CHEMICAL CHARACTERISTCS AND MICROBIAL DIVERSITY OF SOILS FROM MENENGAI CRATER IN KENYA

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ABSTRACT. *A total of 98 soil samples collected from Menengai geothermal site located in Nakuru, Kenya were analyzed for their chemical and microbial components. In region A, phosphorus, sodium, nitrogen and carbon were deficient, region B and C, phosphorus and Manganese, while in D the deficient minerals were phosphorus, sodium, nitrogen and carbon. Toxic minerals were calcium and magnesium in regions A, B, C and D. The most prevalent bacteria were* Escherichia coli *with a mean of* $(3.35x10^7)$ *while the least were* Bacillus sp. $(1.40x10^5)$*. Among the fungi, the most dominant were* Aspergillus nomius *with a mean of* $4.42x\ 10^4$ *with the least being* Panicillium sacculum $(8.09x10^3)$*. As much as geothermal exploration is important in boosting the country's energy reserves, there is a need to rehabilitate the caldera so as to restore the microbial composition of the region.*

KEYWORD. Diversity, crater, geothermal, Menengai, microbial, chemical.

INTRODUCTION

Microorganisms are found in all environments owing to their small size and easy dispersal and the ability to grow and multiply under anaerobic conditions (Dhiva *et al.,* 2016). In addition, their metabolic versatility and flexibility to utilize a broad range of nutrients, and the ability to tolerate and colonize otherwise unfavourable locations such as geothermal environments help them to thrive in many environments. Microorganisms that grow in extreme environments which include but are not limited to hot deserts, polar regions, geothermal areas, are referred to as extremophiles (Abdullah *et al.,* 2016).

Geothermal sites provide a unique ecological site to different microbes (Commichau *et al.,* 2013). Although the sites are deficient in carbon and other nutrients, geothermal ecosystems harbor diverse microbial communities which can adapt themselves in the extreme conditions witnessed in these regions (Galperin *et al.,* 2012). The microorganisms

help in recycling and transformation of various nutrients. The microorganisms of geothermal sites are essential in the productivity, conservation, and rehabilitation of geothermal ecosystems (Martins *et al.*, 2013). The importance of bacteria in promoting of halotolerant plants have been studied (Sunita and Kanwar, 2016). Culturable bacterial distribution in volcanic soils has been reported (Kumar *et al.,* 2014). Volcanic soils sites shows diversity of microbes such as bacteria and fungi. Although a lot of studies have been carried out on microbial components of volcanic areas, little is still known about the microbial diversity of soils in volcanic calderas. Soil samples from four regions in Menengai caldera were collected and analyzed for their chemical and microbial components.

MATERIAL AND METHODS

The study area

The study was carried out in Menengai crater which lies in the North of Lake Nakuru. It rises to a height of 2,278 m above sea level, and is a product of a volcanic eruption that occurred about 200,000 years ago. The crater is considered a dormant volcano with the occurrence of a high temperature geothermal resource, manifested mainly by steaming grounds at a temperature of 88^{o}C. After the eruption, the sides of the volcanic crater collapsed inwards forming a large hole in the crust, called a caldera (Figure 1). Menengai caldera occupies an area of 90 km2 and a diameter of 12 km. It is the second largest caldera in Africa after Ngorongoro in Tanzania. Menengai caldera is located in Rongai and Nakuru North Sub-counties at 35^{o} 28', 35^{o} 36'E, and 0^{o} 13', 1^{o} 10'S (Omenda *et al.,* 2000).

Soil sample collection

Soil samples were separately collected from the top 5cm using a sterile spatula from the 8 sampling points in each region. The samples for each region were mixed to make a composite sample. The composite samples were placed in sterile plastic bags and transported to the department of biological sciences, Egerton University for analysis.

Chemical analysis of the soil

The soil samples were suspended in distilled water. Soil pH was determined according to the procedure described by Martins *et al.,* (2013). The pH meter was standardized using a standard solution provided. Immediately before immersing the electrode(s) into the sample, the sample was well stirred with a glass rod. The electrode(s) were placed into the soil slurry solution and the beaker was gently turned to make good contact between the solution and the electrode(s). Organic carbon was determined using the wet oxidation method (Ramanadevi *et al.,* 2013).

