Journal of Ecobiotechnology 2019, 11: 13-17
doi: 10.25081/jebt.2019.v11.5506
http://updatepublishing.com/journal/index.php/jebt

Research Article

ISSN: 2077-0464

Impact of brewery wastewater sludge on microbiological quality of agricultural soil

C. Ike Christian[1], C. Odika Prince[2]*, E. Okorie Peace[3]

[1]Department of Biological Sciences (Microbiology Unit), Rhema University, Aba, Nigeria, [2]Department of Chemical Sciences (Biochemistry Unit), Rhema University, Aba, Nigeria, [3]Department of Biochemistry, Michael Okpara University of Agriculture, Umudike, Nigeria.

ABSTRACT

Impact of brewery wastewater sludge on microbiological quality of agricultural soil was studied using standard methods. Different concentrations of brewery wastewater sludge were added to soil sample collected from abandoned farm land to produce test soil samples A to C; and a control (without sludge). The samples were allowed to stay for 80 days with exposure to same environmental condition. Standard methods were deployed to isolate and group organisms from the soil samples. Important microbes such as *Streptococcus sp.*, *Klebsilla sp.*, *Proteus sp.*, *Vibrio sp.*, *Shigella sp.*, *Micrococcus sp.*, *Pseudomonas sp.*, *Enterobacter sp.*, *Escherichia sp.*, and *Bacillus sp* amongst others were isolated. The isolated organisms and their loads were more on the test soil samples against the control. These could be indication of the impact of the brewery sludge on the soil. Organisms isolated and grouped have one or more beneficial role to play with relevance to agricultural soil. This study has revealed the impact of brewery wastewater sludge on microbiological quality of agricultural soil.

KEYWORDS: Brewery sludge, microbiological quality, agricultural soil

Received: March 24, 2019
Accepted: May 01, 2019
Published: May 03, 2019

***Corresponding Author:**
C. Odika Prince
Email: prince.odika@yahoo.co.uk

INTRODUCTION

Apart from turning raw materials into furnished goods, the industries are also known to turn out chunk of wastes as industrial wastes [1]. These wastes when improperly disposed pollute the environment adversely [2]. Brewery wastewater sludge is amongst the wastes turned out as industrial wastes in brewery industry [3]. Brewery wastewater sludge is generated from brewing industry [2,3] by discharging 70% of the intake water as effluent [4,5] Analysis of brewery wastewater sludge has reviewed important elements such as nitrogen, phosphorus, potassium as well as volatile fatty acids and others nutrients [6].

Fertilizers and organic manures are materials used to improve the fertility of the soil for better crop yield. However, the quest to fashion out a more eco-friendly method of improving agricultural soil fertility for better yield has led to the possible utilisation of brewery sludge [7]. Brewery sludge can be applied directly to agricultural soil or it can be composted as organic manure before utilizing for plant growth [8]. Existing studies on brewery sludge [8-18] have addressed certain areas. However, the studies on impact of brewery sludge in agricultural soil with special emphasis to microbes and environment are scanty. This study investigated the impact of brewery wastewater sludge on microbiological quality of agricultural soil using a case of abandoned farmland.

MATERIALS AND METHODS

Sludge and Soil Sample Collection

The sludge was collected from the Wastewater Treatment Plant (WWTP) of Nigerian Breweries Plc. Aba, Abia State, Nigeria. Soil sample used for this study were collected from an abandoned, refuge dump farmland in Eboh Lane in Isiala Ngwa North Local Government of Abia State, Nigeria. The samples were transported to Rhema University laboratory for further treatment before usage.

