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African Journal of Environmental Science and Technology

ARTICLES

Full Length Research Paper

Leaching behaviour of azoxystrobin in sandy loam soil

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The mobility of azoxystrobin in sandy loam soil was studied under continuous and discontinuous flow conditions in soil columns under laboratory conditions at two application rates (50 and 100 µg), with simulated rainfall of 300 mm. Residues of azoxystrobin in soil and leachate were estimated by gas-liquid chromatography and confirmed by gas chromatography-mass spectrometry. Though maximum concentration of azoxystrobin was found in the top 10 cm layer under both continuous and discontinuous flow conditions but azoxystrobin residues under continuous flow conditions were recorded down to a depth of 15-20 cm and under discontinuous flow conditions residues, were recorded at depths of up to 20 and 25 cm for the single and double dose treatments, respectively. The retention of azoxystrobin residues was greater under continuous flow conditions at the higher dose, whereas a low retention of azoxystrobin residues at the lower dose and under discontinuous flow conditions was observed. The low mobility of azoxystrobin in soil indicated that it represents a low risk to groundwater systems. Leachate fractions were free from azoxystrobin residues.

Key words: Leaching, azoxystrobin, sandy loam soil, column, residues.

INTRODUCTION

Pesticides are one of the major technological developments of twentieth century. Whether natural or synthetic, they have toxicological significance and pose a potential risk when they persist in the environment. The indiscriminate use of pesticides has given rise to many problems viz. persistence of toxic residues in the environment, development of resistance in insect pests and their subsequent resurgence. To protect crops from severe damage, many pesticides have been formulated to minimize any losses (Krishnaiah et al., 1976; Mishra and Singh, 1976; Mishra, 2002). As the use of pesticides by farmers is the main method used to combat the

problem of insects/pests, farmers often apply them either at high doses or using multiple sprays. This way, improper and injudicious use of pesticides, besides posing health threat to the farm workers, can also leave harmful pesticide residues on the crops and in the soil. Recent studies have detected fungicides and their primary transformation products (TPs) in streams, precipitation, groundwater, and bed sediment (McConnell et al., 1998; Wauchope et al., 2004; Scribner et al., 2006). In some areas of the U.S, this may represent the first time that fungicides have been included in crop management practices. All fungicides are at risk of losing

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all or part of their effectiveness due to development of fungicide resistant fungi (Brent and Hollomon, 1998).Soil, an important component of the environment, can act as a sink for agricultural pesticides. Such treatments may suppress soil micro flora and hence affect soil properties. The pesticides present in soil sometimes act as a source of contamination for subsequent crops. From soil, pesticide residues can reach water bodies by leaching and runoff. The main processes potentially affecting the ultimate fate of pesticides in soil are retention by soil materials (involving adsorption/desorption processes), transformation processes (biological and chemical degradation), and transport (through soil, atmosphere, surface water, or ground water) (Saltzman and Yaron, 1986; van der Hoff and van Zoonen, 1999). Fungicidal natural products, which can be obtained from a wide variety of sources, including plants, bacteria and even fungi, are a particularly attractive source of new leads due to their structural diversity (Godfrey, 1995; Copping, 1996). Azoxystrobin (methyl (E)-2-{2-[6-(2-cyano-phenoxy) pyrimidin-4-yloxy] phenyl}-3-methoxyacrylate) is a synthetic biodegradable strobilurin fungicide. It is a broad-spectrum systemic soil applied fungicide. At room temperature, it is a white crystal solid with a melting point between 118 and 119°C. Its solubility in water at 25°C is 6 mg l^{-1} (Anonymous, 2003). It is absorbed through the roots and translocate to stems and leaves via xylem, or through leaf surfaces to the leaf tips and growing edges. The mode of action is by inhibition of mitochondrial respiration in fungi. It also inhibits mycelial growth, along with spore production and germination. It is active at very low doses against a wide range of fungal pathogens. Laboratory studies show that azoxystrobin is moderately persistent in soil in the absence of light and moderately mobile in soil profile. Therefore, the present investigation was carried out to obtain information on the mobility and leaching behaviour of azoxystrobin in packed soil columns of a sandy loam soil from Northern India under different flow conditions.

MATERIALS AND METHODS

Chemicals and reagents

All the solvents used for this study were of analytical grade. The concentration of the azoxystrobin active ingredient present in the acetone extracts were quantified using gas- liquid chromatography (GLC). The analytical solvents used (acetone, dichloromethane and hexane) were procured from Merck, Darmstadt, Germany. Sodium chloride (ASC reagent grade ≥ 99.9 %) was also obtained from Merck, Darmstadt, Germany. All of the solvents used were redistilled before use in glass apparatus and their suitability was ensured by running reagent blanks before each analysis. The stock solution of azoxystrobin fungicide was prepared at concentration of 100 µg ml^{-1}; and further diluted to prepare working standards.

Preparation of standard solution

A standard 1 mg ml^{-1} stock solution of azoxystrobin was prepared in acetone. The standard solutions required for plotting a calibration curve (2.00, 1.50, 1.00, 0.50, 0.25 and 0.10 µg mL^{-1}) were prepared from stock solution by serial dilution using n-hexane. All standard solutions were stored at 4°C.

Instruments

Analysis of azoxystrobin was carried out on gas liquid chromatograph (GLC) Shimadzu Model GC-2010) equipped with ^{63}Ni electron capture detector (ECD) supplied by M/s Shimadzu, Kyoto, Japan. Confirmation of azoxystrobin was carried out on a gas chromatograph (GC-MS/MS Model Agilent 7890) coupled with mass detector (Mass 7000 GC/MS Triple Quadrupole).

Experiment

The leaching experiment was conducted under laboratory conditions. A sandy loam soil was collected from Research Farm, CCS Haryana Agricultural University, Hisar which had no previous history of pesticide application. Soil was air-dried in the shade, ground and sieved through 2-mm sieve. Commercial formulation (Amistar 23SC) was used for the leaching experiment. Plexi glass columns (90 cm × 5 cm internal diameter) fitted with a perforated sieve covered with filter paper (Whatman No. 1) was used. Each column was sequentially filled with soil up to a height of 60 cm with a bulk density of 1.50 g cm^{-1} of sandy loam soil. This equated to 152 g of sandy loam soil per column each time. The process was repeated until each column was uniformly filled to a height of 60 cm. The experiment was conducted with triplicates and a blank. Before packing, the filter paper was kept at the perforated distal end of the column to allow only the passage of leachates. The azoxystrobin formulation was dissolved in deionized water and simultaneously applied to the top 5 cm of the soil in the column at doses of 50 and 100 µg as single and double dose respectively. After application of the azoxystrobin formulation, the columns were irrigated with 50 mm of distilled water (equivalent to 300 mm rain) at a time interval of 24 h under continuous flow conditions and after one week under discontinuous flow conditions. During leaching 2-3 drops of toluene solution were added to each column to monitor microbial growth. Residues of azoxystrobin were estimated at different soil depths: 0-5, 5-10, 10-15, 15-20, 20-25 and 25-30 cm and in the leachates. Three leachate fractions were collected from each treatment. Columns were then cut into two equal halves and the soil was sampled in 5 cm segments. Segments from the same column were pooled for use of residue analysis.

Estimation of residues

Residues of azoxystrobin were quantified on a gas chromatograph (GC) (Shimadzu Model GC- 2010) equipped with capillary column HP-1 (30 m× 0.32 mm i.d× 0.25 µm film thickness of 5% diphenyl + 95% dimethyl polysiloxane). Other GC parameters were as follows: Temperature (°C): Injection port (300°C), Detector (320°C); Carrier gas (N$_2$) flow was maintained at 60 and 2 ml min^{-1} through column with split ratio 1:10. Retention time (R$_t$) observed for azoxystrobin was 6.442 min.

Confirmation by GC-MS

Confirmation of the presence of azoxystrobin was achieved using a gas chromatograph mass spectrometer (GC-MS) in single ion monitoring mode. A capillary column (30 m × 250 µm × 0.25 µm film thickness) was used for confirmation of these residues.The GC-MS operating conditions were as follows: oven (program) initial

Table 1. Per cent recoveries of azoxystrobin in sandy loam soil and water.

Substrate	Level of Fortification (mg kg^{-1})	Azoxystrobin (% Recovery*±SD)	Mean (% recovery)
Sandy loam soil	0.01	84.40±2.40	
	0.10	85.90±2.65	85.47
	0.25	86.10±1.99	
Water	0.01	89.20±2.13	
	0.10	90.80±2.00	91.58
	0.25	94.75±1.97	

*Average of three replicates.

Table 2. Leaching behaviour of azoxystrobin in sandy loam soil under continuous flow conditions.

Soil depth (cm)	Residues* (µg)				
	Single dose (50 µg) ± SD	Per cent retention	Double dose (100 µg) ± SD	Per cent Retention	Mean
0-5	22.105±0.006	52.38	43.093±0.002	50.98	32.599
5-10	18.007±0.006	42.65	27.277±0.006	32.27	22.642
10-15	2.100±0.036	4.98	10.156±0.005	12.01	6.128
15-20	BDL	-	4.005±0.005	4.74	2.003
20-25	-	-	BDL	-	-
Mean	10.553		21.133		

*Average of three replicates. CD at 5% level of significance : Soil depth= 0.011; Dose = 0.008 ; Soil depth x dose= 0.015.

temperature of 70°C holding for 2 min followed by ramping at 25°C min^{-1} to 150°C and holding for 0 min, then ramping by 3°C min^{-1} to 200°C and holding for 0 min, then ramping at 8°C min^{-1} to 280°C and held for 1 min; injector temperature 280°C. Helium was used as a carrier gas with a flow rate of 1ml min^{-1}. The injection volume was 2 µl with split ratio of 1:10. On the basis of above information a programme was developed in product ion monitoring mode with molecular mass 403.4 in azoxystrobin at four different collision energies of 15, 20, 25 and 30 and MS$_1$ range starting from 150 and MS$_2$ range ending at 350. The precursor ion of azoxystrobin at m/z 343.8 was found to break completely and showed fragmentation peak of product ion at m/z 329 with retention time R$_t$ of 41.786 min (Figures 1and 2).

RESULTS AND DISCUSSION

Efficiency of the method

In the present investigations, recovery experiments were carried out to establish the reliability and validity of the analytical method and to determine the efficiency of the extraction and cleanup procedures for the soil and water. The soil and water control samples were spiked at 0.01, 0.10 and 0.25 mg kg^{-1}, respectively, and processed by following the methodology as described above. Mean

recoveries of azoxystrobin in soil were found to range from 84.46 to 86.10% and in water ranged from 89.20 to 94.75% (Table 1). The average recovery values from all the fortified samples were in excess of 85%. Therefore, the results have been presented as such without applying any correction factor. Limit of detection (LOD), limit of quantification (LOQ), precision and accuracy parameters were derived using the guidelines described by Thompson et al. (2002). Accordingly, the limit of quantification (LOQ) was 0.01 mg kg^{-1} and limit of detection (LOD) was 0.003 mg kg^{-1}.The overall results of azoxystrobin at different soil depths are presented in (Tables 2 and 3). The results show that azoxystrobin leached down to depths of 15 and 20 cm under continuous flow conditions and 20 and 25 cm under discontinuous flow conditions, at single (T$_1$) and double (T$_2$) doses under 300 mm rainfall conditions. The highest concentration of azoxystrobin was found at 0-5 cm depth in both the application rates, and was higher in theT$_2$ dose than the T$_1$ dose. Azoxystrobin leached down to a depth of 0-10 cm in both the doses. Several factors such as adsorption of the pesticide by the soil particles, water solubility of the pesticide, volume of leachate, pH and soil texture can influence the leaching of the pesticide
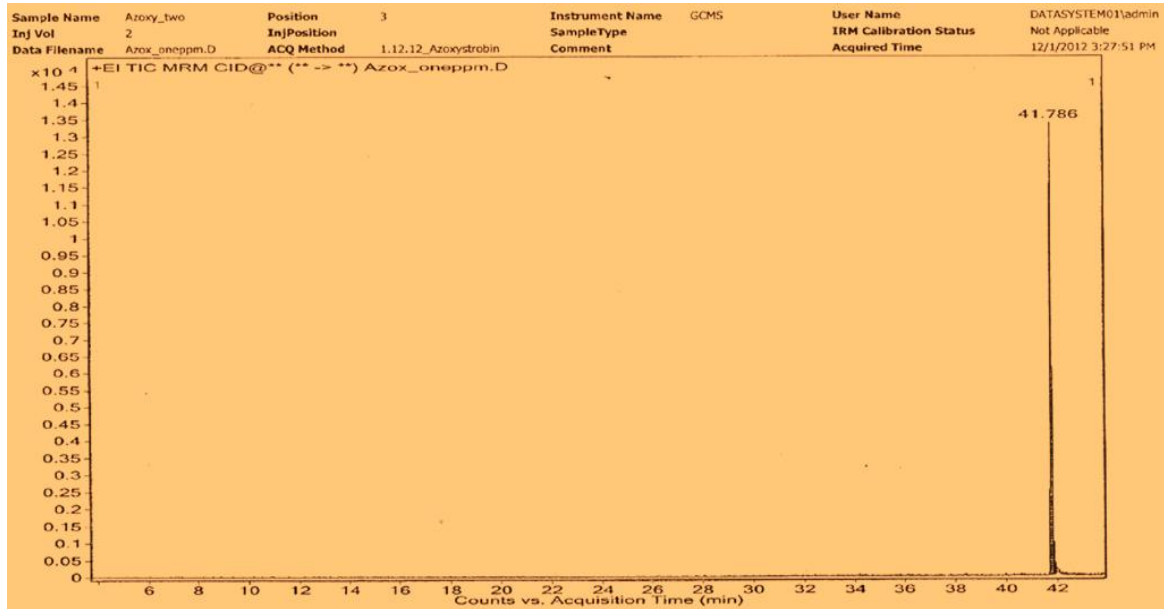
Figure 1. Retention time of azoxystrobin in MRM mode.
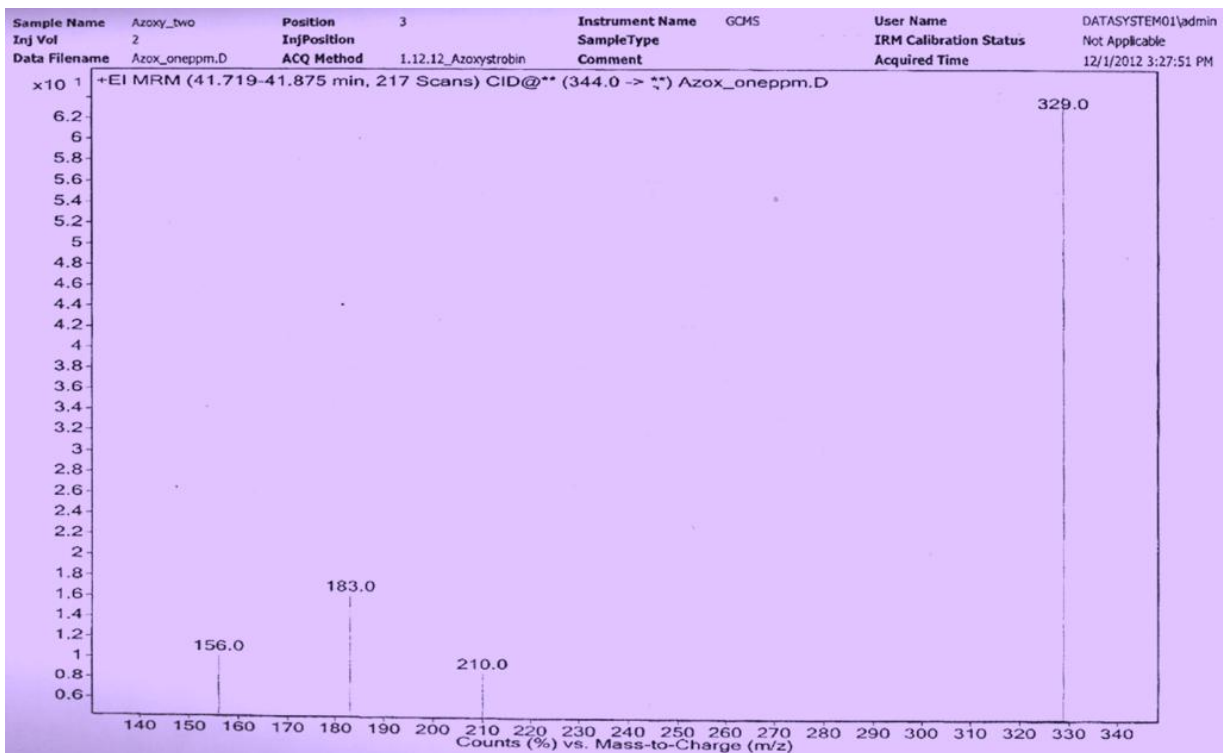
Figure 2. Mass to charge ratio (m/z) counts (%) of azoxystrobin at collision 20 in MRM Mode.

through the soil (Kidd and James, 1991; Crisanto et al., 2000; Halimah et al., 2004). Recovered amount of azoxystrobin residues at various soil depths were analyzed statistically.