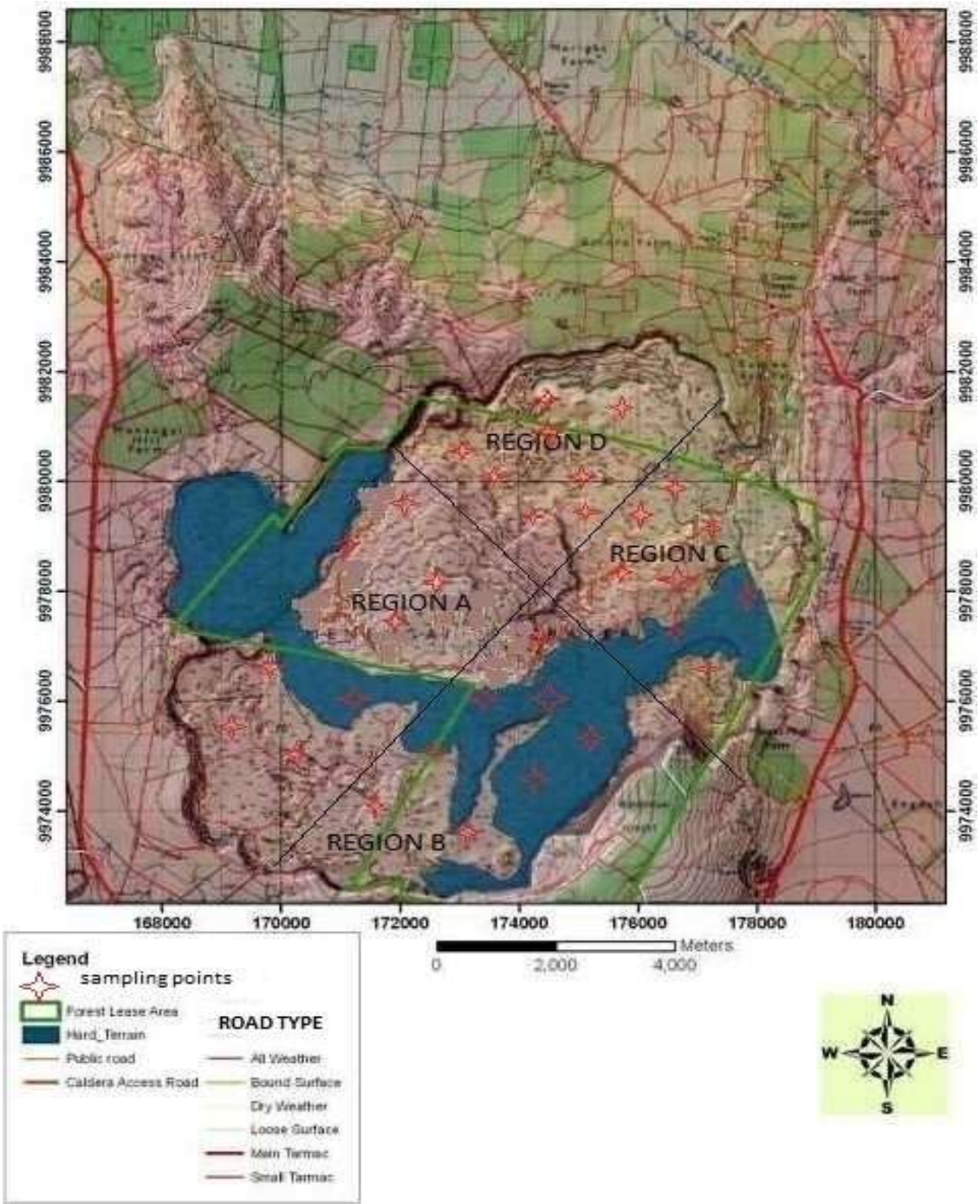

Figure 1: Map of Manengai crater caldera and its environs (Mariita, 2003)

