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Development of Ceramic Water Filter Clays Selection Criteria

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DEVELOPMENT OF CERAMIC WATER FILTER CLAYS SELECTION CRITERIA

BY

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ABSTRACT

This study assesses the impact of clayey materials' properties on biofilm formation within the context of point-of-use water treatment systems such as ceramic water filters (CWFs). CWFs were manufactures using clayey materials from different countries by mixing with sawdust and water, and then fired in a kiln. Due to the influence of clayey properties on the quality and duration of CWFs, this study focused to establish a standardization process for clayey selection criteria for ceramic filter factories around the world. To do this, well-established geosciences, environmental and geotechnical engineering methodologies were used. Physical characteristics of clayey materials can be determined through grain size analysis, and liquid limit and plastic limit tests. Mineralogical composition can be determined using X-ray diffraction (XRD) analysis. ICP-MS analysis identifies metals in sawdust fired ashes. *Pseudomonas Fluorescens Migula* was used as model organism to assess biofilm formation on both clayey materials and CWFs.

Three clayey materials from Guatemala, Canada, and Guinea-Bissau were selected for this study. The Guatemalan clayey belonged to poorly-graded sand with silt contains four identifiable minerals: quartz, muscovite, montmorillonite and albite, and its CWF contains quartz, muscovite, and albite. The Canadian clayey was mainly made of quartz, muscovite, and kaolinite and defined as poorly-graded sand, however, its CWF contains quartz, muscovite and hematite. The clayey material from Guinea-Bissau contains quartz, kaolinite, dickite, and montmorillonite and belongs to poorly-graded sand, and its CWF was made of quartz and hematite, respectively. The average biofilm formation coverages for Guatemala, Canada, and Guinea-Bissau clayey

materials were 20.02% ± 6.65%, 19.27% ± 4.59%, and 9.88% ± 5.01%, respectively, while average biofilm formation coverages for Guatemala, Canada, and Guinea-Bissau CWFs are 13.08% ± 4.12%, 10.39% ± 5.05%, and 8.50% ± 5.35%, respectively. 11 elements including Na, Mg, K, Cr, Mn, Fe, Co, Ni, Cu, Zn, and As were identified and quantified in sawdust ashes after firing process. High concentrations of Cr, Ni, Cu, Zn metals in general hinder biofilm formation, while Na, Mg, and Fe can accelerate biofilm formation, thus incorporation of ash can impact final CWF bulk geochemistry. Compared to previous studies, our study showed similar trends when *P. fluorescens* were used on diverse materials; biofilm formation on Canada clayey material containing kaolinite was higher than on Guinea-Bissau clayey material, which contained montmorillonite. Moreover, in Guatemala clayey, albite contained Na^+, which can be exchanged with H^+ in the culture medium to increase bacterial attachment on the positively charged mineral surface. Muscovite has a high bacterial adhesion, which promotes biofilm formation in Guatemalan and Canada clayey materials. Montmorillonite decreases biofilm formation in Guinea-Bissau clayey material, but does not play a decisive role in Guatemalan clayey material. Heavy metals in sawdust ashes have the potential to lower biofilm formation on CWFs in general, when compared to initial materials—this is supported at least for Guatemalan and Canadian samples. Our data suggest that the presence of muscovite in the CWF material causes significant differences in biofilm coverage, as shown by pairwise analysis of (1) Guatemalan (+ muscovite) and Guinea-Bissau (- muscovite) CWFs and (2) Canadian (+muscovite, +hematite) and Guinea-Bissau (- muscovite, + hematite) CWFs. However, the small observed differences of biofilm formation between clayey

material and CWF of Guinea-Bissau cannot be fully explained by heavy metal loading through firing with ash incorporation or montmorillonite loss coupled to hematite ingrowth, due to initially lower biofilm coverage on related clayey material.

This study showed the importance of the determination of mineral composition of clayey materials for the manufacturing of CWFs. Mineral composition have an important effect on the promotion or hindrance of biofilm formation, therefore impacting the performance of CWF.