Sludge and Soil Mixture

At the laboratory, the soil sample was separated into four samples (three test specimen as test soil samples A to C; and a control) and each test soil sample was homogenously mixed with different concentration of the brewery sludge. For property mixing, each test sample had two subsets (T and J), contained 20 kg of soil to 5 kg of sludge; Group B, which also has two

subsets (T and J), contained 20 kg of soil to 10 kg of sludge; Group C, with two subsets (T and J), contained 20 kg of soil to 15 kg of sludge; the control soil sample, was mixed with no sludge at all. All the samples were allowed to stay for 80 days while exposing them equally to same environmental condition. The two subsets for each test soil sample were pulled together as one sample before analysis.

Microbiological Isolation, Identification and Grouping

Bacterial isolates were identified by carrying out series biochemical test as stipulated by Holt [19]. Total heterotrophic bacterial counts (THBC), total viable bacterial count (TVBC), total coliform bacterial count (TCBC), total nitrifying bacterial count (TNBC) and total fungal count (TFC) were determined using the methods of Prescott et al. [20] and Barnett and Hunter [21].

RESULTS AND DISCUSSION

The roles of soil microorganism have been noted [22-23], and their function in soil has been explained earlier [24] like changing of nutrients from inaccessible to usable forms by bacteria. Microbes isolated and identified from the soil samples after 80 days are presented in Tables 1-4. Microbes such as *Streptococcus sp.*, *Klebsilla sp.*, *Proteus sp.*, *Vibrio sp.*, *Shigella sp.*, *Micrococcus sp.*, *Pseudomonas sp.*, *Enterobacter sp.*, *Escherichia sp.*, and *Bacillus sp.*, were isolated from control sample. *Pseudomonas sp.*, *Bacillus sp.*, *Staphylococcus sp.*, *Micrococcus sp.*, *Lactobacillus sp.*, *Streptococcus sp.*, *Klebsilla sp.*, *Vibrio sp.*, *Salmonella sp.*, *Escherichia sp.*, *Citrobacter sp.*, *Proteus sp.*, *Enterobacter sp.*, and *Shigella sp.*, were isolated from test soil sample A.

Pseudomonas sp., *Bacillus sp.*, *Staphylococcussp.*, *Micrococcus sp.*, *Lactobacillus sp.*, *Lactobacillus sp.*, *Klebsilla sp.*, *Salmonella sp.*, *Escherichia sp.*, *Citrobacter sp.*, *Acinetobactor sp.*, *Serratia sp.*, *Proteus sp.*, *Enterobacter sp.*, and *Vibrio sp.*, were isolated from test soil sample B. *Bacillus sp.*, *Staphylococcus sp.*, *Lactobacillus sp.*, *Streptococcus sp.*, *Achromobacter sp.*, *Klebsilla sp.*, *Salmonella sp.*, *Escherichia sp.*, *Acinetobactor sp.*, *Serratia sp.*, *Proteus sp.*, *Enterobacter sp.*, *Vibrio sp.*, *Shigella sp.*, *Flavobacterium sp.*, *Citrobacter sp.*, *Micrococcus sp.*, and *Pseudomonas sp.*, were isolated from test soil sample C. The brewery sludge may have influence the increased isolates from the test soil samples against the control. These microorganisms become important when their individual functions and relevance to agricultural soil are considered [25-27]. *Flavobacterium sp.* has been known to increase the length of rots significantly [28]. *Bacillus sp.* has been known to increase the uptake of cadmium and significantly increases root and shoot dry weight [29]. It also stimulates plant growth and decreases Cr^{6+} content [30,31]. Generally, all the individual organisms isolated has one or more beneficial roles to play in agricultural soil.