Significant differences in the recovered amount of azoxystrobin at various depths were observed at both application rates. Irrespective of soil depth, residue levels were significantly lower following single dose applications

Table 3. Leaching behaviour of azoxystrobin in sandy loam soil under discontinuous flow conditions.

| Soil depth (cm) | Residues* (µg) | | | | |
	Single dose (50 µg) ± SD	Per cent retention	Double dose (100 µg) ± SD	Per cent Retention	Mean
0-5	20.538±0.007	48.74	41.131±0.003	48.70	30.835
5-10	13.107±0.006	31.11	24.200±0.005	28.66	18.654
10-15	6.421±0.003	15.24	12.605±0.006	14.92	9.513
15-20	2.071±0.004	4.92	5.017±0.002	5.94	3.544
20-25	BDL	-	1.498±0.007	1.77	0.749
25-30	-	-	BDL	-	-
Mean	8.427		16.890		

*Average of three replicates. CD at 5% level of significance : Soil depth = 0.010; Dose = 0.006; Soil depth x dose = 0.014.
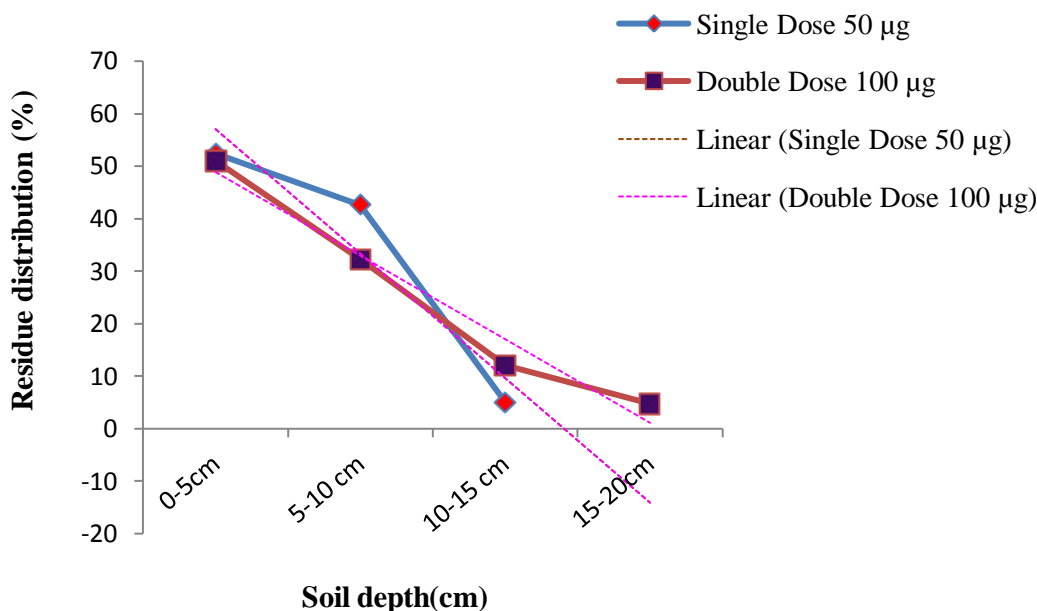
Figure 3. Per cent distribution of azoxystrobin in sandy loam soil under continuous flow conditions.

compared to double dose applications. Per cent azoxystrobin retention at 0-20 and 0-25 cm depth in sandy loam soil is shown in Figures 3 and 4. Azoxystrobin was retained between 100.00 and 99.99% in the single and double dose treatments, respectively under continuous flow conditions. In contrast, approxi0-mately 48.74 and 48.70% of the compound was retained in the 0-5 cm core of soil in the single and double dose treatments under discontinuous flow conditions, showing very low mobility of this fungicide in sandy loam soil. The retention of azoxystrobin in soil cores was higher under discontinuous flow conditions than under continuous flow condition at both azoxystrobin dosages. None of the leachate fractions contained any pesticide residues at

both dosages. Hence, azoxystrobin seems to be of minimal risk to groundwater.

The present results are in agreement with earlier reports. Gupta and Gajbhiye (2004) studied the leaching behaviour of thifluzamide in alluvial soil under laboratory conditions. The study reveals that thifluzamide was moderately mobile in alluvial soil. Only small amounts (<1%) were recovered from leachate fractions, whereas, major portion remained in 0-15 cm soil depth.However, our findings differ from some previously published observations. These differences may result from a number of different parameters including different matrices and conditions like field capacity moisture, submerged conditions, flooded and non-flooded, organic
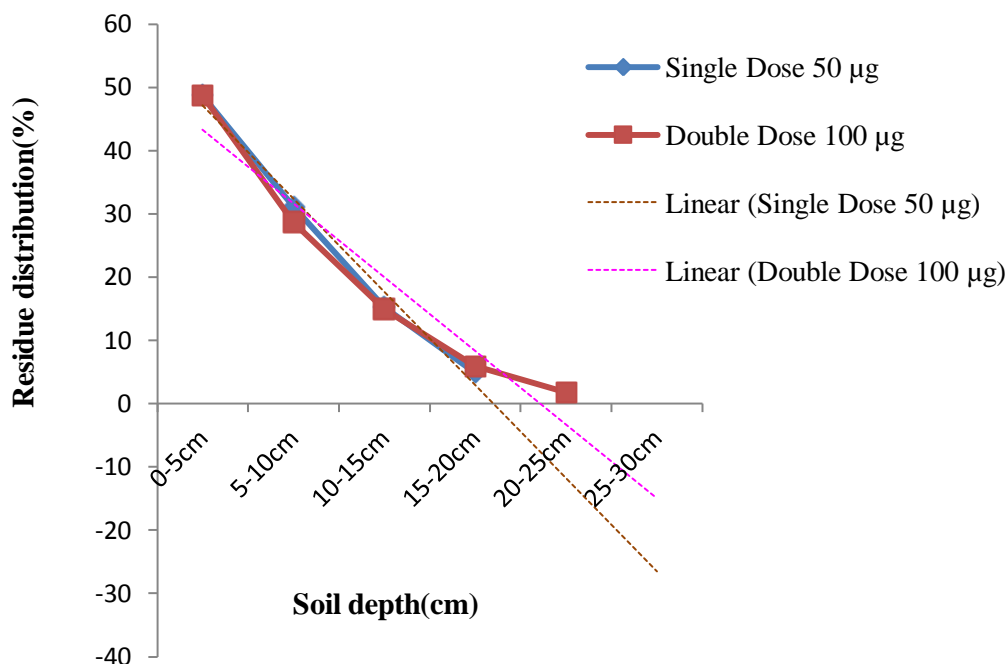
Figure 4. Per cent distribution of azoxystrobin in sandy loam soil under discontinuous flow conditions.

matter.

Bending et al. (2006) reported variability in the degradation rates of the pesticides like isoproturon, azoxystrobin and diflufenican, in sandy loam and clay loam soils. No change in the rate of degradation after 8 months, and the amount of azoxystrobin remaining in the soils ranged from 15.4 to 50.9% at Wellesbourne, and between 29.9 and 49.3% at Kirton. Ghosh and Singh (2009) also reported that azoxystrobin was immobile in a sandy loam soil but its mobility increased with the amount of percolating water added. Following water percolation equivalent to 126 mm of rainfall, azoxystrobin leached down to 5-10 cm depth and nearly 90% of applied fungicide was retained in the top 0-5 cm layer, whilst percolation equivalent to 362 mm rainfall resulted in azoxystrobin leaching down to 10-15 cm soil depth and 50% azoxystrobin leached down to the 5-10 cm soil section. However, under discontinuous flow conditions, a major portion of azoxystrobin (47% of the time zero applied azoxystrobin) was recovered from the 0-5 cm layer, whilst 13% (22% of the azoxystrobin recovered at the end of study) was present in the 5-10 cm layer.

Jørgensen et al. (2012) carried out the study to estimate leaching of the fungicide azoxystrobin and one of its primary degradation products R234886 major fraction at four agricultural research fields (one sandy and three loamy) in Denmark and observed that neither azoxystrobin nor R234886 were detected at the sandy site, but did leach through loamy soils. Azoxystrobin was generally only detected during the first couple ofmonths

following application and R234886 leached for a longer period of time and at higher concentrations (up to 2.1 μgL^{-1}).

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Full Length Research Paper

Characterization of Dabagi clay deposit for its ceramics potential

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A clay deposit in Gwandu Town of Kebbi State, Nigeria has been evaluated for its potential as industrial raw material. The chemical analysis was carried out using X-ray florescence spectrometery while physical property tests such as porosity, bulk density, linear shrinkage, thermal shock resistance, cold crushing strength and refractoriness were done using standard techniques. The result of chemical analysis indicated that the clay was composed of silica (SiO_2), 64.50%; alumina (Al_2O_3), 16.30%; iron oxide (Fe_2O_3), 14.20%; calcium oxide (CaO), 0.2%; potassium oxide (K_2O), 0.74%; titanium oxide (TiO_2), 1.71% and other oxides in traces. The chemical analysis suggests that the clay deposit is mainly made of kaolinite and free quartz. Result of the physical tests shows that the clay has an apparent porosity of 28.46%, bulk density of 1.81 g/cm^3, linear shrinkage of 6.80%, thermal shock resistance of seven cycles, loss on ignition test (L.O.I) of 4.46%, cold crushing strength of 14138 Nm^{-2} and estimated refractoriness of 1349°C.

Key words: Dabagi clay deposit, characterization, x-ray fluorescence and technological tests, Kaolinite, ceramic applications.

INTRODUCTION

Clay is a natural earthy, fine - grained material that acquires plasticity on being mixed with limited quantity of water (Velde, 1992; Idenyi and Nwajagu, 2003). From a chemical or mineralogical standpoint, clay is a complex aluminosilicate compounds containing attached water molecules, which have their origin in the chemical and mechanical disintegration of rocks, such as granites (Nwajagu, 2005). Clay occurs most abundantly in nature in solids, sediments, sedimentary rocks and hydro-thermal deposits (Velde, 1992). One basic property of or other polar ions into their structure. This is called the swelling property. Based on this property, Velde (1992) broadly classified all clays into swelling and non-swelling type. Swelling clays are called smectites. The important

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property according to Velde (1992) is the basic composition and structure of the clays; and this is used to further classify the clay minerals into: Kaolinite group ($Al_2Si_2O_{10}$ (OH_6)): this group has one silica and one alumina unit stacked in alternating fashion (1:1 lattice type). No ion or water molecules can enter the adjacent layers, and only the external surfaces determine their colloidal properties.

Owing to its relatively large particles and low specific surface, kaolinite exhibits less plasticity, cohesion and swelling as compared to other clay minerals. Kaolin is an important and widely used industrial mineral which is refined from kaolinite. It is a naturally occurring minerals of the clay family and may contain a number of impurities such as quartz, feldspar, tourmaline, limestone, zircon, etc. which were derived from the parent rock. It is a weathering product of silicate rocks which is whitish, earthy to dull with plastic touch.

The characteristics and chemical composition of a kaolin deposit usually determines its industrial utilization. Kaolin is one of the most valuable of the industrial clays which is used in most manufactured products. Prominent uses include paper filling and coating; paint, plastic, adhesive and ink pigment; rubber reinforcing agent, ceramic raw materials for porcelain, dinner ware, tiles and enamels, catalyst for petroleum cracking and auto exhaust emission catalytic control devices; cosmetics base; and digestive coating remedy (RMRDC, 2010); Bentonite: bentonite belongs to the group of clays whose technical properties are controlled by the proportion of montmorillonite, a sub-group within the smectitic clays. It is clay derived from deposits of weathered volcanic ash. Bentonites are hydrated aluminosilicates, which composed predominantly of the clay mineral montmorillonite. They are composed of a 3-tier structure with alumina silica sheets' sandwiched between tetrahedral silica units. A simplified formula for montmorillonte is Al_2O_3. $4SiO_2.H_2O$, the other minerals that could be found in bentonite in small content are chrystobalite, biotite, chalcedony, calcite, pyrite, dolomite and plagioclase. There are three principal types of bentonite namely: natural sodium bentonite or sodium montmorillonite, natural calcium bentonites or calcium montmorillonite; and sodium activated bentonites or sodium activated montmorillonites.

Natural sodium bentonite as the name suggests, occurs with sodium as the predominant exchange cation. They are characterized by high swelling, high liquid limit and high thermal durability. It is usually used for drilling mud. The vast majority of the montmorillonites occurring in abundance worldwide is of the calcium type and is referred to as calcium bentonite. Much lower swelling and liquid limit values compared to natural sodium bentonite, characterize them.

Calcium bentonite is used as a bleaching agent in cooking oil industries, bleaching agent in lubricant oil recycling, as a catalyst, absorber, filler, etc.

Bentonite has a wide range of industrial uses. The physical and chemical properties of bentonite make it an important industrial mineral, which has widespread application in various industrial sectors such as foundry sand bond in iron and steel foundries and in iron ore pelletizing in metallurgy; this is probably the largest use for bentonite, as insulator in civil engineering as an efficient materials for drilling mud (because the gel-like suspension it forms in water), as bleaching clay in oil refining, clarifying and decolourising, filtering agent for clarifying wine, beer and treating waste water, Ingredient in cosmetics, animals feeds and pharmaceutical (RMRDC, 2010).

Glass sand/quartz: silica sand/quartz constitutes one of the most readily available geological materials used in industries and factories such as glass manufacturing companies. Silica sand/quartz are said to consist of high optimal percentage of silicon oxide (SiO_2) which is a very good chemically stable element and it remains almost the same no matter the series of cycles it may have gone through, either in transportation or re-deposition. Quartz is silica occurring alone in pure state. Silica/glass sand on the other hand are products of weathering, erosion and transportation by rivers or/and the sea. Naturally occurring silica sands may contain some undesirable impurities like accessory haematite, rutile and dolomite etc. The glass sand is used in the production of various glass products: which include sheet glasses, for windows, bottles, mirrors, optical instruments, chemical apparatus, electrical insulation and condensers, pipe, doors, crucibles, automobile and aircraft bodies, filters and building blocks. They are also used for making abrasives and for gravel parking in the petroleum industries (Nwoye, 2009).

Clay products are versatile industrial material that have amazing variety of uses and applications in ceramics porcelain, dinner wares, glasses, refractory bricks, burnt bricks and architectural tiles and ceramic (Akudinobi, 2006). Important uses have also been found for clays in the rubber industry where its reinforcing potential has been exploited since the early part of 21th century, particularly those with tolerable presence of silica (which serve as reinforcing component in rubber compounding) (Akudinobi, 2006).

The important properties of clay are plasticity, colour, strength, drying and firing shrinkages. The percentage of the minerals oxide (Fe_2O_3, MgO, CaO, Na_2O etc.) in the clay ultimately determine the areas of applications of the clay such as in bricks, floor tiles, paper etc. while the quantity of the alkali metal oxides (Na_2O, K_2O, CaO etc) indicates their suitability for ceramic products Nnuka and Agbo (2000).

Nigeria has appreciable distribution of industries engaged in metal production and other process industries, and hence, there is need for raw - material to

Table 1. Chemical composition of Dabagi clay compared with standard clay for industrial applications: Chester (1973) and Grimshow (1971).

Composition	Dabagi clay	Ceramics	Refractory brick	High melting clay	Glass	paper	Paint
SiO_2	64.50	60.50	51.70	53-73	80-95	45.0-45.8	45.3-47.9
Al_2O_3	16.30	26.50	25-44	16-29	12-17	33.5-36.1	37.9-38.4
Fe_2O_3	14.26	0.5-1.2	0.5-2.4	1-9	2-3	0.3-0.6	13.4-13.7
CaO	0.26	0.18-3	0.10-20	0.5-2.6	4-5	0.03-0.60	0.03-0.60
K_2O	0.74	-	-	-	-	-	-
TiO_2	0.17	-	-	-	-	-	-
CuO	0.03	-	-	-	-	-	-
V_2O	0.08	-	-	-	-	-	-
LOI	4.46.	8.18	8-18	5-14	-	-	-

support their growth. Clay products such as ceramic wares, burnt bricks and floor tiles are cheaper and more durable building materials than cement particularly in Nigeria.