ACKNOWLEDGMENTS

Today is the day: writing this note of thanks is the finishing touch on my thesis, after an intensive period of ten months. This period provides me not only the knowledge in the scientific arena, but also a rapid improvement on a personal level. I would like to reflect on the people who have supported and helped a lot throughout this period, because writing this thesis has had a big impact on me.

I would like to first express my special thanks of gratitude to my thesis advisor Vinka Oyanedel-Craver of the Department of Civil and Environmental Engineering at University of Rhode Island, who gave me the golden opportunity to do this wonderful research on the topic development of ceramic water filter clays selection criteria. Whenever I ran into a trouble spot or had a question about my research or writing, the door to Prof. Craver office was always open. I appreciate that she can arrange a meeting regularly with me once even twice per week to help me and guide me in scientific study. When I made mistakes, and didn't know how to handle it, she can always steer me in the right direction whenever she thought I needed it. Moreover, she is a prototype to teach me how to be a real scientist with persistence, courage, hardworking character, and curiosity.

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PREFACE

This thesis has been used as Manuscript Format and will to be submitted to the Journal *Science of the Total Environment*.

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PUBLICATION STATUS

This manuscript is prepared for the submission to the Journal *Science of the Total Environment*.

MANUSCRIPT

Introduction

An estimated 663 million people worldwide do not have access to an improved source of drinking water.[1] Contaminated water is one the major causes of illness and death globally.[2] Diarrheal diseases, due to consumption of unsafe water, cause an estimated 1.87 million deaths per year specially among children.[2] Additionally, in many developing countries, millions of women spend several hours a day collecting water from distant and often polluted sources.[1] Providing access to safe water means not only finding effective water purification techniques, but also to do it in an affordable manner. Ceramic water filters (CWFs) are a feasible water treatment alternative for developing communities.[3] CWFs have proved to improve the microbiological quality of drinking water and reduce the burden of diarrheal diseases at the household level.[4] Moreover, CWFs are an easy-to-use and durable product.[5]

CWFs are made from locally sourced clayey materials, mixed with water, as well as a burnout material such as sawdust, corn husk, or rice husk. These clayey materials are first brought to a ceramic water filter factory, then mixed with a burnout material (such as sawdust and rice husk) and water. Burnout material is typically added at 15-20% by weight. The clayey-sawdust-water mix is pressed up to 1000 psi using a hydraulic press to give the desired shape to the filter. Finally, the molded filter is fired in a kiln at a temperature over 1000°C for 12 hours. During the firing process, the burnout material volatilizes and produces a porous ceramic material, which is ideal for the filtration of bacteria such as *Vibrio cholera*, *Shigella*, and other water-borne

pathogens.[6,7] Several reports have shown that CWFs have been successfully deployed in developing communities, reducing the occurrence of gastrointestinal diseases.[8,9] CWFs can be impregnated with antimicrobial compounds to prevent biofilm formation, which can reduce the useful operational time of the device.[6]

Previous studies have demonstrated that CWFs with antimicrobial agents such as silver nanoparticles, or a polymer based quaternary amine functionalized silsesquioxane (poly(trihydroxysilyl) propyldimethyloctadecyl ammonium chloride (TPA) have largely improved microbiological removal rate, thus increasing the water quality, compared with ceramic water filters without antimicrobial agents.[10] Yet, no studies have focused solely on the impact of the clayey materials characteristics, which might also influence the microbiological removal performances. Previous studies have shown differences in term of microbiological removal among filters manufactured in various geographical locations.[11] Clayey materials, as the main raw materials to manufacture the CWFs, are obtained from local deposit to reduce costs.[10] However, it is expected that the physical, chemical, and mineralogical properties of clayey materials vary among geographical locations, and therefore the quality of CWFs could vary as well.[12-14] Mineral composition is known to impact biofilm formation. Clayey materials containing the feldspar mineral albite ($NaAlSi_3O_8$), with exhibit charged surfaces that may interact with biofilm development.[15] Kaolinite ($Al_2Si_2O_5(OH)_4$) and dickite ($Al_2Si_2O_5(OH)_4$) promoted biofilm formation. Montmorillonite ($(Na,Ca)_{0.33}(Al,Mg)_2(Si_4O_{10})(OH)_2 \cdot nH_2O$) promoted biofilm formation to a lesser extent than kaolinite. Mineral composition is thus a variable to consider when assessing the performance and life span of CWFs. In other words,