Results of microbiological load of studied soil samples after 80 days are represented in Table 5. From the Table, THBC ranged from 2.10 x 10^5 – 4.50 x 10^5 CFU/g, TVBC ranged

Table 1: Control

Probable organism	Gas	H_2S	Butt	Slant	Oxidation and Fermentation	Citrate	Maltose	Sucrose	Lactose	Glucose	Fructose	Manitol	Urease	Starch Hydrolysis	Voges Proskeaur	Methyl Red	Indole	Catalase	Oxidase	Spore staining	Motility	Cellular Morphology	Gram Reaction
Streptococcus sp.	-	-	A	A	F	-	G	-	-	-	G	-	-	+	-	-	-	+	-	-	-	Cocci	+ve
Klebsilla sp.	+	-	A	K	O/F	+	-	-	AG	AG	A	-	+	-	+	+	-	+	-	-	-	Rod	-ve
Proteus sp.	+	+	A	A	F	+	G	G	A	A	-	A	-	-	+	+	+	+	-	-	+	Rod	-ve
Vibrio sp.	+	-	A	K	O/F	+	-	-	A	A	G	AG	-	-	+	+	-	+	+	-	+	Rod	-ve
Shigella sp.	+	-	A	K	O/F	-	A	-	A	-	A	A	-	-	-	+	+	+	-	-	-	Rod	-ve
Micrococcus sp.	-	-	A	K	O	+	G	-	-	-	-	A	-	-	+	+	-	+	-	-	-	Cocci	+ve
Pseudomonas sp.	-	-	K	K	O	-	G	+	G	-	G	A	-	-	+	+	-	+	+	-	+	Rod	-ve
Enterobacter sp.	+	-	A	K	F	+	-	-	G	-	-	A	-	-	+	-	-	+	-	-	+	Rod	-ve
Escherichia sp.	+	-	A	A	F	-	-	-	A	A	A	-	-	-	-	-	+	+	-	-	+	Rod	-ve
Bacillus sp.	-	-	A	A	F	-	G	-	-	-	G	A	-	+	+	-	-	+	-	+	+	Rod	+ve

-ve = Gram negative; +ve = Gram positive; + = positive; - = negative; A = Acid; G = Gas; O = oxidation; F = Fermentation; K = Alkaline, TSI = Triple sugar iron agar.

Table 2: Bacterial isolates from test soil sample A

Probable organism	Gas	H_2S	Butt	Slant	Oxidation and Fermentation	Citrate	Maltose	Sucrose	Lactose	Glucose	Fructose	Manitol	Urease	Starch Hydrolysis	Voges Proskeaur	Methyl Red	Indole	Catalase	Oxidase	Spore staining	Motility	Cellular Morphology	Gram Reaction
Pseudomonas sp.	-	-	K	K	O	-	G	+	G	-	G	A	-	-	+	+	-	+	+	-	+	Rod	-ve
Bacillus sp.	-	-	A	A	F	-	G	-	-	-	G	A	-	+	+	-	-	+	-	+	+	Rod	+ve
Staphylococcus sp.	-	-	K	K	F	+	G	-	-	-	-	A	-	-	-	-	-	+	-	-	-	Cocci	+ve
Micrococcus sp.	-	-	A	K	O	+	G	-	-	-	-	A	-	-	+	+	-	+	-	-	-	Cocci	+ve
Lactobacillus sp.	+	+	A	A	O/F	-	-	-	AG	G	-	-	-	-	-	-	-	+	-	-	-	Rod	+ve
Streptococcus sp.	-	-	A	A	F	-	G	-	-	-	G	-	-	+	-	-	-	+	-	-	-	Cocci	+ve
Klebsilla sp.	+	-	A	K	O/F	+	-	-	AG	AG	A	-	+	-	+	+	-	+	-	-	-	Rod	-ve
Vibrio sp.	+	-	A	K	O/F	+	-	-	A	A	G	AG	-	-	+	+	-	+	+	-	+	Rod	-ve
Salmonella sp.	+	+	A	K	O/F	+	G	A	A	A	A	-	-	-	-	+	-	+	-	-	+	Rod	-ve
Escherichia sp.	+	-	A	A	F	-	-	-	A	A	A	-	-	-	-	-	+	+	-	-	+	Rod	-ve
Citrobacter sp.	+	+	A	A	F	+	A	A	A	A	A	-	-	-	-	+	-	+	-	-	+	Rod	-ve
Proteus sp.	+	+	A	A	F	+	G	G	A	A	-	A	-	-	+	+	+	+	-	-	+	Rod	-ve
Enterobacter sp.	+	-	A	K	F	+	-	-	G	-	-	A	-	-	+	-	-	+	-	-	+	Rod	-ve
Shigella sp.	+	-	A	K	O/F	-	A	-	A	-	A	A	-	-	-	+	-	+	-	-	-	Rod	-ve