The Kjeldahl method (Khursheed *et al.*, 2014) was used to determine total nitrogen. Phosphorus was extracted by the Bray and Kurtz-1 method and determined spectrophotometrically (Osakwe, 2014). Available potassium and sodium content in the soil were determined by using turbiditimetric methods; calcium was determined by titration with standard KMnO4 solution, magnesium by precipitation in alkaline medium as magnesium ammonium phosphate (Guo *et al.*, 2014). Manganese was analyzed by Atomic Absorption Spectrophotometer using GBC Avanta version 1.31 by flame Automization. (Egejuru *et al.*, 2014)

Microbial isolation and identification

The microorganisms were isolated using serial dilution plate method. About 1 g of the sample was weighed and suspended in 9 mL of distilled water to make 10-1 to 10-3 dilutions. Using a micropipette, 1 mL aliquots of each concentration were added to sterile petri dishes containing 15 mL of sterile sabouraud dextrose agar for fungi, and nutrient agar for bacteria isolation in triplicate. Incubation was carried out at 37oC for 24 h (bacteria) and 28oC for 4 days (fungi). Growth of the organisms were counted using colony counter, and the microbial loads were calculated in CFU/mL. To obtain pure cultures, the microbes were sub-cultured by streaking on new solidified media. The pure isolates were stored in slant bottles for characterizations using colony morphology and biochemical characteristics.

Data analysis

Data were presented using tables. All the data analysis were carried out using Statistical Package of Social Sciences Software (SPSS) software version 17.0 software and Microsoft 2010 spreadsheet. One way ANOVA was used in comparing the means.

RESULT AND DISCUSSION

Chemical characteristics of soil from Menengai crater

The pH ranged between 6.2 in region D to 5.52 in region C, phosphorus from 15.53 in region C to 4.15 in region A, potassium from 1.50 in region C to 0.35 in region A, nitrogen from 0.29 in region C to 0.10 in region A, calcium from 16.56 in region B to 3.45 in region B, magnesium from 2.56 in region B to 2.35 in region D, manganese from 0.51 in region B to 0.31 in region A, sodium from 0.15 in region C to 0.00 in region D, carbon from 2.50 in region C to 1.05 in region A (Table 1). The Ca: Mg ratio varied from 21.78 in region D to 15.61 in region B. In region A, phosphorus, sodium, nitrogen and carbon were deficient, region B and C, phosphorus and manganese while in D the deficient minerals were phosphorus, sodium, nitrogen and carbon. Toxic minerals were calcium and magnesium in region A, B, C and D.

Table 1: Chemical properties of soil samples from Menengai crater.

Chemical properties of soil	Region A	Region B	Region C	Region D	Recommended Levels	
					Low	High
pH	6.01	5.62	5.52	6.20	5.50	7.00
Phosphorus (mg kg^{-1})	4.15	15.00	15.53	5.00	20.00	100.00
Potassium (cmol kg^{-1})	0.35	1.23	1.50	0.33	0.20	1.50
Nitrogen (%)	0.10	0.24	0.29	0.18	0.20	0.80
Calcium (cmol kg^{-1})	3.45	16.56	15.34	4.84	2.00	10.00
Magnesium (cmol kg^{-1})	2.40	2.65	2.59	2.35	1.00	3.00
Manganese (cmol kg^{-1})	0.31	0.51	0.45	0.34	0.10	1.00
Sodium (cmol kg^{-1})	0.02	0.10	0.15	0.00	0.10	2.00
Carbon (%)	1.05	2.40	2.50	1.84	2.00	5.00
Ca: Mg ratio	20.14	15.61	16.00	21.78	4.00	7.00

Identification

Identification of the isolates based on cultural characteristics, colony morphology, motility and biochemical characteristics are presented in Table 2 and 3. The bacteria isolates were identified as *Geobacillus sp*., *Bacillus sp*., *Escherichia coli* and *Proteus mirabilis*, while the fungi were *Fusarium oxysporum*, *Aspergillus nomius*, *Penicillium sacculum (*Plate 1), *Mucor sp*. and *Rhizopus sp*.