There are vast deposits of clay spread across every region in Nigeria, though their properties differ from one site to another on account of geological differences. The present economic state imposes the need for sourcing of local material to meet the increasing demands. This study therefore is to ascertain the chemical composition and mechanical/physical properties of Dabagi clay deposit in Gwandu town of Kebbi State of Nigeria so as to highlight its ceramic value, economic potential and encourage its immediate industrial application/ uses.

MATERIALS AND METHODS

Sample preparation

Stratified random sampling was used. 10 m sample area was divided into ten smaller units and from each unit three samples were collected randomly at a depth of 10 cm, mixed and homogenized. Cone and quartered method was employed until a required (representative) sample was obtained. The coning and quartering means pouring of the clay sample on a flat surface such that it formed a cone which was divided into four equal parts by using a straight edge.

The two alternate quarters are mixed again for further quartering. The representative fractions for the sample was later crushed and grounded to produce 60 mesh (BS). The crushed sample was used for analysis.

Chemical analysis

The chemical composition of the clay was determined using X-ray florescent (XRF). 1 g of clay sample was added to 10 g of anhydrous lithium tetra borate ($Li_2B_4O_7$) acting as a fluxing agent. The constituent were mixed inside a cleansed crucible and heated up to 500°C for 8 min and allowed to cool at room temperature to obtain the fused sample which was used for the analysis. The chemical composition of the sample is presented in Table 1.

Loss on ignition test (L.O.I)

This is the quantity of chemically combined H_2O (and sometimes organic matter content) in inorganic materials (Udochukwu, 2007). Fifty grams of the clay sample was dried in an oven at 110°C and cooled in the desiccator. A porcelain crucible was cleaned, dried and weighed (M_1) to nearest 0.001 g. The dried sample was introduced into the crucible and crucible together with the clay sample ions weighed (M_2) to an accuracy of 0.001 g. The crucible containing the sample was placed in a muffle furnace and heated to a temperature of 900°C for 3 h, were cooled in a desiccator and then weighed (M_3). The loss on ignition was calculated from Equation 1):

$$LOI = \frac{M2-M1}{M2-M3} \tag{1}$$

Apparent porosity test

Apparent porosity is the ability of the clay materials to be impervious to gasses and liquids. Pores are formed as water and gasses are given off during firing process (Nwajagu, 2005). Specimen measuring 5 x 5 x 4 cm was prepared from a conveniently made clay brick; it was dried in an oven at 110°C and fired in a furnace at interval of 100°C for every 10 min till the temperature of 900°C was attained. The fired specimen was cooled and then transferred into a desiccator and dried weight (D) was recorded. The specimen was then transferred into a 250 ml beaker; water was then introduced into the beaker until the tested specimen was completely immersed in the water. The specimen was allowed to soak in boiled water for 30 min being agitated from time to time to assist to release trapped air bubbles. The specimen was then transferred into empty desiccator to cool. The soaked weight (W) was recorded. The specimen was weighed suspended in water using beaker place on balance. This gave suspended weights (S). The apparent porosity was calculated using Equation 2:

$$\text{Apparent porosity} = \frac{W-D}{W-S} \times 100\% \tag{2}$$

Bulk density test

Bulk density is the mass per unit volume of the clay ignoring the

Table 2. Physical properties of Dabagi Clay compared with standard clay for industrial applications (Omowumi, 2000).

Sample description	Apparent porosity (%)	Bulk density (g/cm^3)	Thermal shock - resistance (cycles)	Cold crushing strength (KN/M^2)	Refractoriness (°C)	Linear shrinkage (%)
Dabagi clay	28.46	1.81	7	14138	1349	6.80
Fire clay	20-30	2.30	20-30	15000	1500-1700	4-10
Siliceous fire clay	23.7	2.0	1	15000	1500-1600	7-10
Ceramics	10-30	2.30	20-30	15000	1430-1717	-
Refractory brick	10-30	2.-30	20-30	15000	1430-1717	-

volume occupied by pores (Idenyi and Nwajagu, 2003). The molded brick of the specimen measuring 5 x5 x4 cm was also prepared and dried for 24 h and then dried in an oven at 110°C cooled in a desiccator and weighed (dried weight) after which the specimen was transferred to a beaker containing water and heated for 30 min to assist in releasing air. The specimen was cooled and soaked weight (W) taken. The specimen was then suspended in water using beaker place on balance. The suspended weight (S) was taken. The bulk density was calculated from Equation 3:

$$\text{Bulk Density} = \frac{\rho W}{W - S}$$

(3)

Where, ρw = Density of water.

Thermal shock resistance test

Thermal shock resistance is the ability of the clay materials to withstand heating and cooling several times before a deep crack appears (Lawal, 2005). The test piece of brick was air dried for 24 h and oven at 110°C for 3 h. The specimen was then fired in the furnace at 900°C for 3 h and allowed to cool. The prepared specimen was then inserted in a furnace which has been maintained at 900°C.

The specimen was removed from the furnace with a pair of tongs and allowed cool for 10 min on firebricks. The specimen was returned to the furnace for further 10 min. The process was continued until the test piece was cracked. The number of cycles of heating and cooling before cracking of the specimen was recorded as its thermal shock resistance.

Linear shrinkage test

Linear shrinkage is a property of the clay which makes it to undergo least structural changes and disintegration while being heated (Lawal, 2005). The clay was moistened with water to the point of wedging (which implies that the moistened clay materials remain packed into a ball in hand until intentionally vibration causes the mixture to flow). The wedge sample was cast into fabricated wood box.

The clay bar was prepared and the original/initial length was marked on the sample. The bar was fired at temperature of 900°C for 3 h. The final length after the clay had been fired was recorded. The linear shrinkage was then calculated using the Equation 4:

$$\text{Linear shrinkage} = \frac{A - B}{A} \times 100\%$$

(4)

Where, A = Initial or original length; B = Final length.

Cold crushing strengths test

Cold crushing strength is the ability of the clay to bear load (Udochukwu, 2007). A cube measuring 50 mm on a flat surface was made from the refractory brick the test piece was fired in a furnace at 900°C and the temperature maintained for 5 h. The sample was then cooled to room temperature. The specimen was placed on a compressive tester and the load was applied axially by turning the hand wheel at a uniform rate until failure occurred. The manometer readings were recorded. Cold crushing strength (CCS) was calculated from Equation 5:

$$CCS = \frac{Maximum \ load \ (KN)}{cross \ section \ area \ (M2)}$$

(5)

Refractoriness test

This is the resistance of the clay to fusion and softening at high working temperatures. It is the maximum temperature clay can withstand with no load applied (Nnuka, 2003). The clay sample was formed into simple cones of 1.27 cm base diameter and height 3.81 cm. The cone was mounted on refractory base along with several other standard cones of the same dimensions and standard compositions, the cones were heated at a rate of 5°C/min in the furnace until the test cone bent over its own weight. After cooling, the test cone was compared with the standard cones. The test material was taken to have the pyrometric cone equivalent (PCE) of the standard cone whose behaviour most resembled that of the test cone.

RESULTS AND DISCUSSION

The results from the experimental work are given in Tables 1 to 2. The chemical results of Dabagi clay show high silica (SiO_2) content of 64.50% which satisfies the standard for the manufacture of refractory bricks, ceramics as well as high melting clay with values 51.70, 60.50 and 53-73% respectively but below the range for glass formulation. The alumina content (16.30%) of Dabagi clay is short of the standard required for ceramics (26.50%), refractory bricks (25-44%), paper (33.5-36.1%) and paint (37.9-38.4%) manufacturing industries as reported by

(Chester, 1973). However, it can still be used in the manufacture of high melting clay and alumino silicate and fibreglasses because they required only 16-29% and 12-17% of aluminum oxide respectively as reported by (Chester, 1973). The alumina content of clay is a strong indicator for its refractoriness. The higher the amount of alumina, the higher is the refractoriness of the clay. The iron oxide content of 14.26% is higher than the standard for refractory bricks (0.5-2.4%). Such level of iron oxide usually imparts reddish colour to clay when fired, so making it attractive as a ceramic raw material Nnuka and Agbo (2000). The high iron oxide content also affects the high temperature characteristics of the clay, such as fired strength. The loss on ignition of Dabagi clay is below the range of 8-18% for ceramic and refractory brick production as reported by Chester (1973) and 5-14% for high melting clay. Loss on ignition values are often required to be low (Omowumi, 2000). This is because of its effect on the porosity of material especially refractory bricks.

The physical tests results of Dabagi Clay show an apparent porosity of 28.46%, which is within the standard for the production of fire clay and siliceous fireclay with 20-30% and 23.7% respectively (Omowumi, 2000). The bulk density of 1.81 g/cm^3 is below the value required for the manufacture of fireclay and siliceous fireclay as reported by Omowumi (2000).

The linear shrinkage of the sample after drying and firing fall within the acceptable value of 4 - 10% for fireclay, High Shrinkage values may result in warping and cracking of the clay and this may cause loss of heat in the finished products.

The thermal shock of the clay sample is below the acceptable values of 20-30 cycles as compared in Table 2. The practical implication of this is that their use IS restricted to lining of cables slag pots.

The refractoriness or temperature reached for the sample was 1349°C. This is lower than the recommended range for the manufacture of fireclay, siliceous fireclay and ceramics industries and this may be due to the low amount of Al_2O_3 obtained for Dabagi Clay.

The cold crushing value obtained for Dabagi Clay was 14138 KN/M^2 which is below the specification for the manufacture of fireclay, siliceous fireclay and ceramics industries.

Conclusion

An experimental study was conducted to investigate the suitability of Dabagi clay as an industrial raw material in view of its chemical and physical properties. The result of the chemical analysis shows that Dabagi clay con-tains aluminum oxide (Al_2O_3) and silica (SiO_2) as major constituent. The apparent porosity and linear shrinkage of the clay are moderately high while the bulk density and

cold crushing strength values are low.

The clay is therefore found to be a source of raw material for the production of refractory bricks, ceramics and high melting clay materials.

Recommendation

Dabagi clay deposit is one of the unidentified clay in Nigeria. This probably IS the first work on this clay deposit, thus, if this clay is exploited and harnessed, it will no doubt provide internal sourcing raw material used in ceramic, paper, high melting clay, refractory bricks etc. there is also the need for a geological survey to determine the extent of the deposit.

Some additives such as kaolin clay or limestone and rice husk ash should also be added so as to improve the properties of the clay.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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African Journal of Environmental Science and Technology

Full Length Research Paper

Heavy metals accumulation in edible part of vegetables irrigated with untreated municipal wastewater in tropical savannah zone, Nigeria

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In this present study, the quality of municipal wastewater used for irrigation of spinach was investigated for its heavy metal build-up. The municipal wastewater used for irrigation and the irrigated spinach samples were collected and analyzed for their heavy metal concentrations. The results indicate that the municipal wastewater used was contaminated with copper (1.90 mg/l), lead (0.09 mg/l) and iron (25 mg/l) and the municipal-irrigated spinach was contaminated with manganese (95 mg/kg) and cadmium (0.03 mg/kg). The results of these investigations were compared with World Health Organization (WHO) and Food and Agriculture Organization (FAO) heavy metal standards for irrigation water quality and permissible levels of metals in food and water. It revealed that the heavy metal concentrations were above the recommended threshold limits. High concentrations of these metals are very detrimental to the health of the inhabitants and crop consumers. Regular monitoring for safe practice is strongly recommended in order to avert terminal diseases in the area.

Key words: Domestic wastewater, irrigation, heavy metals, soil, vegetables.

INTRODUCTION

Agriculture uses about 70% of water withdrawals, therefore, it is expected that in times and regions of water scarcity, farmers would turn to domestic or urban waste water as a water source (FAO, 2010). In recent times, farmers use wastewater to irrigate their crops. This wastewater contains large amount of organic materials, some inorganic elements and/or substantial amount of toxic heavy metals (Zavadil, 2009; Arora et al., 2008; Lone et al., 2003), non essential heavy metals which

when present in large amount could be transferred to animal and human beings through food chain (Lone et al 2003). The presence of these substances are harmful to human health because of the non biodegradable nature of heavy metals and their potential to accumulate in different parts of the body (Lawal and Audu, 2011; Arora et al., 2008). Similarly, toxic metals may be absorbed by vegetables through several processes and finally enter the food chain at high concentrations which are capable

Table 1. Heavy metals in soil (mg/Kg) at Soje.

Parameter	Mn	Mg	Cu	Zn	Pb	Fe	Cd
Plot average	3.20	6.85	31.00	25.46	10.00	60.96	0.05
Before irrigation	2.01	10.93	26.32	22.00	6.25	33.10	0.04
Control	1.98	10.95	26.85	22.79	8.97	40.00	0.01
Max. limits	-	-	60.50	370.50	2129.0	-	2.25

of causing serious health risks to consumers (Kihampa et al., 2011). Also, accumulation of highly toxic metals (Cd) even at low concentrations in food and water leads to the buildup of cadmium in kidneys and may likely lead to kidney diseases (Latif, 2009). Other effects of metal toxicity may include damage or reduce mental and central nervous function, lower energy levels, and damage to blood composition, lungs, kidneys, liver and other vital organs (Kihampa et al., 2011). Indeed, it was estimated that 10% of the world's population consumes foods irrigated with wastewater (Finley, 2008). However, these factors: climate, atmospheric deposition, the nature of the soil on which the plants are grown and the degree of maturity of the plants at the time of harvesting can influence the concentration of heavy metals on and with plants (Farooq et al., 2008, Muchuweti et al., 2005). Similarly, Nazif et al. (2006) explained that the fate and effects of pollutants discharged into a particular water body will depend not only on the amount of polluting substances emitted but also on the hydrological, physical, chemical and biology conditions characterizing the water body concerned.

Farmers in Soje took advantage of the continuous flow of water at their vicinity, and were unaware of the quality and the implications of using contaminated water for crop cultivation. Thus, this study aimed at quantifying the content and quality of heavy metals in domestic wastewater used for irrigation of vegetables in Soje, in the soil and edible part of spinach.

METHODOLOGY

Study area

The study site selected for this study is the Soje irrigation scheme. Soje irrigation scheme is located in Soje, few kilometers from Minna railway station, Minna, Niger state, Nigeria. Niger state is situated at the North Central part of Nigeria. It lies in the savannah zone of the tropics between latitude 8°10'N and 11°30' N and longitude 3°30' E and 7°30' E. The study area has two main seasons, the rainy and the dry (harmattan). The rainy season begins in April and ends in October while the harmattan starts in November and ends in March. Soje has an average annual rainfall of 103.3 mm, average annual temperature of 22.5°C and average annual relative humidity of 50.3%. The source of water for Soje irrigation scheme is the wastewaters from Minna Township. The water flows through unlined channels. The farmers take advantage of it continuous flow for irrigation. Investigations revealed that the irrigation scheme was set up by farmers in the area and the area is about 7.5 ha.

Collection of samples

Three plots sizes of 1.803 x 2.44 m were marked out for the purpose of this study. Spinach (*Spinacia oleracea*) was planted on the marked out plots during the dry season of 2010. Soil, wastewater and vegetable samples were sampled.

Composite soil samples made up of five soil samples per plots were collected from a depth of 0 to 25 cm before planting and after the growing period from two irrigated plots and a control plot. The soil samples were air-dried, and crushed to pass a 2 mm mesh sieve. 0.5 g of the finely ground soil samples were digested using 2 ml technical grade trioxonitrate (v) (HNO_3) in beakers at 95°C for 1 h after which hydrogen peroxide (H_2O_2) was added. After cooling, the samples were decanted and diluted with MilliQ water to 10 ml mark for analyses.

The plastic bottles for the wastewater collection were thoroughly washed with detergent and rinsed with tap water before taking the bottles to the study site for sample collection. The bottles were rinsed with the waste water samples before collection. Wastewater samples used for the irrigation of the spinach were collected in 2 l plastic bottles from the Soje unlined channel and transported to the laboratory for heavy metal analysis. These samples were collected in three replicates.

The edible part of the spinach were randomly selected and collected from the plots. The collected samples were stored in labeled polythene bags and then transported to the laboratory for preparation and treatment for analysis. A total of 90 samples were collected from the two plots and the control. The samples were washed with distilled water to eliminate suspended particles. The leafy stalks were removed from the samples; they were sliced and dried on a sheet of paper to eliminate excess moisture. The dried samples were weighed and oven dried at 60°C to a constant weight. The oven dried samples were ground in a mortar until it passed through a 60 mm mesh sieve. A mixture of 2 HNO_3 to 1 $HClO_4$ in a conical flask was used for wet digestion for about 2-3 h on a sand bath, 10 ml of HCL was then added after which the digested samples were filtered with a 0.45 μm pore size cellulose nitrate membrane filter paper. The filtered samples were made up to 100 ml with distilled water and stored for analysis using atomic absorption spectrophotometer.

