria* from Rhizosphere Soil Contaminated with Liquid Waste in Arba Minch University Abaya Campus

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Abstract: **The study was conducted in Arba Minch University, Abaya Campus, on liquid waste contaminated soils. The objective of our study is to isolate and characterize rhizobacteria from Rhizosphere soil contaminated with liquid waste and its associated risk factor on the health of students. The data was collected by using observational check list and Questioners. The samples were collected by using purposive sampling techniques. A total of 3 samples were collected from contaminated rhizosphere by liquid waste, the result obtained revealed that the mean bacterial count from the total sample was 167250 cfu/ml. there was a significance difference of microbial load between nutrient agar ,MacConkey agar and mannitol salt agar. Total 183 colonies were counted; from them staphylococcus and streptobacillus were isolated randomly and characterized by using Biochemical test. The contaminated soil sample has Gram negative bacteria. The data which is collected from questionnaires and observational checklist showed that improper disposal of liquid waste was nearer to the female student dormitory, that cause communicable diseases on the health of the students so the college should take serious action to treat the waste to minimize the risk.**

Keywords: **bacteria, sample, soil, rhizosphere, rhizobacteria, waste.**

1. INTRODUCTION

BACKGROUND OF THE STUDY:

The rhizosphere inhabiting microorganisms compete for water, nutrients and space and sometimes improve their competitiveness by developing an intimate association with plant (Hartman *et al*., 2009). These microorganisms play important roles in the growth and ecological fitness of their host. An understanding of the basic principles of rhizosphere microbial ecology, including the function and diversity of microorganisms that reside there, is necessary before soil microbial technology can be applied in the rhizosphere .The root of plant interact with a large number of different microorganisms, with these interaction being major determinant of the extent of phytoremendation (Glick, 1995).Soil is dynamic living matrix and it is not only a critical resource in agricultural and food security but it is also toward maintenance of all life process. The current public concerns about the side effects of agrochemicals, there is an increasing interest in improving the understanding of cooperative activities among plants and rhizosphere microbial populations so there is an urgent need of biological agents is accepted worldwide. The use of plants growth promoting rhizobacteria is better alternative to solve the problem. The functioning of associative plant-bacterial symbiosis in heavy metal-polluted soil can be affected from the side of both the micro partner (plant-associated bacteria) and host plant. Soil microbe play significant role in maintenance of soil structure, detoxification of noxious chemicals, and control plant pests and plant growth. Here we review different mechanisms commonly used by the beneficial Rhizosphere bacteria to influence plant-

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growth and health in the natural environment. Microorganisms in the soil are in constant state of flux and play an active role in recycling of organic waste nitrogen, phosphorus cycle, mineralization, other phenomenon and also produce several metabolites useful for humans (Agrios, 2005).

PGPR are the bacteria that colonize in rhizosphere region of plant roots and enhance plant growth by different mechanism (Schroth and Hancock, 1982). Root colonization is influenced by biotic factors such as genetic traits of Host plant. The colonizing organism as well as a biotic factors such as soil humidity, soil pH, temperature etc…PGPR enhances plant growth by phosphate Solubilization, IAA production, siderophore production, biological nitrogen fixation, HCN production, cytokines and gibberellic acid production (Glick, 1995;Ramamoorthy *et al*., 2011) opined that PGPR increase germination percentage, seedling vigor, root and shoot growth, total biomass of plants, seed weight, early flowering grains, fodder and fruit yield, that can serve as a source as "Bio" fertilizers (Boddey and Dobereiner, 2000). One of the mechanism used PGPR affect growth is antagonism against phytopathogenic microorganism by production of siderophore (Scherand Baker, 1982).this research provide environmental friendly approach of rhizobacteria that are found in contaminated soil with liquid wastes.

Objective of study:

General objective:

To isolate and characterize rhizobacteria from rhizosphere soil contaminated with liquid waste.

Specific objectives:

To isolate rhizobacteria from rhizosphere soil contaminated with liquid waste

To identify Gram positive and Gram negative bacteria from soil contaminated with liquid waste

To identify rhizobacterial load from rhizosphere soil

To assess associated risk factors on students' health

Statement of the problem:

Liquid wastes are any water born wastes discharges from cafeteria, shower, and laundry are unwanted or hazardous. The habits of open field disposal of these waste plays a role in the contamination of soil and it can be significant health risk. Environmental pollution increase as the density of people increases, unsanitary environments are favorable for the outbreak and spread different types of communicable disease. The study was conducted to examine the disposal of liquid waste in Arba Minch University, Abaya Campus. There is no conventional waste water collection and treatment system in the campus; this may create a high prevalence rate of waterborne disease and high effect on plant growth by reducing soil fertility.