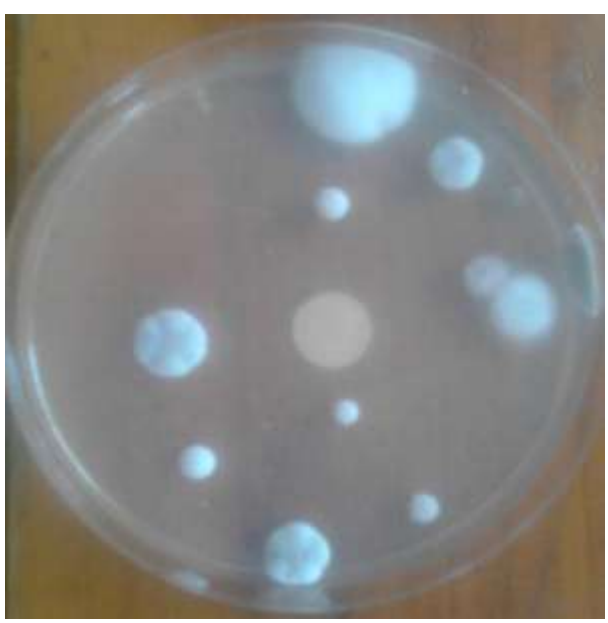

Figure 2: Pure culture of *Penicillium sacculum* growing on sabouraud dextrose agar.

Table 2: Cultural and morphological characteristics of microorganisms isolated from the soils of Menengai crater

Microorganism		**Cultural characteristics**	**Morphological characteristics**
Bacteria	*Geobacillus sp.*	Rod shaped cells, 1 µm diameter	spores that are slightly ovoid
	Bacillus spp.	large, flat colonies	The endospores were oval or round, cylindrical.
	Escherichia coli	rod-shaped cells, flagellated	No spore formation
	Proteus mirabilis	Swarming non-hemolytic colonies	No spore formation
Fungi	*Fusarium oxysporum*	Rapidly growing wooly to cottonly lemon and yellow	Multicellular distinctive sickle shaped macro coniclia.
	Aspergillus nomius	Very common colours of colony (black and white)	Conidia borne in 360 arrangements covering the upper 2/3 of the conidiophores.
	Penicillium sacculum	Large fluffy white colonies almost covering the whole surface	Non – septate branched hyphal enlarge at the apex to form cornidophorex.
	Mucor sp.	Cream white/large fluffy white colonies almost covering the whole surface	Sporangium comes out directly from the hypha
	Rhizopus sp.	Large fluffy white milky colonies which later turns black as culture ages.	Non-septate hyphal with upright sporagioshere connected by stolon and rhizoids, dark pear-shaped sporagium on hemispherical columella.

Table 3: Biochemical characterization of microorganisms isolated from the soils of Menengai crater.

Microorganism		Citrate	Urease	Motility	MR	VP	Indole
Bacteria	*Geobacillus spp.*	+	+	+	+	–	+
	Bacillus spp.	+	+	+	+	–	–
	Escherichia coli	+	–	+	+	–	+
	Proteus mirabilis	+	+	+	+	–	–
Fungi		**Carbohydrate assimilation**	**Spore formation**	**Amino acid assimilation**	**Motility**	**Hydrolysis**	**Lipase Activity**
	Fusarium oxysporum	+	–	+	–	-	+
	Aspergillus nomius	+	–	+	–	–	+
	Penicillium sacculum	+	–	+	–	–	+
	Mucor sp.	+	+	–	–	–	–
	Rhizopus sp.	+	+	+	–	–	–

Key: +: Positive; -: Negative, MR: Methyl Red test, VP: Voges- Proskauer test.