RESULTS AND DISCUSSION

Spinach was cultivated in soil samples with heavy metal as presented on Table 1. Generally, agricultural soils have low background levels of heavy metals; contaminations are mainly through fertilizer application, irrigation with partially treated or untreated sewage. This was reported by Farooq et al. (2008). The result shows that the soil in Soje is not polluted with Mn, Mg, Cu, Zn, Pb, Fe and Cd. There is need to protect the soil from contamination through regular monitoring.

Table 2. Average concentration of elements in domestic wastewater (mg/l).

Parameter	Wastewater	WHO limit
pH	6.40	6.5-8.5
Mn	0.44	-
Mg	0.22	30
Cu	1.90	1.0
B	1.66	-
Zn	1.28	3
Pb	0.09	0.01
Fe	25.0	0.3

Table 3. Heavy metal concentrations in spinach.

Parameter	Mn	Mg	Cu	Cd	Pb	Fe
Irrigated plot 1	1.22	95	1.45	0.033	0.060	3.93
Irrigated plot 2	1.22	95	2.00	0.030	0.030	3.99
Mean concentration	1.22	95	1.73	0.032	0.045	3.96
Control plot	0.64	97	1.09	0.017	0.020	2.21
WHO&FAO*	-	-	40	0.2	0.3	-
USDA*	-	79	-	-	-	2.71
WHO**	6.61		10.00	0.02	2.0	150.0

Permissible levels in food; **Critical level of metal ions in edible portion of vegetables (Lone et al., 2003).

Analysis of waste water

The average concentration of heavy metal content of domestic wastewater used for irrigation purposes at Soje irrigation scheme is shown in Table 2. The pH of the domestic waste water has a low pH, which is not desirable. Low pH value could decrease the solubility of certain essential elements such as selenium and at the same time increase the solubility of many other elements such as Fe, Al, B, Cu, Cd, Hg and Mn (Akan et al., 2008). The concentrations of the heavy metals in the domestic effluent are in the following order of decreasing magnitude Fe > Cu > B > Zn > Mn > Mg > Pb. The concentrations of Fe, Cu, B and Pb were all above the safe limit for WHO standards for domestic wastewater quality for irrigation. Although, Pb is the lowest in concentration of 0.09 mg/l, but it is about the most toxic metals found in aquatic ecosystem. Boron is essential for plant growth in small quantity; the wastewater contained high levels of boron and the toxicity could be observed on older plants by yellowing or drying of the leaf tissue at the tips and edges (Abbott and Hasnip, 1997).

Metal concentration in spinach

The heavy metal concentrations in spinach are shown in Table 3. The concentrations of heavy metals in edible part of spinach vary from metal to metal. The trend of accumulation in the spinach show an order of decreasing magnitude from Mg to Cd (Mg > Fe > Cu > Mn > Pb > Cd). Magnesium had the highest content in spinach sample with cadmium being the lowest of all the metals analyzed. Vegetables are known to naturally accumulate heavy metals from either wastewater or dumpsite (Arora et al., 2008). The Mg content in the spinach far exceeded the permissible levels in food according to the USDA standards. It was also observed that spinach has a good potential for Fe bioaccumulation. Cu, Cd and Pb concentrations were all below the permissible levels allowed in food. Lone et al. (2003) reported that long term exposure to Cd in food and water leads to build up of Cd in kidney causing kidney problems.

Conclusion

Routine monitoring of heavy metal contents of treated, partially treated or untreated domestic wastewater used for irrigation of edible crops are very vital to ensure safe practice, safety to both the health of humans and animals in particular and the environment in general. The quality of soil can also be changed by the application of sewage to farm lands. This study reveals that domestic wastewater

depended upon by farmers at Soje for irrigation of their crops is contaminated with lead, copper and iron. Consumption of these food crops can lead to serious health issues such as cancer. The spinach samples were highly contaminated with magnesium. Excess of magnesium is unlikely to inhibit crop growth though it causes deficiency of calcium and potassium which are essential nutrients of plant (Abbott and Hasnip, 1997).

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Full Length Research Paper

Spatial distribution, chemistry and subsurface temperatures of geothermal springs in Nkhata bay, Malawi

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This study investigated the spatial distribution, chemistry and subsurface temperatures of geothermal springs in Nkhata Bay, Malawi, which lies in the Western Branch of the East African Rift System. Seventeen geothermal springs were identified and mapped in the District. Eleven of these geothermal springs were purposively sampled for chemistry study on the basis of their spatial distribution representativeness and accessibility for reassessment. Two water types were hydrochemically delineated: sodium-sulfate (Na-SO_4) and sodium-sulfate-bicarbonate (Na-SO_4-HCO_3). Additionally, several mineral species were inferred to have been in contact with the geothermal spring waters in the district: calcite, aragonite, dolomite, gypsum, anhydrite, halite, fluorite, goethite, siderite and haematite. The thermal spring waters were undersaturated with respect to aragonite, calcite, dolomite, gypsum, anhydrite, halite and fluorite. However, four geothermal springs were supersaturated with respect to goethite, haematite and siderite. Although all the geothermal springs were undersaturated with fluorite (CaF_2), all contained higher F^- concentrations (from 2.21±0.06 to 10.27 ± 0.25 mg/L) than the World Health Organization (WHO) recommended limit for drinking water (1.5 mg/L). This suggests that fluoride does not precipitate as CaF_2 in the springs but mobilizes freely in the thermal waters without forming other complexes. The cadmium levels in four thermal springs exceeded the Malawi Bureau of Standards (MBS) and WHO safe limit for human consumption (0.005 mg/L). Furthermore, the iron levels in three springs exceeded the WHO and MBS safe limit (0.20 mg/L). Evidently, the chemistry of geothermal springs in Nkhata Bay shows that their utilization for drinking is not suitable. The Giggenbach Triangle revealed that all but one of the springs had fully equilibrated waters. Hence, chemical geothermometers were used to estimate their original subsurface temperatures. Information about these temperatures is required for the assessment of possible geothermal power stations. Amongst the geothermometers used, the Na/K/Ca and Na/K geothermometers offered better and more robust estimates (P>0.01) of geothermal subsurface temperatures. These geothermometers indicated that the original subsurface temperatures were in the ranges of 85 - 209 and 112 - 280°C, respectively.

Key words: Geothermal springs, spatial distribution, chemistry, subsurface temperatures.

INTRODUCTION

Most geothermal springs in the world occur in rift systems, more particularly, in areas along the tectonic plate boundaries where magma tends to rise towards the earth's surface (GEO, 2007). Malawi, which lies in the
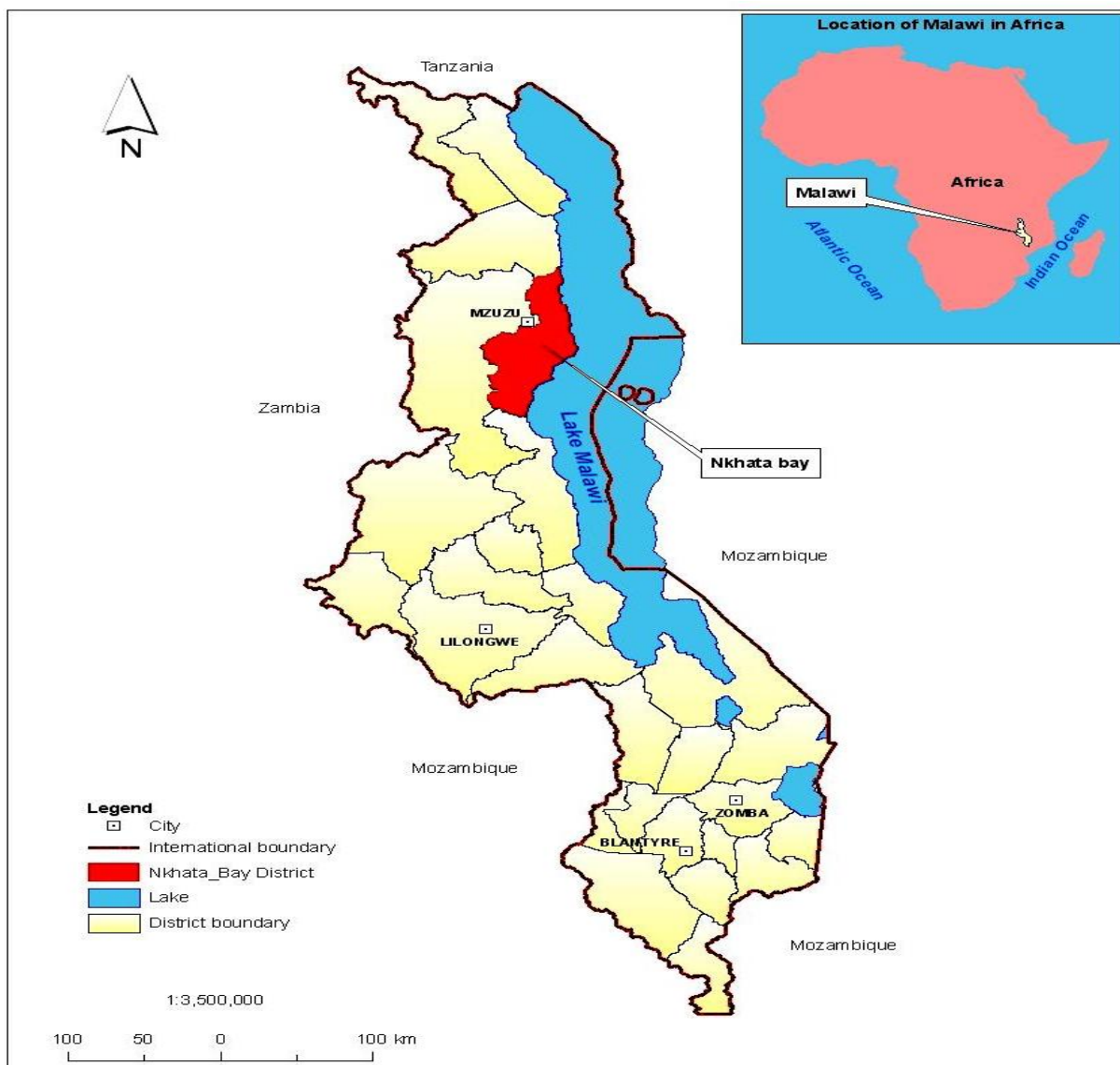
Figure 1. Location of Nkhata Bay, Malawi.

Western Branch of the East African Rift System, has thus many geothermal springs across the country from north to south (Dulanya, 2006).

The presence and distribution of geothermal springs in some parts of Malawi such as Chitipa, Karonga, Rumphi, Nkhotakota, Machinga, Mulanje, Zomba and Chikwawa Districts have been documented and their chemistry described by Harrison and Chapusa (1975), Ray (1975), Kalindekafe (2003) and Dulanya (2006), among others. Nkhata Bay located in the rift-valley floor in the north-eastern part of the country, along Lake Malawi (Figure 1), has numerous geothermal springs but lack research details of their spatial (Appendix 1) distribution and chemistry.

This study was therefore undertaken to investigate the spatial distribution, chemistry and subsurface temperatures of geothermal springs in Nkhata Bay, Malawi, recognizing the important roles similar geothermal resources play in many different parts of the world including boosting tourism in the country, mineral extraction, balneology and production of geothermal electricity.

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MATERIALS AND METHODS

Locations of geothermal springs

Geothermal springs were identified in Nkhata Bay using indigenous information. The Universal Transverse Mercator (UTM) coordinates of the thermal springs were recorded using Global Positioning System (GPS) receiver. These UTM coordinates were superimposed on the electronic (scanned) map of Nkhata Bay obtained from Malawi National spatial Data Center, geo-referenced and digitized in the ArcView 3.3.Software.

Thermal springs chemistry

Water sampling

Eleven thermal springs were purposively sampled in order to study their chemistry. Sampling was done in December, 2007. Grab sampling was used in collecting water samples from geothermal spring-heads. Samples were collected in triplicates using 2 L polyethylene bottles and labelled accordingly. Samples meant for metal or cation analyses were acidified on the spot with concentrated nitric acid (1.5 mL HNO_3 per litre of collected water samples) according to APHA (1989).

Analytical procedures

Physico-chemical and chemical parameters

The physico-chemical parameters such as temperature, pH and electrical conductivity were measured in the field and chemical parameters, Na, K, Ca, Mg, Fe, Al, Cd, SiO_2, Pb, Cr, F^-, Cl^-, NO_3, PO_4^{3-}, HCO_3^- and SO_4^{2-}, were determined in the laboratory according to APHA (1989).

1. Quantitative analysis of physico-chemical and chemical parameters

Determination of electrical conductivity (EC): The electrical conductivity of water samples was determined in the field using WPA - CM 35 conductivity meter. The electrode of the meter was rinsed before dipping into subsequent water samples to prevent inter-sample contamination (APHA, 1989).

Determination of temperature: A silver thermometer (graduated up to 300°C) was used to determine temperature at the spring-heads of the geothermal springs *in situ*. The thermometer was immersed in the thermal water and readings were taken on the spot according to APHA (1989).

Determination of pH: The pH of water samples was determined in the field at sampling points using a glass electrode Kent EIL 7020 pH meter. Prior to the pH determination, the meter was calibrated using pH 7 and 4 buffering solutions, respectively. The meter's electrode was rinsed with distilled water before determining the pH of any subsequent sample to prevent inter-sample contamination (APHA, 1989).

2. Analysis of metals/cations

The nitric acid digestion method was used for metal/cation determination (APHA, 1989). A well-homogenized unfiltered sample (100 ml) was transferred to a 250 ml flask and 5 ml of concentrated

nitric acid was added to it. The mixture was then brought to slow boiling and evaporated on a hot plate until the volume was reduced to 15-20 ml. Concentrated nitric acid (5 ml) was added to the solution and the flask covered with a watch glass before heating it to obtain a gentle refluxing. The heating continued whilst adding 5 ml portion of nitric acid until the solution became light coloured clear solution.

Finally, concentrated nitric acid (2 ml) was added to dissolve any remaining residues and later the solution was quan-titatively filtered into 100 ml volumetric flask and diluted to the mark (APHA, 1989). The preparation of standard solutions for analysis of metals in water samples were done briefly as follows: For each of the metals, an aliquot of 10 mL taken from 1000 ppm standard stock solution were diluted with distilled water in a 100 mL volumetric flask to prepare an intermediate stock solution (100 ppm). Then, appropriate volumes of the intermediate stock solutions (0.0, 2.0, 4.0, 6.0, 8.0, 10.0, and 20 mL) were diluted with distilled water in 100 mL volumetric flasks to prepare working standard solutions of 0.0, 2.0, 4.0, 6.0, 8.0, 10.0 and 20.0 ppm, respectively. Absorbances of working standard solutions and water samples were determined using Atomic Absorption Spectrophotometer (AAS), Buck Scientific model no. 200A.

Cadmium (Cd): A standard stock solution (1000 ppm) was prepared by dissolving cadmium metal (1.00g) in concentrated nitric acid (4ml) before diluting the resultant solution with distilled water in a 1-L volumetric flask. Absorbances of the working standard solutions and samples were read at 228.8 nm (APHA, 1989).

Chromium (Cr): A standard stock solution (1000 ppm) was prepared by dissolving anhydrous potassium dichromate (2.83 g) in distilled water (200mL). Concentrated nitric acid (2 mL) was added to the solution before diluting with distilled water in a 1-L volumetric flask. Absorbances of the working standard solutions and samples were read at 357.9 nm (APHA, 1989).

Lead (Pb): A standard stock solution (1000 ppm) was prepared by dissolving lead nitrate (1.60 g) in distilled water (200 mL). Concentrated nitric acid (2 mL) was added to the solution before diluting with deionised water in a 1-Litre volumetric flask. Absorbances of the working standard solutions and samples were obtained at 283.3 nm (APHA, 1989).

Iron (Fe): A standard stock solution (1000 ppm) was prepared by dissolving iron wire (1.00 g) in 1+1 nitric acid water (50 mL). The standard solutions used in the final determination were 0.00, 0.5, 1.0, 2.0, 5.0 and 10.0 ppm. Absorbance of the working standard solutions and samples were read at 248.3 nm (APHA, 1989).