mineralogical composition has been reported as a factor that can affect the performance of CWFs,[11,16] however, to our knowledge, no study has systematically assessed this claim.

The main goal of this study is to elucidate the impact of mineral composition on the microbiological removal on CWFs using a well-established geosciences, environmental and geotechnical engineering methodologies with the purpose of establishing a standardization process for clayey material selection criteria for ceramic filter factories around the world.

Material and Methods

12 clayey samples were provided by the Potter without Borders organization (PWB) and one sample was obtained from the Ixtatan Foundation, Guatemala. The information for all 13 samples are listed as below in Table 1:

Table 1 *Sources of Clayey Minerals*

Name	Source Country, City	Coordinates	Source Name
Indonesia	Indonesia	None	PWB
Tanzania	Tanzania	None	PWB
Nicaragua	Nicaragua	None	PWB
Mozambique	Nampula, Mozambique	None	PWB
Guayaquil	Guyaquil factory site	2° 7'7.48"S, 79°58'59.41"W	PWB
Biyo Mire Black	Hargeisa, Somalia	9°31'49.80"N, 44° 6'52.16"E	PWB
Biyo Mire Red	Hargeisa, Somalia	9°31'36.77"N, 44° 7'1.60"E	PWB
Guinea Bissau Black	Safim, Guinea Bissau	11°58'40.31"N, 15°37'50.13"W	PWB
Guinea Bissau Red	Safim, Guinea Bissau	11°58'40.31"N, 15°37'50.13"W	PWB
Guinea Bissau Factory	Safim, Guinea Bissau	11°58'40.31"N, 15°37'50.13"W	PWB
Nova Scotia #1	Lantz, Canada	44°58'46.15"N, 63°28'28.30"W	PWB

Nova Scotia #2	Bridgetown, Canada	44°50'22.41"N, 65°16'43.72"W	PWB
Guatemala	Guatemala	15°49'55.16"N, 91°28'44.24"W	Ixtatan Foundation

In our study, three clayey samples are chosen to manufacture for ceramic water filters and to study their further characteristics because of their maximal geographical span, available clayey samples amounts, and their different mineral compositions. Two raw clayey samples, provided by Potter without Borders organization, are used to manufacture ceramic water filters locally, in Canada (Bridgetown Elementary School Driveway, Bridgetown, 44°50’22.41’’N, 65°16’43.72’’W), and Guinea-Bissau (Safim, 11°58’40.31’’N, 15°37’50.13’’W). A third sample from Guatemala was obtained from the Ixtatan Foundation and was collected from San Mateo Ixtatan, Huehuetenango, Guatemala (15°49'55.16"N, 91°28'44.24"W).

Clayey material characteristics.

Physical properties. Grain size distribution analysis and liquid and plastic limit testing of the soil were selected to determine physical characteristics of soils, and classify soils. Grain size distribution analysis quantifies particles size category and provides the information necessary for classifying the soil in accordance with the Unified Soil Classification System (USCS, Table 2) using the Standard Test Method for Particle-Size Analysis of Soils (ASTM D422) and Standard Test Method for Amount of Material in Soils Finer Than the No. 200 (75 µm) Sieve (ASTM D1140). The liquid and plastic limit tests provide information regarding the effect of water content on the mechanical properties of soil, the effects of water content on volume change and soil consistency in accordance with Standard Test Methods for Liquid Limit, Plastic Limit, and Plasticity Index of Soils (ASTM D 4318).