Isolation of microbes from the soils of Menengai crater

Among the bacteria, *Geobacillus sp.* ranged from $7.40x10^5$ in region D to $2.22x10^2$ in region C, *Bacillus sp.* from $5.34x10^5$ in region D to$1.43x10^1$ in region C, *Escherichia coli* from $9.19x10^7$ in region B to$5.26x10^3$ in region D, *Proteus mirabilis* from$3.34x10^7$ in region C to $1.10x10^1$ in region A. The fungus *Fusarium oxysporum* ranged from $2.20x10^4$ in region C to $1.50x10^1$ in region D, *Aspergillus nomius* from $2.40x10^4$ in region B to $2.40x10^1$ in region D, *Penicillium sacculum* from $2.10x10^4$ in region C to $1.60x10^2$ in region D, *Mucor sp.* from $2.20x10^4$ in region C to $2.30x10^1$ in region D, *Rhizopus sp.* from $3.49x10^4$ in region C to$1.96x10^2$ in region D (Table 4). The bacterial ($F= 0.95$, $P= 0.44$) and fungal ($F=29.83$, $P=0.00$) isolates in this study varied significantly.

Table 4: Microbial colony forming units from soil samples obtained from Menengai crater

Microorganism		Region A	Region B	Region C	Region D	Mean
Bacteria	*Geobacillus sp.*	$5.60x10^4$	$5.00x10^2$	$2.22x10^2$	$7.40x10^5$	**$1.99x10^5$**
	Bacillus sp.	$2.32x10^4$	$5.70x10^1$	$1.43x10^1$	$5.34x10^5$	**$1.40x10^5$**
	Escherichia coli	$3.59x10^4$	$9.19x10^7$	$4.15x10^7$	$5.26x10^3$	**$3.35x10^7$**
	Proteus mirabilis	$1.10x10^1$	$5.32x10^6$	$3.34x10^7$	$2.10x10^2$	**$9.68x10^6$**
Fungi	*Fusarium oxysporum*	$3.40x10^2$	$1.50x10^4$	$2.20x10^4$	$1.50x10^1$	**$1.09x10^4$**
	Aspergillus nomius	$9.30x10^1$	$2.40x10^4$	$1.90x10^4$	$2.40x10^1$	**$4.42x10^4$**
	Penicillium sacculum	$2.10x10^2$	$1.10x10^4$	$2.10x10^4$	$1.60x10^2$	**$8.09x10^3$**
	Mucor sp.	$4.20x10^2$	$1.20x10^4$	$2.20x10^4$	$2.30x10^1$	**$8.66x10^3$**
	Rhizopus sp.	$2.80x10^3$	$2.27x10^4$	$3.49x10^4$	$1.96x10^2$	**$1.52x10^4$**

DISCUSSION

The chemical characteristics of soils obtained the current study are typical of volcanic calderas. The results concur with a previous study carried out in Manikaran, Himachal Pradesh (Sunita and Kanwar, 2016). According to Waithaka *et al*., (2015), this could be attributed to formation of soils of menengai from solidification of magma. However the low phosphorus content observed in this study differed with a study carried out elsewhere (Jugran *et al*., 2015) which may be due to differences in the parent material from which the magma was formed prior to volcanic activity (Gagliano *et al*., 2014).

Although Menengai crater presents a hostile environment for microbial growth, the number of microbial isolates was high (Table 4). This could be explained by the capability of microbes to change their genetic constitution so as to suite different environments.

According to Sharma *et al.,* (2013) microorganisms are in a constant mission of making themselves better so as survive in the ever changing microbial niches.

The high numbers of *Bacillus sp.* witnessed in this study agree with a study previously carried out by Ramanadevi *et al*., (2013). This could be attributed to the ability of *Bacillus sp*. to form spores which are resilient to changing and environmental conditions (Connor *et al*., 2010). In contrast, the number of *E. coli* isolates in this study was higher than those obtained in Japan (Guo *et al.,* 2014). The presence of humans and animals in national reserves leads to increased isolation of E. coli (Dhiva *et al.,* 2016) which may have led to the differences.

A study carried out in Nigeria by Umar *et al.,* (2016) obtained higher levels of *Proteus mirabilis* than the current study. This could be attributed to differences in the samples from which the bacteria were isolated. *Proteus mirabilis* is essentially a pathogen, hence its isolation from environmental samples largely originates from poor disposal of human wastes (Umar, *et al*., 2015).

On the other hand, the isolation of *Fusarium oxysporum* agreed with a study carried out elsewhere (Rohilla and Salar, 2012). This could be attributed to the type of samples from which the fungus was isolated coupled with the fact that the fungus is a spore former (Das and Anitha, 2011). However the values of *Aspergillus nomius* and *Penicillium sacculum* obtained in this study were lower from those that were obtained from India (Mamta *et al*., 2013). This difference may have resulted from differences in environmental conditions from the two study areas.