Sodium (Na): A standard stock solution (1000 mg/L) was prepared by dissolving sodium chloride (2.542 g) in 1 L volumetric flask and diluted to volume with the distilled water. The standard solutions used in the final determination were 0.0, 2.0, 4.0, 6.0, 8.0, 10.0, and 20 mg/L. Absorbances of the working standard solutions and samples were read at 589.0 nm (APHA, 1989).

Potassium (K): A standard stock solution (1000 ppm) was prepared by dissolving potassium chloride (1.907 g) dried at 110°C and later diluted to 1 L with distilled water. The standard solutions used in the final determination were 0.00, 0.5, 1.0, 2.0, 5.0 and 10.0 ppm. Absorbances of the working standard solutions and samples were read at 769.9 nm (APHA, 1989).

Calcium (Ca): The standard calcium (Ca) stock solution (1000 mg/L) was prepared by adding distilled water (50 mL) to calcium carbonate, $CaCO_3$, (2.497 g) and adding drop-wise concentrated hydrochloric acid, HCl, (10 mL) to complete dissolution. The solution was subsequently diluted to the mark in 1000-ml volumetric

flask. The stock solution was used to prepare an intermediate stock solution (100 mg/L) from which the working standard solutions were prepared. The standard solutions used in the final determination were 0.00, 0.5, 1.0, 2.0, 5.0 and 10.0 mg/L. Absorbances of the standard solutions and samples were read at 422.7 nm on the AAS (APHA, 1989).

Determination of magnesium (Mg): A standard stock solution of Mg was prepared as follows: 1.000 g of magnesium ribbon was dissolved in a minimum volume (5mL) of (1+1) HCl. The solution was diluted to 1 L with 1% (v/v) HCl. The stock solution was used to prepare an intermediate stock solution (100 mg/L) from which the working standard solutions were prepared. The standard solutions used in the final determination were 0.00, 0.5, 1.0, 2.0, 5.0 and 10.0 mg/L. Absorbances of the working standard solutions and samples were read at 285.2 nm on the AAS (APHA, 1989).

Analysis of aluminum (Al): Aluminum concentrations in geothermal spring water samples were determined colorimetrically using UV-VIS spectrophotometer (APHA, 1989). Samples (2 mL each) and aluminum (Al) standards (10 mL each) were separately pipetted into 100-mL flasks. The following were added into each of the standards and samples: 1+199 thioglycolic acid (10mL) (prepared from 5 mL of the acid diluted to 1000 mL), solochrome cynine solution (10 ml, 0.075%) and ammonium acetate solution (pH 6). Solochrome cynine solution (0.075%) was prepared as follows: solochrome cynine analar (0.75 g) was dissolved in 200 mL of deionised water; sodium chloride (25 g), ammonium nitrate (25 g) and concentrated nitric acid (2 mL) were added into the solochrome cynine solution. This solution was then filtered through 542 Whatman paper (without washing) and was left to stand for overnight before use. Ammonium acetate solution (pH6) was prepared as follows: acetic acid (570 mL) was mixed with distilled water (1130 mL); then ammonium solution (570 mL) was carefully added; the resultant solution was mixed, cooled and adjusted to pH 6 with ammonia using a pH meter. The samples and the standards were topped to the 100-ml mark with distilled water. The aluminum (Al) standards were made from intermediate Al standard solution (100 mg/L) prepared from Al stock solution (1000 mg/L) as follows: Al wire (1.000 g) was dissolved in a minimum amount of (1+1) HCl, a small drop of mercury was added as a catalyst. The solution was filtered to remove mercury and then diluted to 1000 mL with 1% (v/v) HCl. Aluminum standard stock solution (10 ml, 1000 mg/L) was pipetted into a 100-mL volumetric flask and diluted to the mark to make intermediate aluminum standard solution (100 mg/L). The aluminum standard concentrations (0, 2, 5, 10, 15, 20 and 25 mg/L) were prepared from intermediate aluminum standard solution (100 mg/L).

This was done by pipetting 0, 2, 5, 10, 15, 20 and 25 mL from the intermediate standard aluminum solution into 100-mL volumetric flasks and diluting to the mark. The standards were run on UV-visible spectrophotometer and a concentration versus absorbance calibration curve for the standards was generated. Then sample absorbances and concentration were read from the colorimeter (UV-Visible spectrophotometer).

3. Determination of anions: Sulfate, nitrate, chloride, fluoride, bicarbonate, phosphate and silica

Analysis of sulfate (SO_4^{2-}): Sulfate (SO_4) in geothermal spring water samples was determined colorimetrically on UV-visible spectrophotometer (APHA, 1989). A calibration curve was gene-rated from standard stock sulphate solution (100 mg/l) which was prepared by dissolving sodium sulphate (1.479 g) in distilled water and diluted to the mark in 1000-ml volumetric flask. Sulfate stan-dard solution series for calibration were prepared from this stock solution by diluting 0, 2.0, 5.0, 10.0, 20.0 and 25.0 ml to 100 mL

to provide the following sulfate concentrations: 0, 2.0, 5.0, 10.0, 20.0, and 25.0 mg/L, respectively.

Then the standard sulfate solutions (100 mL) of concentrations 0, 2.0, 5.0, 10.0, 20.0, and 25.0 mg/L were transferred into 250-mL Erlenmeyer flask. A buffer solution (25.0 mL) was added and, while stirring, a spatulaful of barium chloride (BaCl₂) was added. After 60 seconds, absorbance was measured at 420 nm on UV-Visible Spectrophotometer. A calibration curve was prepared by relating absorbance of standards with their concentrations. Sulfate concen-tration in geothermal spring water samples was determined as follows: samples (100 mL) were each transferred into 250-ml Erlenmeyer flasks. A buffer solution (20.0 mL) was added into each sample and, while stirring, a spatulaful of BaCl₂ was added.

After 60 s, absorbance was measured at 420 nm on UV-Visible spectrophotometer. In order to make correction for sample colour and turbidity present in the original sample, blanks from which BaCl₂ was withheld were prepared and run. The sulfate concentrations in the samples were determined directly by referring to the calibration curve after subtracting blank absorbance from sample absorbance.

Determination of nitrate (NO_3^-): A nitrate Ion-Selective Electrode (ELIT 8021), on a Mettler Toledo Seven Multimeter, was used to determine nitrate concentration in the geothermal spring water samples (APHA, 1989). Prior to sample reading, the electrode and meter were calibrated using sodium nitrate (NaNO₃) standard concentrations (1.0, 2.0, 5.0, 7.0 and 10.0 mg/L) prepared from the intermediate NaNO₃ standard (100 mg/L). This intermediate standard was prepared from 1000mg/L stock solution, which was again prepared by dissolving NaNO₃ (1.371g) which was dried at 105°C for 4 h. The standard concentrations were prepared by pipetting the following volumes from the intermediate NaNO₃ standard solution (100 mg/L) respectively: 1.0, 2.0, 5.0, 7.0, and 10 mL. These volumes were diluted to the mark in 100-ml volumetric flasks. Then the standards and samples (50 mL each) were carefully pipetted into 250-ml beakers separately. Ammonium sulfate ((NH₄)₂SO₄) (1.0 mL, 2.0 M) was added to these standards and samples as ionic strength adjustment buffer (ISAB) in order to ensure stable conditions before reading on the meter. Then after 20 min, NaNO₃ standards (1.0, 2.0, 5.0, 7.0 and 10.0 mg/L) were read from the meter as a means of electrode/ meter calibration. The nitrate concentrations of the samples were read from the meter.

Determination of chloride (Cl^-): A chloride Ion-Selective Electrode (ELIT 8261) on a Mettler Toledo Seven Multi meter was used to determine chloride concentrations in the geothermal spring water samples (APHA, 1989). Before sample reading, the electrode and meter were calibrated using NaCl standard concentrations (1.0, 2.0, 5.0, 7.0, 10.0, 15.0 and 20.0 mg/L) prepared from the intermediate NaCl standard (100mg/L). The intermediate standard was prepared from 1000mg/L stock solution, which was again prepared by dissolving NaCl (1.649 g) dried at 110°C for 4 h. The standard concentrations were prepared by pipetting the following volumes from the intermediate NaCl standard solution (100 mg/L) respectively: 0, 2.0, 5.0, 7.0, 10.0, 15.0 and 20.0 ml. These volumes were diluted to the mark in 100-ml volumetric flasks. Then the standards and samples (50 ml each) were carefully pipetted into 250-ml beakers separately. Sodium nitrate (1 ml, 2% v/v, 5M) was added to 50ml of individual samples and standards, as Ionic Strength Adjustment Buffer (ISAB), to stabilize conditions before taking readings. Then after 20 min, NaCl standards (0, 2.0, 5.0, 7.0, 10.0, 15.0 and 20.0 mg/L) were read from the meter as a means of electrode/ meter calibration. Thereafter, the nitrate concentrations in the samples were read from the meter.

Determination of fluoride (F^-): The fluoride ion-selective electrode Thermo Orion Ion Plus (9609BN) on a Mettler Toledo Seven Multimeter was used to determine fluoride concentration in the

geothermal spring water samples against the sodium fluoride (NaF) standard curve (APHA, 1989). The NaF standards (0, 2.0, 5.0, 10.0, 20.0 and 25 mg/L), prepared from intermediate NaF standard (100 mg/L), were used to calibrate the electrode and meter. This intermediate standard was prepared from NaF stock solution (1000 mg/L), which was again prepared by dissolving NaF (2.210 g) dried at 105°C for 6 h. The standard NaF concentrations were prepared by pipetting the following volumes from the intermediate NaF standard solution (100 mg/L) respectively: 0, 2.0, 5.0, 7.0, 10.0, 15.0 and 20.0 ml.

These volumes were diluted to the mark in 100-ml volumetric flasks. Total Ionic Strength Adjustment Buffer (TISAB) (25 mL) was added to 25 mL of individual samples and standards to adjust pH and reduce complex interferences before fluoride concentration reading in both standards and samples on the meter. Then after 20 min, NaF standards (0, 2.0, 5.0, 7.0, 10.0, 15.0 and 20.0 mg/L) were read from the meter as a means of electrode/ meter calibration. The nitrate concentrations of the samples were obtained directly from the meter.

Determination of bicarbonate (HCO_3^-): Bicarbonates were determined using titrimetric method with hydrochloric acid (APHA, 1989). Hydrochloric acid (HCl) (0.02 M) was prepared by pipetting concentrated HCl (8.3 mL, 1.18 S.G) into 1000-mL volumetric flask and diluted to the mark with distilled water. Exactly 200mL of this stock solution was diluted to 1000 mL with distilled water which was standardized against sodium carbonate (Na_2CO_3, 0.0100 M).
Na_2CO_3 (0.0100 M) was prepared as follows: anhydrous sodium carbonate (1.0599 g) previously dried at 250°C for one hour was dissolved in 300ml of distilled water, diluted to 1000-mL mark and stored in a polyethylene bottle. Then the prepared Na_2CO_3 solution (25.0 mL, 0.0100 M) was transferred into a 250-mL beaker, distilled water (25 mL) was added, and titrated with hydrochloric acid (0.02 M) to phenolphthalein end point. The resultant solution was boiled gently for 3-5 min, cooled to room temperature and titrated to bromocresol green/methyl red end-point.

The volume of the hydrochloric acid titrant used was (S), and concentration, M, of the hydrochloric acid was calculated as follows:

$$Molarity (M) = \frac{0.01 \times 25.0 \times 2}{S}$$

Samples were titrated in triplicates as follows: a sample of volume, V, (25 mL) was transferred into a 250-ml conical flask, phenolphthalein indicator solution (4 drops) were added and titrated with standard hydrochloric acid of concentration, M, until pink colour was just discharged.

The volume of titrant used was recorded as (P). Bromocresol green/ methyl red indicator (four drops) was added and titration was continued to a light pink colour. Total volume of titrant used was recorded as (T).
The bicarbonate concentration was calculated as follows:

$$HCO^{3-} \ mg/L = \frac{(T-2P) \times M \times 61000}{V}$$

Determination of phosphate (PO_4^{3-}): The phosphate (PO_4^{3-}) standards were used to generate a calibration curve in the UV-Visible Spectrophotometer from which the PO_4^{3-} concentrations in geothermal spring water samples were read (APHA, 1989).
The standard PO_4^{3-} solution (50 μg/mL) was prepared as follows: 3 g of potassium dihydrogen orthophosphate (KH_2PO_4) was dried on a watch glass at 105-110°C for overnight and cooled in a dessicator. KH_2PO_4 (0.9594 g) was weighed out and dissolved into a 500-Ml volumetric flask with distilled water. This was 1 mg/ml PO_4^{3-}

solution (standard stock solution).
For analysis, 5 ml of 1 mg/mL PO_4^{3-} solution was diluted to 100 ml with distilled water. This was 50 μg/mL PO_4^{3-} solution (intermediate standard solution). The PO_4^{3-} standard concentrations (0, 0.05, 0.1, 0.2, 0.3, 0.4 mg/L) were prepared from this intermediate standard solution by pipetting the following volumes into 100-mL volumetric flasks respectively and diluting them to the mark: 0, 1, 2, 4, 6 and 8 mL. Then the geothermal spring water samples (1 mL) were each pipetted into 100-mL beakers and into each beaker, HNO_3 (2 mL) and 5 mL of perchloric acid ($HClO_4$) were added. The resultant solution was heated on a hot plate until 2mL of solution remained (about 30 min) and then allowed to cool. HNO_3 (2 mL) and $HClO_4$ (5 mL) were added and the solution was again heated on the hot plate until 1 mL of solution remained. The solution was then allowed to cool.
Distilled water (20 mL) was added and the solution was heated gently (for 10 min, but it was not allowed to boil). The solution was decanted into a 100-ml volumetric flask and distilled water (70 mL) and reducing solution (20 mL) were added and the solution was diluted to volume with distilled water. This was left overnight before reading. The absorbance of the blank, standards and samples was measured at 555 nm.

Determination of silica (SiO_2): The first step was preparation of silica (Si) standards for derivation of a calibration curve from which Si concentration in the geothermal spring water samples were to be read on the UV-Visible spectrophotometer (APHA, 1989). From standard stock Si solution (1000 mg/L) (purchased), 5ml were pipetted into 500-mL volumetric flask and diluted to volume with distilled water.
Si standard volumes of 0, 2, 5, 10, 15 and 20 mL were measured from standard Si stock solution (0.01 mg/mL) using a 50-ml burette into 100-ml volumetric flasks. These standards contained the following concentration respectively: 0, 0.02, 0.05, 0.10, 0.15, and 0.20 mg/mL. Samples (1 mL) each were pipetted into clean nickel crucibles. Sodium hydroxide (2 g) was added and dissolved. The solution was heated over an open flame, starting with a small flame and gradually increasing the flame until the bottom of crucible was just red. Heating was continued for 3 min. Then the contents were removed and cooled. The crucible was then put into a 500-mL crucible polypropylene beaker. Hot distilled water was added into the crucible to dissolve the melt. The crucible was then removed, rinsed with hot distilled water and allowed to cool. The solution was then transferred into a 250-ml volumetric flask and diluted to the mark with distilled water. The solution was filtered through filter paper into a 500-ml propylene beaker. (First 10-20 mL solution was discarded).
The filtered solution was transferred to a plastic bottle to serve as sample stock solution. To sample stock solutions (1 ml) pipetted into 100-mL volumetric flasks in triplicates, P-nitrophenol indicator (1 drop) was added and acidified with drops of HCl (1+4) until the colour changed from yellow to colourless. The solution was diluted to about 50mL. To the mixture, HCl (1+4) (1 mL) and 5% ammonium molybdate solution (4 mL) were added and mixed thoroughly, allowed to stand for 10 min and 10% v/v tartaric acid solution (5 mL) and ascorbic acid solution (2 mL) were added. The resultant solution was diluted to the mark with distilled water, allowed to stand for 30 min at room temperature and absorbance and concentration of Si measured at 650nm wavelength. The SiO_2 were calculated from Si results.