-ve = Gram negative; +ve = Gram positive; + = positive; - = negative; A = Acid; G = Gas; O = oxidation; F = Fermentation; K = Alkaline, TSI = Triple sugar iron aga.

Table 3: Bacterial isolates from test soil sample B

Probable organism	Gas	H_2S	Butt	Slant	Oxidation and Fermentation	Citrate	Maltose	Sucrose	Lactose	Glucose	Fructose	Manitol	Urease	Starch Hydrolysis	Voges Proskeaur	Methyl Red	Indole	Catalase	Oxidase	Spore staining	Motility	Cellular Morphology	Gram Reaction
Pseudomonas sp.	-	-	K	K	O	-	G	+	G	-	G	A	-	-	+	+	-	+	+	-	+	Rod	-ve
Bacillus sp.	-	-	A	A	F	-	G	-	-	-	G	A	-	+	+	-	-	+	-	+	+	Rod	+ve
Staphylococcus sp.	-	-	K	K	F	+	G	-	-	-	-	A	-	-	-	-	-	+	-	-	-	Cocci	+ve
Micrococcus sp.	-	-	A	K	O	+	G	-	-	-	-	A	-	-	+	+	-	+	-	-	-	Cocci	+ve
Lactobacillus sp.	+	+	A	A	O/F	-	-	-	AG	G	-	-	-	-	-	-	-	+	-	-	-	Rod	+ve
Lactobacillus sp.	-	-	A	A	F	-	G	-	-	-	G	-	-	+	-	-	-	+	-	-	-	Cocci	+ve
Klebsilla sp.	+	-	A	K	O/F	+	-	-	AG	AG	A	-	+	-	+	+	-	+	-	-	-	Rod	-ve
Salmonella sp.	+	+	A	K	O/F	+	G	A	A	A	A	-	-	-	-	+	-	+	-	-	+	Rod	-ve
Escherichia sp.	+	-	A	A	F	-	-	-	A	A	A	-	-	-	-	-	+	+	-	-	+	Rod	-ve
Citrobacter sp.	+	+	A	A	F	+	A	A	A	A	A	-	-	-	-	+	-	+	-	-	+	Rod	-ve
Acinetobactor sp.	-	-	K	K	O	-	-	-	A	A	A	A	-	-	-	+	-	+	-	-	-	Rod	-ve
Serratia sp.	-	-	A	K	F	+	G	G	-	-	A	+	-	-	+	-	-	+	-	-	+	Rod	-ve
Proteus sp.	+	+	A	A	F	+	G	G	A	A	-	A	-	-	+	+	+	+	-	-	+	Rod	-ve
Enterobacter sp.	+	-	A	K	F	+	-	-	G	-	-	A	-	-	+	-	-	+	-	-	+	Rod	-ve
Vibrio sp.	+	-	A	K	O/F	+	-	-	A	A	G	AG	-	-	+	+	-	+	+	-	+	Rod	-ve
Flavobacterium sp.	-	-	A	K	O	-	AG	AG	-	A	-	G	-	-	-	-	-	-	-	-	-	Rod	-ve

-ve = Gram negative; +ve = Gram positive; + = positive; - = negative; A = Acid; G = Gas; O = oxidation; F = Fermentation; K = Alkaline, TSI = Triple sugar iron agar.