Table 2 *Unified Soil Classification System (USCS)*[17]

Coarse-Grained Soils					
% passing No.200	% of C.F. Passing No.4	% passing No.200			USCS Name
<50%	>50%	0-5%	Cu >6 and 1<Cc<3 ?	yes	Well-graded sand
				no	Poorly-graded sand
		5-12%	Dual classification		Poorly-graded sand with silt
					Poorly-graded sand with clay
					Well-graded sand with silt
					Well-graded sand with clay
		12-50%	PI > 0.73(LL-20)% ?	yes	Clayey sand
				no	Silty sand
	<50%	0-5%	Cu >4 and 1<Cc<3 ?	yes	Well-graded gravel
				no	Poorly-graded gravel
		5-12%	Dual classification		Poorly-graded gravel with silt
					Poorly-graded gravel with clay
					Well-graded gravel with silt
					Well-graded gravel with clay
		12-50%	PI > 0.73(LL-20)% ?	yes	Clayey gravel
				no	Silty gravel
PI: Plastic Index, LL: Liquid Limit, The coefficient of uniformity, C_u is a crude shape parameter and is calculated using the following equation: $C_u=D_{60}/D_{10}$, where D_{60} is the grain diameter at 60% passing, and D_{10} is the grain diameter at 10% passing. The *coefficient of curvature*, C_c is a shape parameter and is calculated using the following equation: $C_c=(D_{30})^2/D_{60}*D_{10}$, where D_{60} is the grain diameter at 60% passing, D_{30} is the grain diameter at 30% passing, and D_{10} is the grain diameter at 10% passing.					
Fine-Grained Soils					
% passing No.200	LL>50% ?	PI > 0.73(LL-20)% ?			USCS Name
> 50%	yes	yes			Fat clay
		no			Elastic silt
	no	yes			Lean clay
		no			Lean silt

Mineralogical composition. X-ray diffraction (XRD) has been used to identify qualitatively the type of minerals presented in the clayey material. For this method, the clayey material were finely ground, dried at 60 °C overnight, homogenized, and sieved through a No.100 (149 µm) sieve, and analyzed using TERRA Portable XRD

from Olympus™ equipment and its protocol, and interpreted using peak-matching software (X-powder, xpowder.com), to give a qualitative analysis of the minerals.[18]

Ceramic water filter preparation.

Clayey materials preparation. Clayey samples were sieved through a 0.149 mm mesh separately. After sieving, a mineral suspension with a concentration of 100 g/L was prepared using deionized water and disaggregated using an ultrasonic bath for 10 min.

Ceramic water filter preparation. Disaggregated clays were then manufactured to 4.7 cm diameter, 1.5 cm thickness, fired disks, which correspond to the most common thickness of ceramic water filters.[10,16] Ceramic water filter disks were manufactured by dry mixing 20% of sawdust sieved through No.100 and No. 325 sieves and 80% of raw clayey material. Then water was added to form a paste, in order to be pressed up to 1000 psi using a hydraulic press, to give the desired shape to the filter. The shaped filter was dried for 3 days at room temperature, and then fired in a muffle furnace. Finally, the air dried filter was fired in a kiln up to 900 °C, first at a rate of 150 °C/h from room temperature to 600 °C, and then at a rate of 300 °C/h to 900 °C, holding this final temperature for 3 hours.[16] Manufactured ceramic water filters were crushed into small pieces, then sieved through 0.149 mesh. Each sieved ceramic water filter material was suspended in deionized water at a concentration of 100 g/L and disaggregated using an ultrasonic bath for 10 min to prepare ceramic water filter suspension solution.

Sawdust ash preparation and analysis.

Sawdust was fired according to the same ceramic water filter firing process. The resultant ashes were analyzed by Method 200.7 standard operation procedure provided by EPA by using Thermo Fisher Scientific X Series II ICP-MS to determine various metals content.[19]

Biofilm formation analysis.