Subsurface temperatures

The subsurface temperatures of geothermal springs in Nkhata Bay were determined using the following cation geothermometers: 1) Na/K chemical geothermometers by Truesdell (1976), Fournier
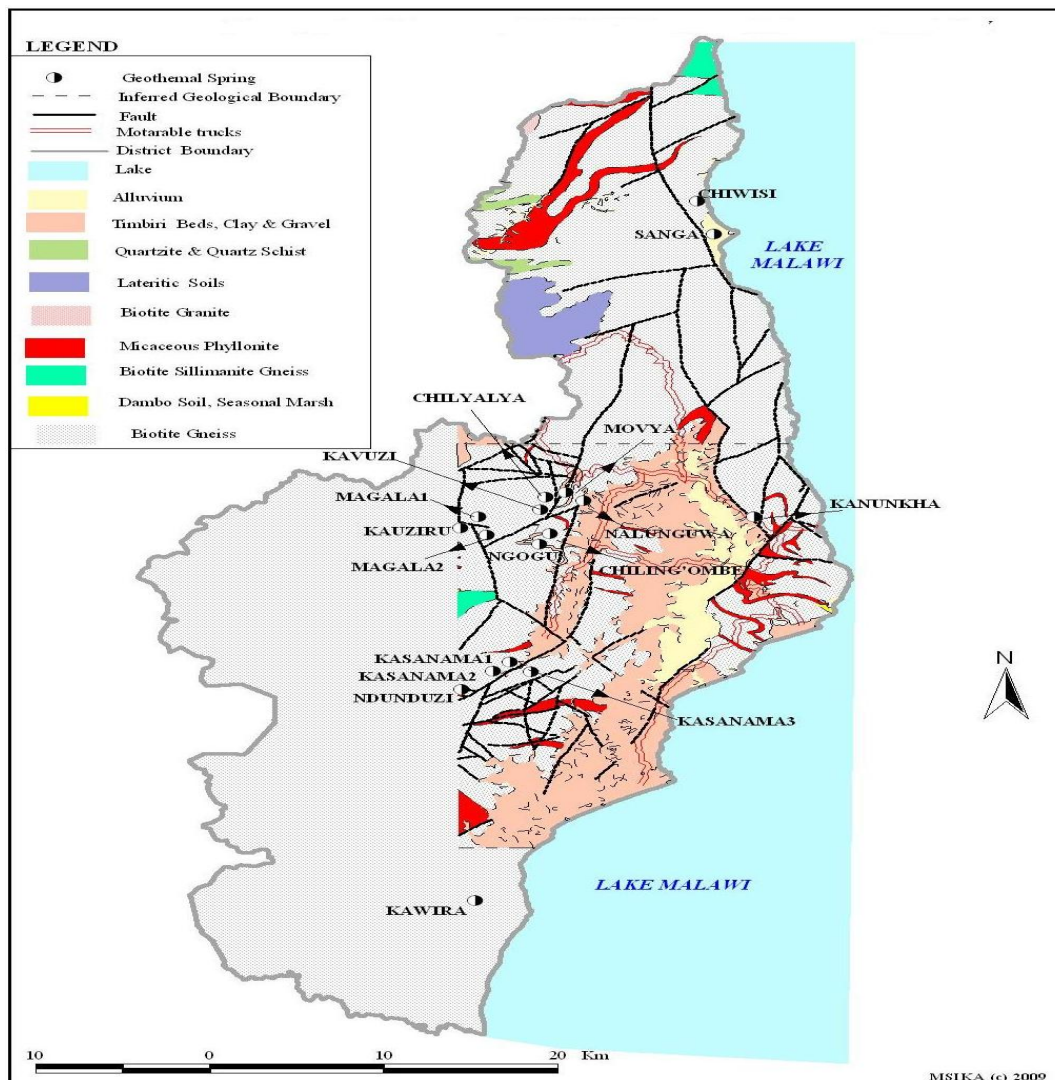
Figure 2. Spatial distribution of geothermal springs in Nkhata Bay, Malawi.

(1979), Arnorson (1983), Giggenbach (1988), and Verma and Santoyo (1997). These geothermometers have been coded as Na/K1, Na/K2, Na/K3, Na/K4 and Na/K5 respectively; 2) Na/K/Ca chemical geothermometer by Fournier and Truesdell (1973); and, 3) K/Mg chemical geothermometer by Giggenbach (1988).

RESULTS AND DISCUSSION

Spatial locations of geothermal springs in Nkhata Bay, Malawi

Seventeen geothermal springs, with surface temperatures ranging from 29±0.3 to 74±0.5°C, were identified and their distribution mapped in Nkhata Bay (Figure 2).

Spatially, 76% of the springs are clustered towards Nkhata Bay West and 94% of the springs characteristically occur along the major faults in the Basement

Complex in the District. Ostensibly, the spatial distribution of geothermal springs in the district is controlled by faulting which possibly provides conduits for hydrothermal convection in the Basement Complex (Figure 2).

Chemistry of geothermal springs

A summary of the chemistry of the thermal springs is provided in Table 1.

Physico-chemical characteristics of the geothermal springs

The surface temperature values of the thermal springs in Nkhata Bay ranged from 74.0±0.5 to 29.0 ±0.3°C. The

Table 1. Some chemical and physico-chemical characteristics of geothermal springs in Nkhata Bay, Malawi.

Parameter (chemical species in mg/l)	Kawira	Kanunkha	Nalunguwa	Chilyalya	Kavuzi	Movya	Chiling'ombe	Ngogu	Magala1	Kasanama1	Kasanama3
Na	97.46±2.98	97.23±4.96	97.26±1.36	88.06±1.87	87.65±5.56	81.57±3.60	96.00±1.79	96.62±7.21	93.53±	96.90±1.70	107.50±3.00
K	3.18±0.037	17.25±0.371	1.76±0.038	1.47±0.011	1.63±0.015	1.54±0.027	1.34±0.003	1.59±0.008	1.73±0.064	2.51±0.057	4.64±0.016
Ca	0.65±0.004	11.09±0.03	1.14±0.007	1.97±0.035	1.63±0.024	1.98±0.025	0.87±0.012	0.86±0.009	1.05±0.003	0.94±0.054	1.89±0.012
Mg	0.33±0.006	9.26±0.049	0.002±0.0002	0.24±0.001	0.17±0.002	0.07±0.002	0.06±0.001	0.15±0.001	0.08±0.002	0.02±0.003	1.32±0.014
Fe	0.66±0.044	27.23±0.103	Not detected (Nd)	Nd	Nd	Nd	Nd	Nd	Nd	Nd	1.40±0.133
Cd	0.033±0.007	0.049±0.007	0.020±0.008	0.023±0.010	Nd	0.001±0.009	Nd	Nd	Nd	Nd	Nd
SiO$_2$	0.16±0.007	0.44±0.020	Nd	Nd	Nd	Nd	0.02±0.007	0.05±0.007	0.08±0.007	0.17±0.003	0.20±0.011
F$^-$	10.27±0.25	7.31±0.10	3.29±0.07	2.78±0.19	2.73±0.03	2.55±0.24	2.47±0.03	3.16±0.17	2.21±0.06	5.67±0.01	5.39±0.14
Cl$^-$	6.49±0.83	14.73±0.56	9.55±0.20	7.62±0.10	8.74±0.27	7.26±0.13	5.64±0.87	5.57±0.12	11.68±0.36	6.17±0.10	5.61±0.17
HCO$_3^-$	67.21±9.60	96.02±13.58	43.21±4.8	76.81±0.00	43.01±6.80	76.81±0.00	52.81±4.80	76.81±0.00	48.01±0.00	52.81±4.80	33.61±4.81
SO$_4^{2-}$	46.02±2.73	106.52±2.10	99.82±0.86	85.86±4.83	93.52±2.35	87.48±2.85	60.55±2.10	123.63±3.98	55.92±0.63	86.98±0.99	53.16±3.14
Ionic Strength	0.0041	0.0070	0.0046	0.0045	0.0045	0.0040	0.0055	0.0039	0.0049	0.0047	0.0041
Temp (°C)	74.0±0.5	53.2±0.5	39.5±1.8	46±0.0	50.3±1.0	41.2±0.3	40.4±0.3	39.0±1.0	48.3±0.6	61.5±1.3	65.0±0.0
pH	8.12±0.09	6.50±15	8.65±0.12	8.23±0.12	7.64±0.04	8.08±0.05	8.27±0.10	8.43±0.11	7.89±0.23	8.36±0.07	8.39±0.14
EC (µS/cm)	5267±153	7183±76	5300±0.0	4767±58	5183±104	4430±20	4917±176	4617±29	4800±0.0	6393±51	6810±193

electrical conductivities ranged from 7183- 4430 µS/cm (Table 1). The pH in the geothermal springs studied was in the range of 6.5-8.65. Water with a pH below 6.5 is considered acidic and a pH above 8.5 is considered basic (GNWT, 2008). Thus, practically all the studied thermal spring waters are of neutral type. The exception is Nalunguwa, which is slightly alkaline (Table 1).

Chemical characteristics

Sodium (Na) represented the highest concen-tration values in almost all the studied geothermal springs, this was followed by sulfate (SO_4^{2-}), bicar-bonate (HCO_3^-), chloride (Cl^-), fluoride (F^-), potas-sium (K), calcium (Ca), nitrate (NO_3^-), magnesium (Mg) and Cadmium (Cd) - in descending order of

abundance. Thus, in terms of cations, Na is followed by K in abundance in the geothermal springs.

However, unlike Na (>80 mg/L), the concentration of K is considerably lower (<10 mg/L) in most thermal springs in Nkhata Bay. With regard to anions, the following is their descending order of abundance (mg/L): SO_4^{2-} > HCO_3^- > Cl^-, > F^- > NO_3^-.

Lead (Pb), chromium (Cr) and phos-phate (PO_4^{3-}) were below detection limits in all the studied geothermal springs. Trilinear Piper plot (Figure 3) was used to delineate the principal hydrochemical characteristics of the thermal springs.

The trilinear piper plot hydrochemically distin-guished two water facies of geothermal springs: 1) sodium - sulfate (Na-SO_4) water type for

Kasanama1 and Nalunguwa geothermal springs and 2) Sodium- sulfate-bicarbonate (Na-SO_4-HCO_3) water type for Kawira, Kanunkha, Movya, Chilyalya, Chiling'ombe, Ngogu, Magala1 and Kasanama3 springs.

Equilibrium conditions and water quality of geothermal springs in Nkhata Bay, Malawi

Several mineral species in contact with the thermal waters and equilibrium conditions (as reflected by the saturation indices (SI)) in the geothermal springs in Nkhata Bay were monitored and analyzed using AquaChem 5.1 software (SWS, 2008).

The following mineral species were inferred to have been in contact with the geothermal
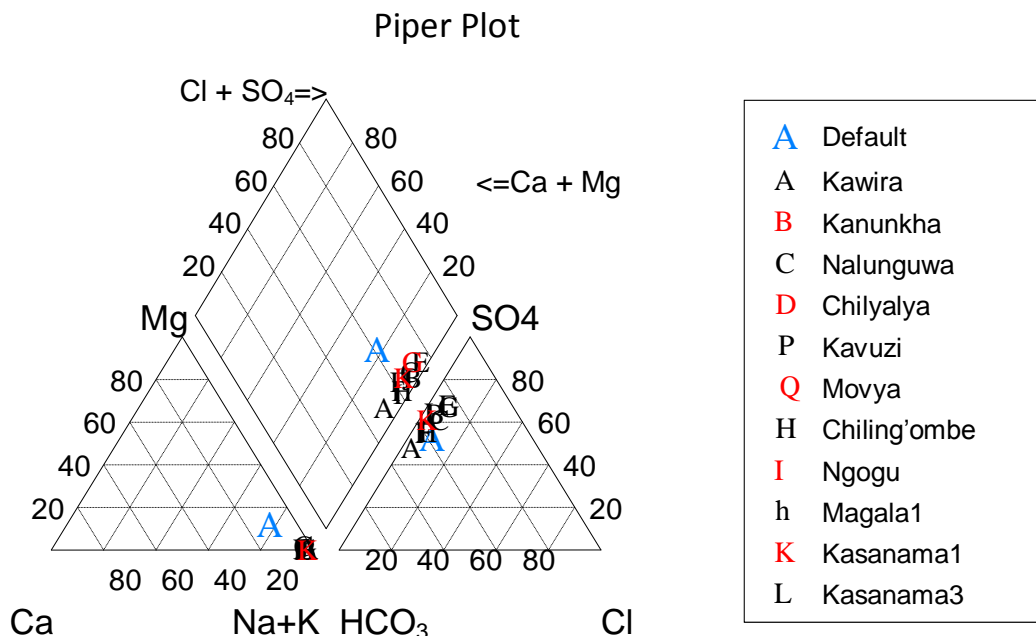
Piper Plot

Figure 3. Principal chemistry of the water facies of geothermal springs in Nkhata Bay.

spring waters in the district: calcite, aragonite, dolomite, gypsum, anhydrite, halite, fluorite, goethite, siderite and haematite.

There was undersaturation (SI<0) in all of the springs with respect to the following mineral species: calcite ($CaCO_3$), aragonite ($CaCO_3$ in its rhombic form, harder and less common or stable than calcite), dolomite ($CaMg(CO_3)_2$), anhydrite ($CaSO_4$), halite or rock salt (NaCl), gypsum ($CaSO_4 \cdot 2H_2O$) and fluorite or fluorospar (CaF_2). However, there was supersaturation (SI>0) with respect to goethite (FeO(OH), haematite (Fe_2O_3) and siderite ($FeCO_3$) in Kanunkha, Kawira, Nalunguwa and Kasanama3 thermal waters.

Water, which is calcite-undersaturated (SI (calcite) <0), is corrosive to the pipes and on the other hand, if the water is calcite-oversaturated (SI (calcite>0), the calcite precipitates out and settles on the pipelines (Purschel, 2006).

Similarly, siderite, goethite and haematite (SI>0) precipitate and settle down in the springs in which they are inferred, unlike calcite, aragonite, halite, gypsum, anhydrite and fluorite (SI<0) which suggestively remain in thermal water column.

Despite fluorite undersaturation in the studied geothermal springs, fluoride concentration (Table 1) was high in all of them (>1.5 mg/L, WHO guidelines for drinking water). Moreover, due to the undersaturation conditions, fluoride cannot precipitate as CaF_2, and so mobilizes freely without forming other complexes in the thermal waters as also observed by Rango et al. (2008). Chronic ingestion of concentrations much greater than 1.5 mg/L is linked with development of dental fluorosis and, in

extreme cases, skeletal fluorosis; high doses have also been linked to cancer (BGS, 2008).

Iron (Fe) levels observed in Kawira, Kanunkha and Kasanama3 thermal springs exceeded the safe limit of 0.20 mg/L set by MBS. High levels of iron oxides may result in development of a benign pneumoconiosis, called siderosis (lenntech, 2006).

The cadmium (Cd) levels observed in Kawira, Kanunkha, Nalunguwa and Chilyalya geothermal springs exceed the safe limit of 0.005 mg/L set by both MBS and WHO. Except Chilyalya which occurs on a hill where no farming takes place, the cadmium levels in other springs are likely to be exacerbated by phosphate fertilizer, which is commonly used in the farming field along the banks since fertilizer is a natural sink of cadmium.

Recent data indicate that adverse health effects of cadmium exposure may occur at lower exposure levels than previously anticipated, primarily in the form of kidney damage but possibly also bone effects and fractures (Järup, 2003).

The EC range of 7183- 4430 µS/cm in thermal waters in Nkhata Bay exceeds the WHO recommended EC value in surface water of 2500 µS/cm.

The pH of most of these thermal waters was within 6.5-8.5 (recommended limit, WHO water quality criteria) except Nalunguwa thermal waters which were slightly alkaline (pH = 8.65).

The extent of equilibrium conditions in the studied geotheothermal springs in Nkhata Bay is been represented in Figure 4. The Gibbenbach Triangle, the triangular K-Mg-Na representation by Giggenbach(1988), allows the verification of the extent to which water-rock
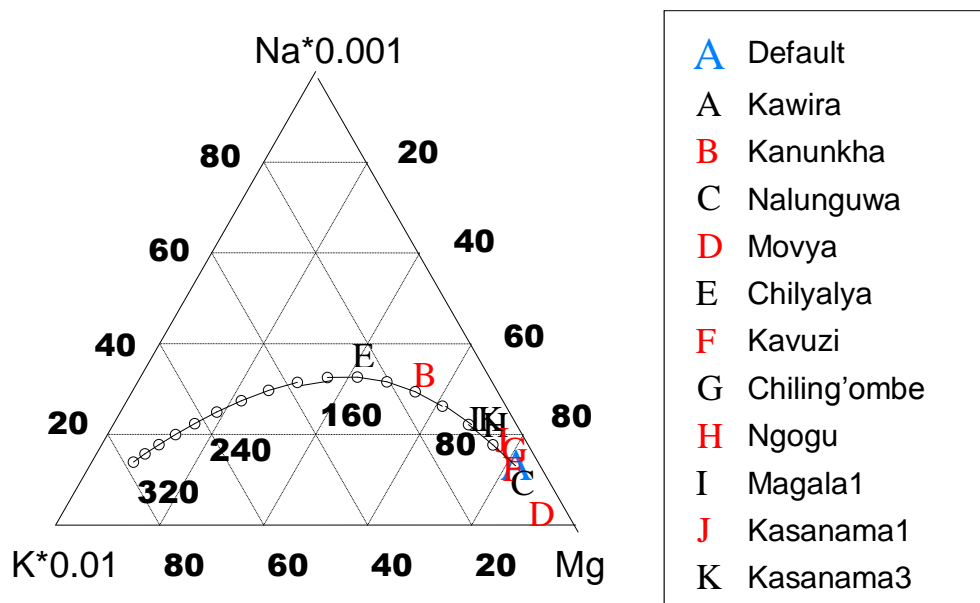
Giggenbach Triangle

Figure 4. Extent of water-rock equilibrium in geothermal springs understudy.

equilibriums have been attained in the reservoirs (SWS, 2008). The triangle (Figure 4) comprises three zones: Immature waters (at the base); Partially equilibrated waters (in the middle); and Fully equilibrated waters (along the curve).