Significance of the Study:

Liquid wastes contain many different types of bacteria and other microorganism originating from human activity. The presence of bacteria in waste is normal and expected but it becomes a problem if the waste is not kept separately from people. There is no research study in the campus before, so this study will give insight information and get scientific attention about liquid waste disposal mechanism of abaya campus. So it will fill the gap and providing further evidence to finding of prior studies, and also identified a few additional factors that worth for further research and validation. And also gives better awareness for the community about health effect of unsanitary environment to minimize the risk.

Scope of the Study:

The study was conducted in ArbaMinch University abaya campus on liquid waste around female dormitory

2. MATERIAL AND METHOD

Description of the study area:

This study was conducted in Abaya Campus liquid waste disposal area, Arba Minch University, Arba Minch town. Arba Minch University is nestled at the foot of Gamo Gofa mountain ranges facing huge abaya lake. Arba Minch is found in

the south nation nationalities and people's regional state (SNNPRS). It is located at 30° 56' north of equator and 37° 44' east. The area is about 2184 hectares at an elevation of 1,285m. It is 505 km South of Addis Ababa in greater east African Rift valley. It is found in Gamo Gofa zone and the second largest town in (SNNPRS) next to Hawassa. It is surrounded by Arba Minch zuria wereda (Philip and Brain, 2009).

Study Design:

Cross sectional study was conducted to identify bacterial load in the soil contaminated with liquid waste and the data collection was conducted from October 2017 to June 2018 in Arba Minch University Abaya campus.

Sample Size and Sampling Techniques:

Three different soil samples were collected by using purposive sampling technique.

Sample and Data Collection:

Primary data collection was used to collect data from Abaya campus students by using Questionnaire and observational check list. The sample was collected from selected area and transport aseptically to Microbiology laboratory. Bacteria were isolated from the collected sample.

Identification and Characterization:

The isolated bacteria was identified and characterized by using simple biochemical tests, such as Gram staining and Catalase test.

Procedure:

Rhizospheric soil collection in the field

Rhizospheric soil sample was collected from the liquid waste disposal area within 30 meter difference. During collection the upper surface of the soil was cleaned and taken up to 5cm depth from three different areas aseptically in sterilized baker, then brought to the microbiology laboratory.

Laboratory Procedure:

1g of soil sample was measured and dissolved in 9ml sterilized distilled water and serially diluted up to 10^{-6} fold, Then from serially diluted soil sample 1ml was transferred to nutrient agar from 10^{-6}-10^{-3} fold And also in mannitol salt agar and macConkey simultaneously using micropipette and spread on the surface of medium by using spreader. Finally all plates were incubated at 30^{0}C for 24 hour.

Description of Bacteriological loads of the soil sample

Colony for each plate was counted by using colony counter after 24hr and described as colony forming unit per milliliters (cfu/ml) by using the following formula

> **No of colonies in original sample** = number of colony counted $*1/d_f*V$
>
> Where:
>
> d_f = dilution factor
>
> **v** = volume of the sample

Biochemical characterization:

Colony morphology, shape, color, form, and growth pattern were recorded after 24h of growth on nutrient agar, mannitol salt agar, MacConkey agar at 30^{0}c. The Gram reaction was performed as described by (Vincent and Humphrey, 1970). Catalase production was checked by placing a drop of H_2O_2 on to the bacterial colony on a glass slide.

Data analysis

Data was analyzed by using simple descriptive statistical methods such as Percentage, table, and figure.

3. RESULT AND DISSCUTION

Result

Bacterial colonies grown on different medium

A total of 183 Rhizobacterial colonies were isolated from rhizosphere of C*ordial africana* and *Vernonia amygdalina* plants soil sample after incubated at 30^oc for about 24 hours.

Table 1: Bacterial colonies grown on different medium

Soil sample	Types of media	Dilution factor	No of colony	No of colonies in original sample(cfu/ml)
Mixed soil sample	Nutrient agar	$_{10}-3$	Too much	Too much
	MacConkey agar	10^{-3}	156	$156*10^3$
	Mannitol salt agar	10^{-3}	3	$3*10^3$
	Nutrient agar	$_{10}-4$	Too much	Too much
	MacConkey agar	$_{10}-4$	21	$21*10^4$
	Mannitol salt agar	$_{10}-4$	-	-
	Nutrient agar	10^{-5}	Too much	Too much
	MacConkey agar	$_{10}-5$	3	$3*10^5$
	Mannitol salt agar	10^{-5}	-	-

The above table shows that the selective media, mannitol salt agar and MacConkey agar have the ability to grow higher number of bacteria selectively

Over all Mean Of bacterial colony counted on all Medias

The mean of bacterial colony on MacConkey and mannitol salt agar for the mixed soil sample described as below in table 2.