Bacteria: *Pseudomonas Fluorescens* Migula (ATCC® 13525™) was selected in this study because it is a model organism commonly used in biofouling study of membranes, therefore known of forming biofilm at the proposed testing conditions.[20] A single colony from a stock culture was inoculated in 500 ml of Erlenmeyer flask containing 100 mL of Lysogeny broth medium (LB medium: Tryptone 10g/L, NaCl 10g/L, and yeast extract 5g/L).[21] Microorganisms in the LB medium grew aerobically on a rotary shaker for 16 hours at 37°C at 110 rpm of agitation and were harvested at mid-exponential growth phase. The cells were pelleted by centrifugation for 15 min at 3000 g and 25°C, and the supernatant removed. The pellets were rinsed with phosphate-buffered saline solution (PBS) by centrifugation for 15 min at 3000 g and 25°C three times.[22] The resulting pellets were resuspended in PBS solution up to 20 mL and bacteria cell concentration was observed using Optical Density at 670 wavelengths (OD670) using a Genesys 10μv Scanning Thermo Scientific® spectrophotometer.

Biofilm formation on mineral surfaces. A modification of a previously proposed method was used to assess biofilm formation on mineral surfaces.[23] First, coverslips (18mm * 18 mm) were treated with a 7:3 (v/v) H_2SO_4: H_2O_2 solution for one hour, then rinsed with deionized water and sonicated for 15 min in an ultrasonic bath. The

washed coverslips were dried at 60°C and stored in a desiccator for later use. For each clayey sample, a suspension with a concentration of 100 g/L was prepared and 0.4 mL of suspension was pipetted onto the coverslip in six different places and then transferred to an oven for 20 min at 120°C. Then coverslip with the bound clayey material was rinsed continuously with deionized water for 20 seconds and then dried at 60°C. The clayey coated coverslip was autoclaved at 121°C for 20 min and placed in sterile polystyrene 6-well plates (Figure 1). Then, 0.25 mL bacterial solution (prepared as described in the previous section) was added on each of the six plates where the clayey material was bound and allow to contact for 10 min in the incubator at 37°C. After that, 4.75 mL LB medium was added to each well to completely cover the coverslip and placed in an incubator for 48 hrs. at 37°C. After the incubation period, the coverslip was removed and rinsed with deionized water three times, then dried by removing the liquid using a paper towel, placed at the edge of the coverslip and placed on clean glass slides for 10 min. This process was performed in triplicate for both for clayey and CWF samples coated coverslip.

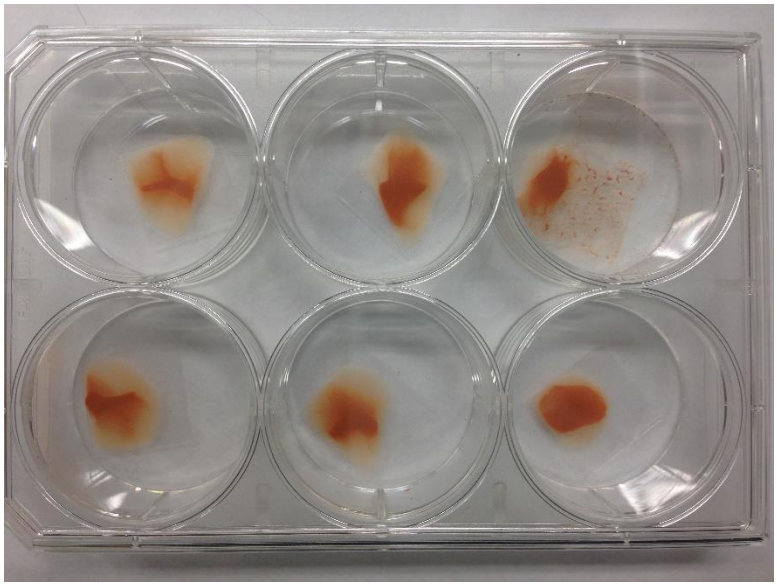

Figure 1 *The clayey material coated coverslips in sterile polystyrene 6-well plates*