Except Movya which reflects immature water, Kavuzi, Chilyalya, Nalunguwa, Chiling'ombe, Ngogu, Magala1, Kawira, Kanunkha, Kasanama1 and Kasanama3 geothermal springs reflect fully equilibrated waters (Figure 4).

Since the Giggenbach Triangle (Giggenbach, 1988) indicates that most of the geothermal springs studied in Nkhata Bay have fully equilibrated waters, their chemistry can reliably be used to calculate the probable subsurface temperatures at which water-rock equilibriums were attained.

Subsurface temperatures of geothermal springs in Nkhata Bay

Three cation geothermometers (Na/K, Na/K/Ca and K/Mg) geothermometers were considered for estimating the sub-surface temperature of the geothermal springs in Nkhata Bay.

Na/K geothermometer usually offers the best chemical indication of high temperatures at depth since the Na/K ratio is low in hot areas and high in cool areas (Elder, 1981). These chemical constituents of the hot water are leached from geological sources in such a manner that at higher temperatures, there is an increased tendency for

K to replace Na in feldspars (Elder, 1981).

The chemical reaction that occurs is reflected in the chemical Equation (1).

$$Na\text{-feldspar} + K \leftrightarrow Na + K\text{-feldspar} \qquad (1)$$

Further, Na/K geothermometers are not affected by any concentration-dilution process since alkali metals are generally aquaphilic and their ratio is not affected by the kinetics of the reservoir (CER, 2007). They can, thus, be indicative of the highest temperature at which the waters have reached at chemical equilibrium with the reservoir rocks.

Evident from Table 2, only cation (Na/K, Na/K/Ca and K/Mg) geothermometers were considered for sub-surface temperature estimation of the geothermal springs in Nkhata Bay. Silica geothermometers were not considered relevant in this study because they gave subsurface temperature values which were too inconsistent to be plausible (Appendix 2).

The values were, thus, too low for most silica geothermometers and too high for one geothermometer. For example; silica (SiO_2) geothermometer models by Truesdell (1976), Fournier (1977) and Verma and Santoyo (1997) gave values which were too low (<0°C) while one model by Rimstidt (1979) gave temperatures values which were too high (> 500 ≤ 40922°C) - the reason being that silica was found in very low concentrations in geothermal springs understudy (Table 1). This was probably because of dilution that might have

Table 2. Sub-surface temperatures of Nkhata Bay geothermal springs estimated using some cation (Na/K, Na/K/Ca and K/Mg) geothermometers.

Geothermal Spring	Measured temperature (°C)	Na/K$_1$ (°C)	Na/K$_2$ (°C)	Na/K$_3$ (°C)	Na/K$_4$ (°C)	Na/K$_5$ (°C)	Na/K/Ca$_1$* (°C)	K/Mg (°C)
Kawira	74	92	137	103	156	142	144	26
Kanunkha	53	259	272	262	283	272	209	26
Nalunguwa	39.5	56	104	68	125	111	114	10
Movya	41	52	100	64	121	107	85	31
Chilyalya	46	58	106	69	126	112	93	29
Kavuzi	50	59	106	70	127	113	86	26
Chiling'ombe	40	42	91	54	112	98	106	26
Ngogu	39	51	99	63	120	106	113	29
Magala1	48	57	105	69	126	112	116	25
Kasanama1	61.5	77	123	89	143	129	131	16
Kasanama3	65	112	154	123	173	159	150	29

*No Mg-correction was done on geotemperature values for Na/K/Ca geothermometer.

occurred as a result of mixing between thermal and ambient ground waters. On the other hand, this could also be due to silica precipitation that might have happened prior to the collection of geothermal water samples for analysis as was also observed in one study conducted by Wahl (1977).

In this study, however, most Na/K geothermometers were used to estimate geotemperatures of the thermal springs, considering that the best chemical indications of high temperatures at depth are given by the Na/K ratio which, for example, is low in hot areas and high in cool areas (Elder, 1981). These chemical constituents of the hot water are leached from geological sources in such a manner that at higher temperatures, there is an increased tendency for K to replace Na in feldspars (Elder, ibid). The chemical reaction that occurs is reflected in the chemical equation:

Na-feldspar + K $\leftrightarrow$ Na + K-feldspar
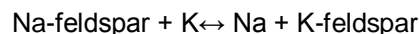
CER (2007) also observes that Na/K geothermometers are not affected by any concentration-dilution process since alkali metals are generally aquaphilic and their ratio is not affected by the kinetics of the reservoir. Therefore, they can be indicative of the highest temperature at which the waters have reached at chemical equilibrium with the reservoir rocks (CER, 2007).

The deep reservoir temperatures in the geothermal springs in Nkhata Bay ranged from 112 to 283°C, estimated using Na/K geothermometers (Table 2). Similarly, Na/K/Ca geothermometer gave a subsurface temperature range of 85 - 209°C in the thermal springs. On the contrary, the K/Mg geothermometer gave a sub-surface temperature range of 10 to 29°C for geothermal springs in Nkhata Bay. The highest geotemperatures were associated with Kanunkha, Kawira and Kasanama3 geothermal springs; the lowest geotemperatures were

associated with Chiling'ombe and Ngogu geothermal springs.

Pirlo (2004) indicated that the better the correlation between the emergence spring temperatures and subsurface temperatures estimated by the geothermometer, the better the geothermometer becomes for efficient use in a particular hydrothermal system.

From the correlation analysis conducted using Genstat 12.0 Software (NAG, 2008), Na/K/Ca geotemperatures showed the strongest positive correlation with measured temperatures of the geothermal springs (r = 0.50) and a geotemperature range of 85 - 209°C has been estimated for geothermal springs in Nkhata Bay.

Similarly, Na/K geotemperatures showed relatively strong positive correlation with the measured (emergence) temperatures of the geothermal springs (r ≥ 0.36, P<0.01).

In particular, Na/K$_4$ geotemperatures displayed the strongest positive correlation with the measured temperatures of the thermal springs (r ≥ 0.39, P<0.01). Using Na/K$_4$ geothermometer, the geotemperature range of 112 - 283°C was estimated for geothermal springs in Nkhata Bay. On the contrary, K/Mg geothermometer that gave the lowest geotemperatures (Table 2), had the weakest positive correlation with the measured temperatures of the geothermal springs (r ≥ 0.03, P<0.01).

Hong et al. (2005) observe that K/Mg geothermometers can only be used for chloride waters as it is sensitive to mixing. The low geotemperatures given by K/Mg geothermometers can be attributed to very low Mg content (<1 mg/L) in most thermal waters understudy (Table 1) possibly due to dilution as a result of mixing between thermal waters and ambient groundwater.

To this end, it seems, therefore, that the Na/K/Ca and Na/K geothermometers offer better and more robust

estimates of original subsurface temperatures of geothermal springs in Nkhata Bay; they both gave significant positive correlation with the emergence temperatures of the springs (p<0.01).

Conclusion

The chemistry of geothermal springs in Nkhata Bay reveals two spring water types: (1) sodium-sulfate (Na-SO4) springs, which include Nalunguwa and Kasanama1 geothermal springs; and, (2) sodium-sulphate-bicabornate (Na-SO4-HCO3) springs, which include Kawira, Kanunkha, Movya, Chilyalya, Ngogu, Chiling'ombe, Magala1 and Kasanama3 geothermal springs. Except Movya which has immature water, all these thermal springs have fully equilibrated waters.

The chemistry of geothermal springs in Nkhata Bay, Malawi, evidently shows that utilization of the thermal waters for drinking is not suitable due to high fluoride concentrations (>1.5 mg/L, WHO guidelines for drinking water) and high EC values (>2500 μScm^{-1}, WHO recommended EC value).

In addition, high concentrations of iron (Fe) (>0.20 mg/L set by MBS) and cadmium (>0.005 mg/L, safe limit set by both MBS and WHO) were determined in several thermal springs in the District, such as Kawira, Kanunkha, Kasanama3, Nalunguwa and Chilyalya. The original subsurface temperatures of Nkhata Bay geothermal springs, estimated by the Na/K/Ca and Na/K geothermometers, ranged from 85 to 209 and 112 to 283°C, respectively. The highest geotemperatures were associated with Kanunkha, Kawira and Kasanama3 geothermal springs; the lowest geotemperatures were associated with Chiling'ombe and Ngogu thermal springs.

The Na/K/Ca and Na/K geothermometers were found to provide better and more robust estimates for subsurface temperatures (geotemperatures) of geother-mal springs in Nkhata Bay as these most significantly (p<0.01) correlated with the emergence temperatures of the thermal springs with correlation coefficient (r) =0.50 and r≥ 0.36 for Na/K/Ca and Na/K geotemperatures, respectively. The estimation of subsurface temperature in the geothermal springs in Nkhata Bay, however, needs re-evaluation by use of more extensive hydrogeoche-mical and geophysical surveys in order to establish whether they can be used for generation of geothermal electricity in Malawi.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Appendix 1. Spatial data for geothermal springs in Nkhata Bay, Malawi.

Geothermal spring	utm coordinates	Village	Traditional authority
Kawira	0610504; 8665862	Chaphulika	Zilakoma
Kanunkha	0635870; 8718574	Chighachang'ombe	Timbiri
Nalunguwa	0620479; 8720782	Chilongozi	Kabunduli
Movya	0618818; 8721976	Chilongozi	Kabunduli
Chilyalya	0618291; 8721782	Chamawoya	Kabunduli
Kavuzi	0618187; 8721622	Chamawoya	Kabunduli
Chiling'ombe	0616070; 8715216	Kachiweya	Kabunduli
Ngogu	0616096; 8714990	Kachiweya	Kabunduli
Kasanama1	0614246; 8697772	Chilipeta II	Kabunduli
Kasanama2	0614163; 8697752	Chilipeta II	Kabunduli
Kasanama3	0615297; 8698416	Chilipeta II	Kabunduli
Ndunduzi	0609352; 8694896	Chilipeta II	Kabunduli
Magala1	0610111; 8716546	Vimaso	Kabunduli
Magala2	0610164; 8716592	Vimaso	Kabunduli
Kauziru	0609554; 8716788	Vimaso	Kabunduli
Chiwisi	0630933; 8761965	Siyalimba	Musisya
Sanga	0632384; 8757501	Kajizinge	Musisya

Appendix 2. Geotemperatures estimated by silica geothermometers for thermal springs in Nkhata Bay, Malawi.

Spring name	Measured Temperature (^oC)	QTZ_1	QTZ_2	QTZ_3	QTZ_4	QTZ_5
Kawira	74	-41.37	-41.82	2089.63	-81.19	-73.09
Kanunkha	53	-21.81	-22.23	40921.95	-46.02	-54.36
Nalunguwa	39.5	-	-	-	-	-
Movya	41	-	-	-	-	-
Chilyalya	46	-	-	-	-	-
Kavuzi	50	-	-	-	-	-
Chiling'ombe	40	-74.00	-74.45	518.75	-154.97	-103.63
Ngogu	39	-59.80	-60.26	881.68	-120.11	-90.45
Magala1	48	-53.05	-53.50	1165.38	-105.11	-84.12
Kasanama1	61.5	-40.35	-40.80	2221.82	-79.22	-72.13
Kasanama3	65	-37.13	-37.57	2747.08	-73.08	-69.06

QTZ_1: Silica geothermometer by Trusdell (1976)

Formula: $T_{SiO2} = \dfrac{1315 - 273.15}{5.205 - \log m}$

QTZ_2: Silica geothermometer by Fournier (1977)

Formula: $T_{SiO2} = \dfrac{1309 - 273.15}{5.19 - \log m}$

QTZ3: Silica geothermometer by Rimstidt (1997)

Formula: $T_{SiO2} = (856/\log m) + 0.0254 - 273.15$

QTZ4: Silica geothermometer by Verma and Santoyo (1997)

Formula: $T_{SiO2} = -44.119 + (0.24469m) + ((-1.7414 \times 10^{-4})\, m^2) + (79.305 \times \log m)$

QTZ5: Silica geothermometer by Fournier (1977)

Formula: $T_{SiO2} = \dfrac{1032 - 273.15}{4.69 - \log m}$

Note: m = concentration of SiO_2 in ppm or mg/L.

Full Length Research Paper

The absorption and scavenging ability of a bacillus in heavy metal contaminated soils (Pb, Zn and Cr)

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A bacterial strain, which was able survive Pb, Zn and Cr -heavy metal compound culture was isolated from the soils of Shanjia Villiage, Qufu City, Shandong Province. An observation of the morphological and biochemical characteristics of the compound and the analysis of 16SrDNA sequence revealed that it was *Bacillus*. Standing liquid culture was used to study the tolerance of Pb Zn Cr -heavy metals compound and the ability to absorb and clean the heavy metal compound. Further study was made to compare the absorbing ability of Cu, Cd and Hg.

Key words: *Bacillus*, heavy metal, Pb, Zn, Cr.

INTRODUCTION

With the rapid development of economy and society, the exploitation of coal resources in China increases gradually and environmental problems caused by coal gangue have become increasingly serious (Shao and Cao, 2002). Coal gangue is the main component of coal slag. According to statistics, the cumulative stacked gangue is more than 40 tons (Guo, 2007), but the annual growth rate is still 1-2 million tons. There are heavy metals such as Cr, Pb, Zn, Cd, Cu and other heavy metal elements in the gangue. When there is accumulation of harmful heavy metals in the soil to a certain degree, it will have toxic effects on soil - plant system, which not only causes soil degradation, decline in crop yields and quality, but also pollutes the surface water and groundwater through runoff and leaching, deterioration of water environment, and

direct contact with food chain and other ways to endanger human health and life. In the toxic heavy metals, pollution in soil ecological system is long-term and irreversible (Liu, 2008); but the problem is not obvious. Therefore, soil ecosystem metal, heavy metal pollution and prevention especially in the international arena have been a difficult and hot research topic (Chi, 2006).

Microbial remediation is the use of microorganisms (bacteria, fungi, indigenous alien gene engineering bacteria) on the metabolism and transformation of pollutants, degradation of pollutants, mainly used for degradation of organic pollutants in soil. Microbial remediation technology does not destroy the soil environment for plant growth, does not form secondary pollution and does not transfer the pollutants to residual

Figure 1. The location map of sampling point.

Figure 2. Soil sampling distribution.

methods to solve the problem of heavy metal contamination in soil. However, because of their own limitations, they have failed to get the ideal method for soil heavy metal management (Tang, 1996; Wang, 1996). In recent years, there are a many researches about heavy metal resistant bacteria, which are mainly concentrated in heavy metals such as Cd, Cu and As. Therefore, the composite metal reports is even less.

This work studied the soil polluted by coal gangue, based on the theory of microbial remediation technology. Isolated strains can fight a variety of heavy metals. This work at the same time fills the gaps in the domestic research of heavy metal pollution in China caused by coal gangue, and shows how to prevent the pollution of the gangue based on background and scientific information.

This test on coal gangue from mountain soil separated strains that have the ability to absorb compositing metals- Pb, Zn and Cr. After the physiological and biochemical reaction of the strains was identified as well as 16SrDNA, an auxiliary research was done for the bacteria to explore their resistance to Cu, Cd and Hg and their ability to remove degradation.

MATERIALS AND METHODS

The source of the bacteria

The experiment soil samples are taken from Shanjia Vilage Coal Mine in Qufu City (Figure 1), Shandong Province.

The distribution point of the samples is shown in Figure 2; with distance of 2, 5, 10, 15, 20 and 25 m. After retrieving six samples, they were mixed fully and set aside for pretreatment, with quartiles.