Table 2: Mean bacterial colony counted from the soil sample

sample	Over all Mean (cfu/ml)
Soil sample	167250

The above table shows that, the mean bacterial colony was 167250cfu/ml from the collected soil sample. This indicates, there was high amount of bacteria found in the soil, which is found around liquid waste disposal area.

Morphological and biochemical characterization

Simple biochemical characterizations were done on randomly selected isolates from a total of 183 rhizobacterial colonies.

Table 3: Morphological and biochemical characteristics of bacterial isolates from contaminated rhizosphere with liquid wastes.

Strain	Isolated from	Gram reaction	Catalase test	shapes	forms	Colors
Staphylococcus	RHS	+	+	Round shaped	Cluster form	Purple

Streptobacillus	RHS	+	+	Large shaped	Rod	Chain	purple

Associated risk factors for the contamination of the soil by bacteria

The associated risk factors for the contamination of soil in this study are indicated in table 4. As indicated in the table, lack of treatment plant, hazardous and untreated wastes disposed in the campus are the major risk factors observed for the contamination of the soil by bacteria.

Table 4: the status of waste disposal area

Observational check list	Yes	No
The waste disposal area is away from the dormitory		√
The waste disposal area is away from the cafeteria	√	
Healthcare liquid waste management practice is found to be satisfactory		√
Quality assurance of liquid waste treatment process		√
Presence of treatment plant		√
Are hazardous and dangerous wastes properly segregated from other wastes, stabilized, treated, and disposed of in accordance with the standards?		√
The Mosquitoes are less in this area	√	

Socio-demographic characteristics of respondents:

All of the 20 respondents who participated in the assessment of associated risk factors were females. The female student's dormitory has four blocks, but the respondents were selected only from the first floor of the two blocks. Because the selected two blocks are found nearer to the waste disposal area. As indicated in table 5 below, all of the respondents (100%) are in the age range of 18-25. Regarding their educational status, most of them (40%) are second year students and most of them (25%) are from biology department.

Table 5: Socio-demographic characteristics of respondents

	Variables	Frequency	Percentage (%)
Age	18-25	20	100
	25-35	-	-
	>35	-	-
Sex	Female	20	100
Educational status(year)	1	1	5
	2	8	40

	3	7	35
	4	4	2o
Department	Physics	3	15
	Chemistry	1	5
	Biology	5	25
	Biotechnology	4	20
	Industrial chemistry	2	10
	Sport	2	10
	Statics	3	15

Table 6: Knowledge of respondents about waste disposal area and related problems

		Frequency	Percentage (%)
Do you know where the waste disposal area is	Yes	20	100
	No	0	0
Do you think that there is a treatment for the waste disposal	Yes	0	0
	No	20	100
Do you think the waste disposal can cause disease	Yes	20	100
	No	0	0
Do you think you have faced disease related with the waste?	Yes	16	80
	No	4	20

Table 6, show that 100% of students know the area of waste disposal and they think there is no treatment before and after disposal. All (100%) of respondents think that the waste has the ability to cause disease, from them 80% think they faced the disease and 20% think don't faced any disease.

Table 7: knowledge of students about the diseases

	Name of the disease	Frequency	Percentage (%)
students who mentioned the name of the disease	Common cold	13	65
	Malaria	9	45
	Typhoid	8	40

Allergy	5	25
Asthma	6	30
Others	6	30

As the table shows, respondents can mention the name of the disease, 65% of them mention common cold, 45% Malaria, 40%Typhoid, 25% Allergy and 30% Asthma

Table 8: knowledge of respondents about the agents of diseases

The Name of agents	Frequency of respondents	Percentage (%)
Bacteria	10	50
Arthropods	4	20
Viruses	10	50
Others	4	20

As the table above shows, the respondents also mentioned the agents for the disease, from the total respondents, 50% mentioned bacteria as agent of disease, 20% Arthropods and 50%viruses.

Diseases that the respondents faced:

Release of untreated liquid waste to the environment could impose problems on the health of students like Common cold, Allergy, Asthma, and others.