In a study carried out in polyhouse agriculture soil of Rajasthan India, Jasuja *et al*., (2013) observed that *Mucor sp.* and *Rhizopus sp.* are common fungi in soils. This may explain the high number isolated in this study because the two study sites experiences high temperatures. Contrary to these findings, Karthik *et al.*, (2012) obtained very low values for the fungi from volcanic agricultural waste dump soil. This may be due to constant spraying of crops grown in the region with fungicides, while as volcanic soils of the current study were obtained from virgin caldera that has never been cultivated.

CONCLUSION

The current study establishes that the chemical characteristics of soils obtained from Menengai geothermal caldera are typical of soils formed from solidification of magma. Some of the mineral elements are at the levels toxic to growth of microorganisms. However, the number of isolated microorganisms suggest that they have developed the necessary mechanism to enable them survive in this hostile environment.

RECOMMENDATION

Currently, there are a lot of activities going on in Menengai crater. Cutting down of trees for charcoal burning is a normal scenario, followed by geothermal exploration and intensive grazing. This may have had a negative impact on the microbial diversity in the soils of this important geographical region. There is a need to rehabilitate the caldera to avoid further negative effects on this important volcanic site.

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CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

REFERENCES

Abdullah M. Al-Dhabaan, K. and Ali H. (2016). Identification of contaminated soil from isolated fungi in Riyadh province. Life Science Journal, 13(2): 123-128.

Connor, N., Sikorski, J. and Rooney, A. P. (2010) Ecology of speciation in the Genus Bacillus. Applied Environmental Microbiology, 76:1349-1358.

Commichau, F. M., Pietack, N. and Stu¨ lke, J. (2013). Essential genes in Bacillus subtilis: a re-evaluation after ten years. Molecular Biosystems, 9: 1068–1075.

Das, M. and Anitha, S. (2011). Mycoremediatio of moocrotophos, International Journal Pharmaceutical Sciences, 2(1): 337-342.

Dhiva, S., Jaishanker Pillai, H.P. and Shinde, V. M. (2016). Isolation and characterization of soil microorganisms for potential biocontrol activity. Internatinoal Journal of Current Research in Biological Sciences, 3(3): 26-29.

Egejuru, A. L., Alessandro, W. D., Tagliavia, M., Parello, F. and Quatrini, P. (2014). Methanotrophic activity and diversity of methanotrophs in volcanic geothermal soils at Pantelleria (Italy). Journal of Biogeosciences, 11: 5865–5875.

Gagliano, A. L., Alessandro, W. D., Tagliavia, M., Parello, F. and Quatrini, P. (2014). Methanotrophic activity and diversity of methanotrophs in volcanic geothermal soils at Pantelleria (Italy). Journal of Biogeosciences, 11: 5865–5875.

Galperin, M. Y., Mekhedov, S. L., Puigbo, P., Smirnov, S., Wolf, Y. I. and Rigden, D. J. (2012). Genomic determinants of sporulation in Bacilli and Clostridia: towards the minimal set of sporulation-specific genes. Journal of Environmental Microbiology, 14: 2870–2890.

Guo, Y., Fujimura, R., Sato, Y, Suda, W., Kim, S., Oshima, K., Hattori, M., Kamijo, T., Narisawa, K. and Ohta, K. (2014). Characterization of Early Microbial Communities on Volcanic Deposits along a Vegetation Gradient on the Island of Miyake, Japan. Journal of Microbes in the Environment, 29 (1):38-49.

Jasuja, J. L., Kumar, G., Rao, K. V. (2013). Screening of pectinase producing microorganisms from agricultural waste dump soil. Asian Journal of Biochemistryand Pharmaceutical Research 2:329- 337.

Jugran, J., Rawat, N. and Joshi, G. (2015). Amylase production by Geobacillus sp. GJA1 isolated from a hot spring in Uttarakhand. ENVIS Himalayan ecology, 23: 120-125.