Table 4: Bacterial isolates from test soil sample C

Probable organism	Gas	H_2S	Butt	Slant	Oxidation and Fermentation	citrate	Maltose	Sucrose	Lactose	Glucose	Fructose	Manitol	Urease	Starch Hydrolysis	Voges Proskeaur	Methyl Red	Indole	Catalase	Oxidase	Spore staining	Motility	Cellular Morphology	Gram Reaction
Bacillus sp.	-	-	A	A	F	-	G	-	-	-	G	A	-	+	+	-	-	+	-	+	+	Rod	+ ve
Staphylococcus sp.	-	-	K	K	F	+	G	-	-	-	-	A	-	-	-	-	-	+	-	-	-	Cocci	+ ve
Lactobacillus sp.	+	+	A	A	O/F	-	-	-	AG	G	-	-	-	-	-	-	-	+	-	-	-	Rod	+ ve
Streptococcus sp.	-	-	A	A	F	-	G	-	-	-	G	-	-	+	-	-	-	+	-	-	-	Cocci	+ ve
Achromobacter sp.	-	-	K	K	-	+	AG	-	-	AG	AG	-	+	+	-	-	-	+	+	-	+	Rod	-ve
Klebsilla sp.	+	-	A	K	O/F	+	-	-	AG	AG	A	-	+	-	+	+	-	+	-	-	-	Rod	-ve
Salmonella sp.	+	+	A	K	O/F	+	G	A	A	A	A	-	-	-	-	+	-	+	-	-	+	Rod	-ve
Escherichia sp.	+	-	A	A	F	-	-	-	A	A	A	-	-	-	-	-	+	+	-	-	+	Rod	-ve
Acinetobactor sp.	-	-	K	K	O	-	-	-	A	A	A	A	-	-	-	+	-	+	-	-	-	Rod	-ve
Serratia sp.	-	-	A	K	F	+	G	G	-	-	A	+	-	-	+	-	-	+	-	-	+	Rod	-ve
Proteus sp.	+	+	A	A	F	+	G	G	A	A	-	A	-	-	+	+	+	+	-	-	+	Rod	-ve
Enterobacter sp.	+	-	A	K	F	+	-	-	G	-	-	A	-	-	+	-	-	+	-	-	+	Rod	-ve
Vibrio sp.	+	-	A	K	O/F	+	-	-	A	A	G	AG	-	-	+	+	-	+	+	-	+	Rod	-ve
Shigellasp	+	-	A	K	O/F	-	A	-	A	-	A	A	-	-	-	+	-	+	-	-	-	Rod	-ve
Flavobacterium sp.	-	-	A	K	O	-	AG	AG	-	A	A	G	-	-	-	-	-	-	-	-	-	Rod	-ve
Citrobacter sp.	+	+	A	A	F	+	A	A	A	A	A	-	-	-	-	+	+	+	-	-	+	Rod	-ve
Micrococcus sp.	-	-	A	K	O	+	G	-	-	-	-	A	-	-	+	+	+	+	-	-	-	Cocci	+ ve
Pseudomonas sp.	-	-	K	K	O	-	G	+	G	-	G	A	-	-	+	+	-	+	+	-	+	Rod	-ve

-ve = Gram negative; +ve = Gram positive; + = positive; - = negative; A = Acid; G = Gas; O = oxidation; F = Fermentation; K = Alkaline, TSI = Triple sugar iron agar.

Table 5: Results of microbial load of soil samples

Load (CFU/g)	Control	Test soil sample A	Test soil sample B	Test soil sample C
THBC ($\times 10^5$)	2.10	3.90	4.50	4.20
TVBC ($\times 10^4$)	1.30	2.50	3.20	2.30
TCBC ($\times 10^3$)	4.20	1.30	1.90	1.10
TNBC ($\times 10^2$)	0.40	0.80	1.00	1.20
TFC ($\times 10^5$)	4.10	5.90	6.50	6.90

Total Heterotrophic Bacterial Count (THBC), Total Viable Bacterial Count (TVBC), Total Coliform Bacterial Count (TCBC); Total Nitrifying Bacterial Count (TNBC); and Total Fungal Count (TFC).

from 1.30 x 10^4 – 3.20 x 10^4 CFU/g, TCBC ranged from 1.10 x 10^3 – 4.20 x 10^3 CFU/g, TNBC ranged from 0.40 x 10^2 – 1.20 x 10^2 CFU/g and TFC ranged from 4.10 – 6.90 x 10^5 CFU/g. Heterotrophic bacteria breakdown carbohydrates and sugars and make them available to the soil [32,33].