Table 9: Diseases that the respondents faced due to the waste

Name of the disease	Frequency of respondents	Percentage (%)
Common cold	10	50
Asthma	4	20
Allergy	2	10

Table 9 shows; from all respondent 50% of students think they attacked by common cold due to the waste, 20% by asthma and 10% by allergy. The result indicated that improper disposal of waste have cause communicable diseases.

4. DISCUSSIONS

This study focused on isolation and characterization of bacteria found in the liquid waste contaminated rhizosphere. Three agars were used to isolate rhizobacteria, from them Nutrient agar is very suitable for the growth of soil bacteria and it's difficult to count the colony but MacConkey and Mannitol salt agar is selective to grow *streptobacillus* and *staphylococcus* respectively. The mean bacterial count, which is obtained from the soil samples is167250cfu/ml. Ubiquitous microorganisms employ strategies to survive in diverse environments, thus controlling the growth of their neighbors. One such organism, bacillus was found to inhibit the growth of *staphylococcus* as a result of these strategies (Sagar, *et al*., 2011).

According to the research conducted at Mexico by (Cruz, *et al*., 2016) they were isolated six selected soil bacteria from the two sample of petroleum contaminated soil with different colonial morphology, Gram behavior of the isolates was

diverse, two isolate were Gram positive *cocci* and the rest of the isolate were 50% Gram positive Bacillus *spp* and 50% Gram negative *Bacillus spp*. All the isolated bacteria were isolated based on its DNA sequence homology analysis as *staphylococcus spp*, *Bacillus* cereus strain, *sporosarcina aquimarina, Achromobacteor spp*, *Peanibacillus spp*, they isolate bacteria in species level and several bacteria's were isolated in their study because petroleum was highly contaminant of soil than domestic waste.

Results lines with earlier studies conducted on bean, carnation by Alstorm, in1991. State that Suppression of many of soil born pathogen by the rhizobacteria strains in various crop have been well documented (Vidyasekaran *et al*., 1997).These Rhizobacterial isolates can be utilized for suppression of soil born pathogen in mustard crop. In this study, assessment of associated risk factor on the health of student related to untreated liquid waste, show that from selected 20 students 16 students think that the waste cause health problem because of soil borne pathogens, According to a report released in May by the American Academy of Microbiology. Complacency about wastewater treatment can be dangerous. The report, titled A Global Decline in the Microbiological safety of water: A call for action, estimate that, worldwide, 80percent of infectious disease may be water related, Scientists believe there may be hundreds of disease causing organisms in wastewater that have yet to be identified.

According to the research conducted in Debre Markos Town, Amhara Reginal state, Ethiopia, by Maru Abebaw in 2014 indicates that the Assessments of house hold wastewater management on environment is inadequate. The waste management and challenge were evaluated by survey of 162 sample respondents, interviews and field observation. The result show that the impact of the liquid waste grouped in to health problem, water and air pollution. Improper liquid waste cause the outbreak of communicable disease on the community, 6.2% of the diagnoses in the town were skin infection disease. Similarly according to interview finding the discharged waste water is not flow and stay long time cause to create offensive odder and the community easy to catch with common cold and the cause of disease of those asthmatic patients. When we compare with our finding the result was similar because from our respondent many of them are attacked by common cold and asthma because of the odor and the waste is found close to female dormitory so the risk is high.

5.   CONCLUSION AND RECOMMENDATION

Conclusion:

The bacterial colonies counted on this study from rhizosphere contaminated with liquid waste demonstrated that the soil is highly contaminated by gram positive bacteria. The contaminated soil was associated with several risk factor and this study show that improper disposal of waste water play a role in the cause of communicable disease. Generally the result of the study revealed that the school administrator don't give attention for the improper disposal of waste, the health of students is at risk because the waste disposal area is nearer to the female students dormitory.

Recommendation:

Based on the result of present study the following recommendations are forwarded;

- Temporary solution is giving awareness to the students as well as the clearer to protect themselves from the disease

- The habits of open field disposal of liquid waste is one of the main causes of soil and water contamination so the school administrator should give attention for proper management of liquid waste

- The dry and liquid waste should be separated

- The College should treat the waste according to their type before disposed to the environment by using physical, chemical and biological treatment method.

- The waste disposal area should be far from the students because unsanitary environments are favorable for the outbreak and spread different types of communicable disease

- Its prefer to show the problem to the person related with environmental waste treatment agencies to get better solution

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