The screening culture of the strains

The preparation of the soil solution

The mixed sample was collected into laboratory cultures domesticated for seven days. 1 g of the mixed sample was used, which was diluted with 99 mL of sterile water. After, it was oscillated for 20 min, and stood still for 20 min. The upper clear liquid of 0.5 mL was taken. 4.5 mL sterile water was added into a test tube and the soil diluents concentration of 10^{-3} was obtained. In accordance with the above method, one can get diluted soil concentration gradient of 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7}.

The preparation of the culture media

Beef extract-peptone medium: 5 g beef extract; 10 g peptone; 5 g NaCl; 15-20 g agar; 1000 mL H_2O; pH 7.2-7.4. Potato medium: 200 g selected high quality potatoes; 20 g glucose; 15-20 g agar; 1000 mL H_2O; natural pH. GAO 1st synthetic culture medium: 20 g soluble starch; 1 g KNO_3; 0.5 g $K_2HPO_4·3H_2O$; 0.5 g NaCl; 0.5 g $MgSO_4·7H_2O$; 15-20 g agar; 0.01 g $FeSO_4$; pH 7.2-7.4.

The culture medium components were configured for preparation and the autoclave was used for sterilization for 20 min under 121°C. After the medium was cooled to about 50°C, 60 mg Pb $(NO_3)_2$, 50 mg $ZnSO_4$ and 80 mg $K_2Cr_2O_7$ were added (every three parts separately) to the medium. It was oscillated and shaken to have a dissolved mix. All the operations were done on bench top.
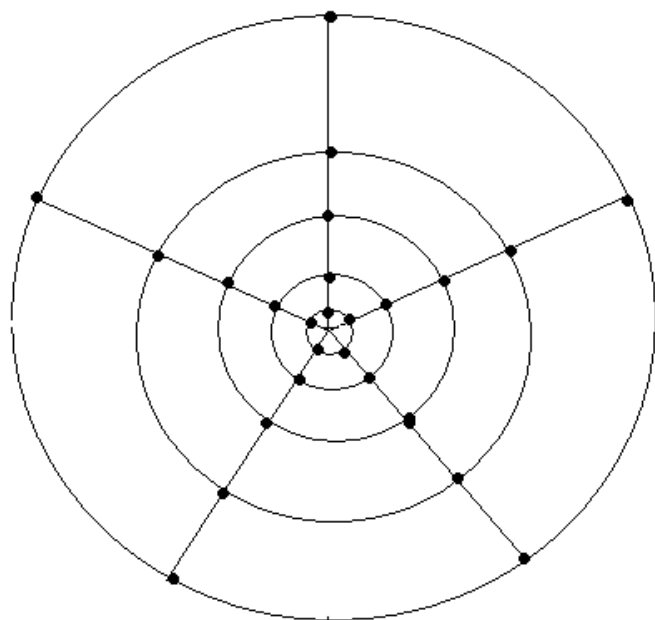
Problems (Zhang and Xia, 2000). It can maximize the removal of pollutants or make contaminants harmless. It interferes less in surrounding environment and has the advantages of good environmental and social benefits (Chen et al., 1997). Bioremediation technology, the rapid rise in recent years, has become a green field of scientific research (Ebbs, 1997; Weon, 2011).

At present, domestic and overseas scholars are launching a lot of research on the coal mine area and soil heavy metal management (Li, 2009; Liu, 2009; Murzaeva, 2004). Domestic and foreign experts have adopted the toxic improver method, using local traditional and chemical

Table 1. The specific microorganisms culture conditions.

Temperature (°C)	Microbiota	Culture medium	Soil solution concentration	Incubation time
37	Germ	Beef extract-peptone medium contains Pb, Zn and Cr	10^{-5}, 10^{-6}, 10^{-7}	24 h
28	Fungus	Potato medium contains Pb, Zn and Cr	10^{-5}, 10^{-6}, 10^{-7}	48 h
28	Actinomycetes	GAO 1th synthetic culture medium contains Pb, Zn and Cr	10^{-4}, 10^{-5}, 10^{-6}	5 day

The screening of the strains

The strains were kept in 9 cm plate for development, using the pouring plate method. The specific culture conditions are in Table 1. Each concentration has three parallel groups, and the growth of the strains was recorded.

Domestication of the strains

Good growth was obtained by training the resistant strains colony with a cross, using the methods of purification. Purification of culture medium and training methods are used to screen the same species. All strains were purified for three times. At last, the individual colony was purified. All the strains were kept in the refrigerator (4°C) for preservation.

The study of the ability of the strains for removing degradation

Heavy metal processing method

The medium component is 1.2.2, but agar was not added. The four concentrations were done at the same time. The concentrations of the bacteria are screened as a benchmark concentration. The concentrations were set ordinarily for five, ten and twenty times and vaccination training was done. The liquid stalling training method was taken for study. The bacteria culture conditions are given in Table 1; the growing strains were not added to the sune-vector.

Research methods

The liquid static method was used for training; and the liquid static training of the bacteria cultures is centrifuged by high-speed freezer in 13000 r/min and 4°C. The concentration of heavy metal was measured from the centrifugal machine supernatant by the atomic absorption photometer.

Identification of the strains

The bacteria strains with the best degradation ability were selected for identification.

Morphological observation of strains

The morphology of the bacteria colony in the beef was observed specially in a flat plate. Mycelial morphology was observed under oil lens by using gram staining, and the stained spore was added for observation.

Physiological and biochemical reactions of the strains

Glucose oxidation fermentation test, oxidase test, starch hydrolysis test, Acetyl methyl methanol test, methyl red (MR) test, catalase test, producing H_2S test, indole test, nitrate reduction test, and so on were done for the selected bacterium.

PCR amplification and sequence analysis of 16SrDNA

16SrDNA sequence analysis : total DNA strain extracted was cultured , as a template, using primers 8f 5'AGAGTTTGATCCTGGCTCAG 3 '20 bp and 1492r 5'GGTTACCTTGTTACGACTT 3 '19 bp; the PCR product was cloned by the Shanghai Biological Engineering Co., Ltd. for purification identification.

Resistance of the strains to Cu, Cd and Hg

Based on the optimal growth conditions, the screening strains can degrade Cu, Cd and Hg as well as composite metals- Pb, Zn and Cr, using the same methods. The benchmark concentration is 0.5 mg/L. To calculate its removal efficiency of Cu, Cd and Hg as compared to the initial removal efficiency experiments, more comprehensive experimental results are drawn.

RESULTS

Eight strains were obtained after screening: three bacteria, three fungi and two actinomyces. The study of the ability of degradation and removal showed that the strain with most powerful removal and degradation ability was bacteria. The strains with the highest percentage of degradation and absorption are three selected bacteria.

Strains identification

Morphological observation of strains

The selected bacteria are round, neat at the edge, ecru white, opaque, gram-positive and are found in aspen. The form is microscopically shown in Figure 3.

Physiological biochemical reactions of the strains

The results of physiological biochemical reactions of the strains are seen in Table 2.
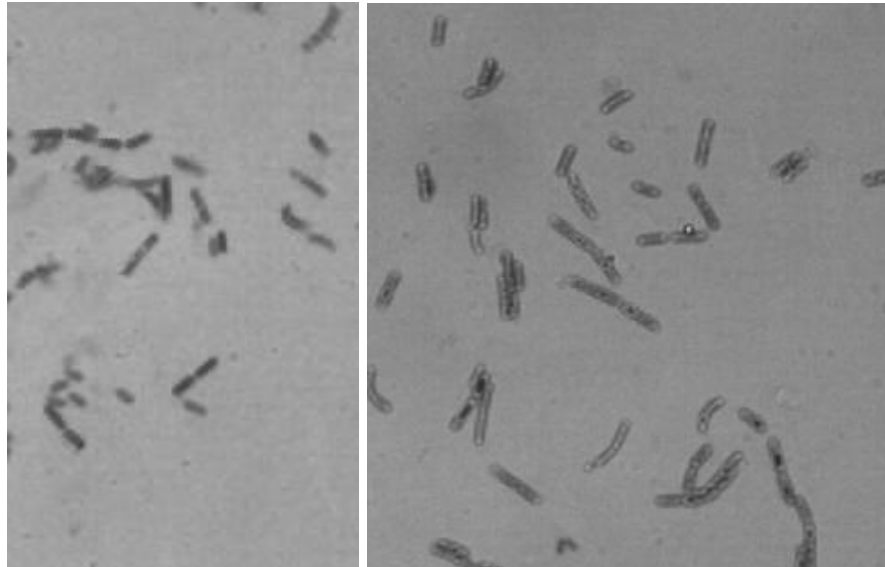
Figure 3. The form observations microscopically.

Table 2. Results of physiological and biochemical reactions of the strains.

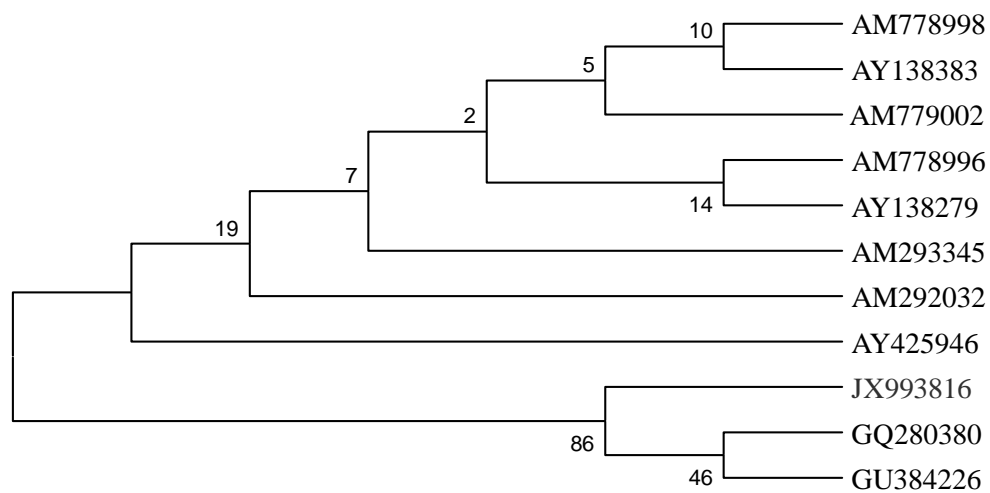| Physiological biochemical reactions	Result
Oxidase	-
Catalase	+
Amylolysis	+
Oxidation fermentation of glucose	+
Indole test	-
Nitrate reducing reaction	+
Methyl red test (MR)	+
Acetyl methyl methanol test	+
Produce H_2S test	-

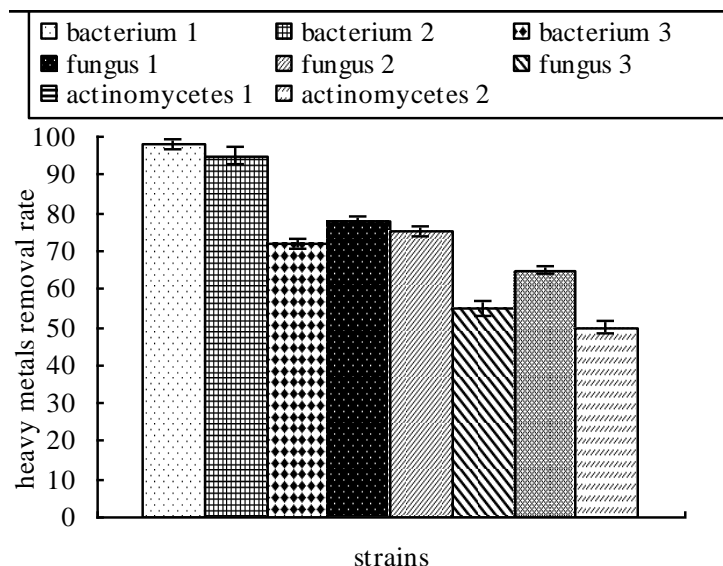**Figure 4.** Evolutionary tree.

Figure 5. Different rates of strains for heavy metals removal. Annotations: This compound metal concentration is of the benchmark concentration (namely 1 times concentration).
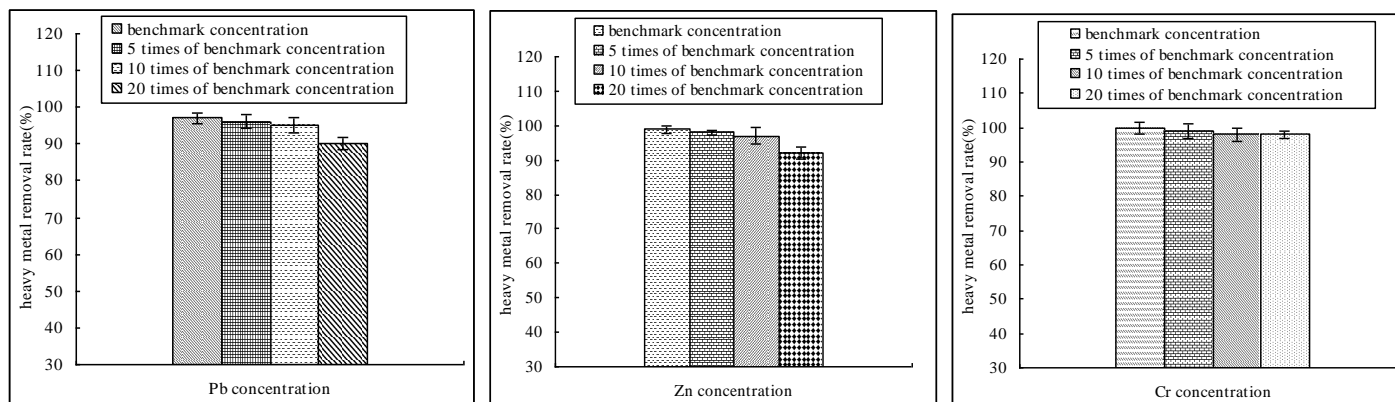

Figure 6. The strain away rate of Pb, Zn and Cr.

Sequence analysis of 16SrDNA

16SrDNA sequence specific to the Shanghai SANGON Biological Engineering Technology Service Co. Ltd selected strain is 1640 bp (GenBank accession number JX993816). BLAST searches for the series, using Clustal W and PHYLIP software to analyze and build the phylogenetic tree (Figure 4).

From the results of the analysis, selected strain and Bacillus homology was 100%. Generally, 16S rDNA sequence homology of less than 98% can be considered to belong to different types; homology less than 93~95% can be considered to belong to different genera (Devereux et al., 1990; Fry et al., 1991). Thus, combining the selected strains based on their morphological, physiological and biochemical reaction characteristics, *Bacillus cereus* is identified.

The ability to degrade and remove Pb, Zn and Cr

The analysis of the ability to degrade and remove different strains of composite metals- Pb, Zn and Cr

Figure 5 shows clearly: (1) Different microorganisms have different ability to degrade and remove heavy metals; the overall performance is: *Actinomyces > fungi > bacteria*. (2) Different strains (bacterium 1, 2, 3) of the same kind of microorganism (such as bacteria) have different degradation and removal ability.

The ability to degrade and remove heavy metal compound

In Figure 6, the separated bacilli all have certain resistance to Cu, Cd and Hg, and have certain Degrada-

tion removal ability. With the increase of metal concentration, the removal rate drops down. From the figure, we can also see that the *bacilli* remove three kinds of metal in the benchmark concentration.

DISCUSSION

In this work, samples are taken from coal gangue mountain soil; and the microbe's resistance to the composite metals- Pb Zn Cr is studied. Bacteria extracted from the soil solution were configured. From the experiment, we can draw the following conclusions:

1) Eight strains are obtained after screening three bacteria, three fungi and two actinomyces. After their morphological, physiological and biochemical reaction observation as well as 16 SrDNA sequence analysis, it is shown that the bacteria are gram positive bacilli. There are different types of media due to differences in nutrients for enrichment and separation of different species of microbes. It does not only improve the separation efficiency of strains and selective enhancement, but also guarantees the reliability and feasibility of the experiment. So it is the premise of the experiment to be carried out smoothly.

2) Experimental results show that different strains that degrade and eliminate heavy metals exhibit different processing capabilities. The ability to process heavy metals is strongest among bacteria, followed by fungi and actinomycetes. In addition, different strains of the same bacteria species also exhibit different processing capabilities. The results show that with increased concentration of heavy metals, their ability to remove those decreases.

3) Further research proves that bacillus also has certain ability to remove Cu Cd Hg.

Conflict of Interests

The author(s) have not declared any conflict of interests.

ACKNOWLEDGEMENT

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