CONCLUSION

The increased number of isolates from test soil samples (soil mixed with brewery wastewater sludge) and increased microbial loads as observed in the present study could be indication that brewery sludge can impact positively on agricultural soil. Bacteria and fungi are emerging indicators of soil condition, and all the isolated and grouped organisms of the present study has one or more beneficial role to play with relevance to agricultural soil. This study has revealed the impact of brewery wastewater sludge on microbiological quality of agricultural soil.

REFERENCES

1. Genner C. Treatment and disposal of brewery effluents. Brewers Guardian. 1988; October. 25-27.
2. Kalatzi E, Sazakli E, Karapanagioti HK, Leotsinidis M. Composting of brewery sludge mixed with different bulking 2016. agents.http://uest.ntua. gr/Cyprus 2016/proceedings/pdf/Kalatzi_et_al_Composting_of_brewery_sludge_mixed_with_different_bulking_agents.pd (Accessed on 2nd, April, 2019).
3. Olowu RA, Osundiya MO, Onwordi CT, Denloye AA, Okoro CG, Tovide OO, Majolagbe AO, Omoyeni OA, Moronkola BA. Pollution status of brewery sewage sludge in Lagos, Nigeria. International Journal of Research and Reviews in Applied Sciences. 2012;10(1):159-65.
4. Vriens L, Van Soest H, Verachtert H. Biological treatment of malting and brewing effluents. Critical Reviews in Biotechnology.1990; 10 (1): 1-46.
5. Kanagachandran K, Jayaratne R. Utilization potential of brewery waste water sludge as an organic fertilizer. Journal Institute of Brewing. 2006; 112(2): 92-96.
6. Ramya N, Srinivas T, Lakshmi KA. Studies on effect of brewery waste water sludge (BWS) on morphology and yield of chilly (*Capsicum annum* L.) plant. International Journal of Pharmaceutical Sciences and Research. 2015; 6(1):405-409.
7. Senthilraja K. Effect of brewery effluent irrigation and sludge application in combination with organic amendments on growth and yield of sesame (*Sesamum indicum* L.). International Journal of Current Microbiology Applied Sciences. 2017; 6(12): 965-977.
8. Stocks C, Barker AJ, Guy S. The composting of brewery sludge. Journal Institute of Brewing. 2002, 108(4), 452–458.
9. Erdem N, Ok SS. Effect of brewery sludge amendments on some chemical properties of acid soil in pot experiments. Bioresource Technology. 2002; 84(3):271-173.
10. Luque O, Bracho O, Maier TW. Utilization of brewery waste water sludge for soil improvement. Techical Quarterly Master Brewers