Karthik, L., Singh, K., Bose, H., Richa, K., Gaurav, K. and Bhaskara, R. (2012). Isolation and characterization of protease producing marine eubacteria. Journal of Agricultural Technology, 8(5): 1633-1649.

Khursheed, A. W., Rajni, Y., Shivom, S. and Krishan, K. U. (2014). Comparative Study of Physicochemical Properties and Fertility of Soils in Gwalior, Madhya.Pradesh. World Journal of Agricultural Sciences, 10 (2): 48-56.

Kumar, M., Yadav, A. N., Tiwari, R., Prasanna, R. and Saxena, A. K. 2014. Deciphering the diversity of culturable thermotolerant bacteria from Manikaran hot springs. Annals of Microbiology, 64: 741-751.

Mamta J., Rashmi S., A. K. Sharma, K. and Anil, P. (2013). Isolation and characterization of Fusarium oxysporum, a wilt causing fungus, for its pathogenic and non-pathogenic nature in tomato (Solanum lycopersicum). Journal of Applied and Natural Science 5 (1): 108-117.

Mariita, N. O. (2003). An integrated geophysical study of the northern Kenya rift crustal structure: implications for geothermal energy prospecting for Menengai area. A PhD dissertation, University of Texas at El Paso, USA.

Martins, L. F., Antunes, L. P., Pascon, R. C., de Oliveira, J. C., Digiampietri, L. A., Barbosa, D., Peixoto, B. M., Vallim, M. A. and VianaNiero (2013). Metagenomic analysis of a tropical composting operation at the Sa˜o Paulo Zoo Park reveals diversity of biomass degradation functions and organisms. PLoS ONE (8) 619-28.

Omenda, P. A., Opondo, K., Lagat, J., Mungania, J., Mariita, N., Onacha, S., Simiyu, S., Wetang'ula, G., and Ouma, P. (2000). Ranking of geothermal prospects in the Kenya rift. Kenya Electricity Generating Company Limited, internal report, 121 pp.

Osakwe, S. A. (2014). Evaluation of Physicochemical Characteristics of Soils in the Flood Disaster Affected Areas of Isoko Region of Delta State, Nigeria. IOSR Journal of Applied Chemistry, 7(5): 24-31.

Ramanadevi, V., Naik, L. S. and Aruna, K. (2013). Isolation and Biochemical characterization of protease isolated from Bacillus sp SVN12. International Journal of Research in Pure and Applied Microbiology, 3(3): 94-101.

Rohilla S. K. and Salar R. K. (2012). Isolation and Characterization of Various Fungal Strains from Agricultural Soil Contaminated with Pesticides. Research Journal of Recent Sciences, 1(2): 297-303.

Sharma, N., Vyas, G. and Pathania, S. (2013). Culturable diversity of thermophilic microorganisms found in hot springs of northern Himalayas and to explore their potential for production of industrially important enzymes. Scholars Academic Journal of Biosciences 1: 165-178.

Sunita, D. and Kanwar, S. (2016). Deciphering the diversity of aerobic culturable thermophiles in hot springs of Manikaran, Himachal Pradesh. International Journal of Farm Sciences, 6(1): 156-162.

Umar, M., Akafyi, D., Abdulkarim, I., Yaya, A. A., and Danasabe, Y. (2015). International Journal of Biological and Biomedical Sciences, 4(10): 063-066.

Umar, M., Yaya, A. A., Yusuf, G., Tafinta, I.Y., Aliko, A.A., Jobbi, D.Y. and Lawal, G. (2016). Biochemical characterization and antimicrobial susceptibility trends of Proteus mirabilis isolated from patients suspected with urinary tract infections attending Sickbay Hospital, Zaria, Kaduna, Nigeria. Annals of Biological Sciences, 4(2):1-8.

Waithaka, P. N, Muthini M. J. and Kebira A. K. (2015). Physico-chemical Analysis, Microbial Isolation, Sensitivity Test of the Isolates and Solar Disinfection of Water Running in Community Taps and River Kandutura in Nakuru North Sub-county, Kenya. The Open Microbiology Journal, 9: 117-124.