Association. 1990;27(1):5–9.
11. Cihat K, Gokhan C, Abdullah B, Ouz BK and Roger H. Effects of beer factory sludge on soil properties and growth in sugar beet (*Beta vulgaris saccharifera L.*), Bioresource Technology. 2003;75-77.
12. Stocks C, Barker AJ, Guy S. The composting of brewery sludge. Journal Institute of Brewing. 2002; 108(4): 452–458.
13. Kumar S, Sharma MP, Sharwan K. Performance evaluation of brewery waste water treatment plant. International Journal of Engineering Practical Research. 2013; 2:105-111.
14. Chaitanyakumar S, Azeem U, Bhupattthi R. Efficiency assessment of combined treatment technologies. Indian Journal of Fundamental and Applied Life Sciences. 2011; 1(2):138-145.
15. Kanagachandran K, Jayaratne R. Utilization potential of brewery waste water sludge as an organic fertilizer, Journal of Institute of Brewing. 2006; 112(2): 92-96.
16. Ahmadi H, Vahid A, Farshad D, Abdolati S. Effect of different levels of nitrogen fertilizer on yield, nitrate accumulation and several quantitative attributes of five Iranian spinach accessions. Journal of Agricultural and Environmental Science. 2010; 8(4): 468-473.
17. Nihal E, Sonay S, Ru OK. Effect of brewery sludge amendments on some chemical properties of acid soil in pot experiments, Journal of Bioresource Technology. 2002; 84: 271-273.
18. Mohimi AO, Ekpo KE, Chukwuedo ME. Economic importance and application options of some industrial sludge conditioned by different treatment methods, African Journal of Biotechnology 2010; 7: 2434-2440.
19. Holt JG. The shorter bergey`s manual of determinative bacteriology. 8th ed. The Williams and Wilkins Company, Batimore, MD, USA.1982.
20. Prescott LM, Harley JP Kelin DA. Microbiology, 6th ed., McGraw Hill, London. 2005; pp.135-140.
21. Barnett HL, Hunter BB. Illustrated genera of imperfect fungi. 3rd ed. Burgess Publishing Company, Minnesota, USA. 1972.
22. Vrieze J.The littlest farmhands. Science. 2015; 349 (6249): 680–683.
23. Christopher J. Living soils: the role of microorganisms in soil health. 2017. http://www.futuredirections.org.au/publication/living-soils-role-microorganisms-soil-health (accessed on 12 Feb., 2019).
24. Jelen BI, Giovannelli D, Falkowski PG. The Role of microbial electron transfer in the coevolution of the biosphere and geosphere. Annual Review of Microbiology. 2016; 70 (1): 45–62.
25. Sylvia DM, Jeffry JF, Peter GH, David AZ. Principles and applications of soil microbiology. Upper Saddle River: Prentice Hall, 1998.
26. Cakmaki ML, Evans HJ, Seidler, R.J. Characteristics of a nitrogen-fixing *Klebsiella oxytoca* isolated from wheat roots. Plant and Soil. 1981; 61: 53–64.
27. Riggs PJ, Chelius MK, Iniguez AL, Kaeppler SM, Triplett EW. Enhanced maize productivity by inoculation with diazotrophic bacteria. Australian Journal of Plant Physiology. 2001; 28 (9): 829–836.
28. Belimov A, Hontzeas N, Safronova VI, Demchinskaya SV, Piluzza G, Bullitta S, Glick BR. Cadmium-tolerant plant growth-promoting bacteria associated with the roots of Indian mustard (*Brassica juncea* L. Czern.). Soil Biology and Biochemistry. 2005; 37: 241–250.
29. Sheng XF, Xia JJ. Improvement of rape (*Brassica napus*) plant growth and cadmium uptake by cadmium-resistant bacteria. Chemosphere. 2006; 64:1036 – 1042.
30. Rajkumar M, Nagendran R, Kui JL, Wang HL, Sung ZK. Influence of plant growth promoting bacteria and Cr^{6+} on the growth of Indian mustard. Chemosphere. 2006; 62: 741–748.
31. Ma Y, Rajkumar M, Freitas H. Inoculation of plant growth promoting bacterium Achromobacter xylosoxidans strain Ax10 for the improvement of copper phytoextraction by *Brassica juncea*. Journal of Environmental Management. 2009;90(2):831-7.
32. James JH. The Role of soil bacteria. Fact Sheet, Agricultural Natural Resources. SAG 13-11 accessed on 5th Jan., 2019.
33. Sylvia DM, Hartel PG, Fuhrmann JJ, Zuberer DA. Principle and Application of Soil Microbiology. 2nd ed. Edited by David M. Sylva, Pearson Prentice Hall. 2005.