Biofilm coverage measurement. After 48 hours of incubation, each sample-coated coverslip was added with 10 µL of a solution with 3:500 (v/v) SYTO™ 9 green fluorescent nucleic acid stain in deionized water. After five minutes' contact with the dye, coverslips were then examined using a Cytoviva® Model V10E microscope under two channels analyzed by QCapture Pro 7™ Software.[24] Channel 1 defined by QCapture Pro 7™ Software is gain 10, offset -400, and gamma 5, which can detect solely *Pseudonoma fluorescens* biofilm, while Channel 2 is set to gain 10, offset 10, and gamma 5 to observe mineral coated attachment percentage on the coverslip. The coverage percentage was calculated through biofilm formation area under Channel 1 divided by clayey material area under Channel 2. In each coverslip, 10 spots were selected and images were captured under the two channels listed previously. ImageJ software Version 1.51h (National Institutes of Health) was used to quantify biofilm coverage on each coverslip. The biofilm coverage was determine for each clayey material or ceramic water filter coated coverslip.

Results

Clayey material characteristics.

Physical properties. According to USCS soil classification: grain size analysis, liquid limit, plastic limit, and plastic index analysis together can determine soil category. The liquid limit, plastic limit, and plastic index of three clayey materials are presented in Table 3. Grain size analysis determined the size of particles. Guatemala samples had 10.78% passing mass through No. 200 sieve, which was less than 50% mass and can be first defined as coarse- grained soil (Figure 2). So do clays from Canada (Figure 3),

and Guinea-Bissau (Figure 4) with 4.92% passing mass and 3.37%, respectively. In coarse-grained soil category, because all three clayey materials passing No.4 sieve percentage are 100% and greater than 50%, they all belonged to a sand category. Moreover, Guatemala sample did not fulfill both uniformity coefficient (C_u) >6 and 1<coefficient of curvature (C_c) <3 and Plastic Index > 0.73(Liquid Limit-20) % criteria and therefore belonged to poorly-graded sand with silt, while Canada sample and Guinea-Bissau sample did not fulfill C_u >6 and 1<C_c<3 criterion and can be both defined as poorly-graded sand. Other nine results of clayey samples are shown in supplementary documents (see Appendices).

Table 3 *Liquid limit, plastic limit, and plastic index of materials from Guatemala, Canada, and Guinea-Bissau*

Clayey minerals	**Liquid limit (%)**	**Plastic limit (%)**	**Plastic Index (%)**
Guatemala	33.99	30.79	3.20
Canada	28.91	19.60	9.31
Guinea-Bissau	33.99	23.23	10.76

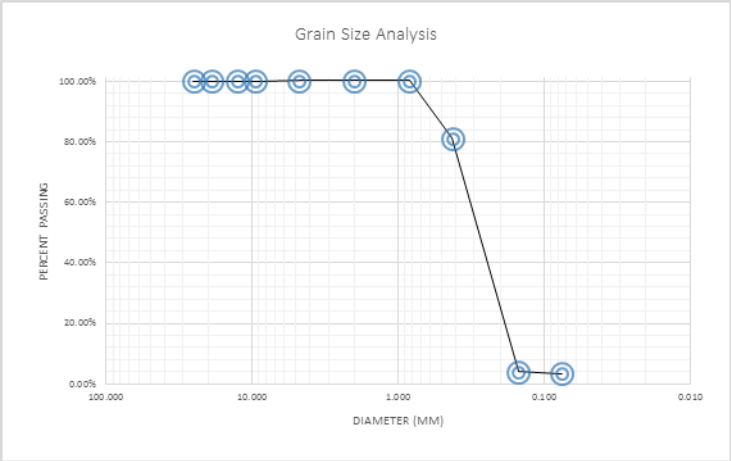

Figure 2 *Grain size analysis of Guatemala material*

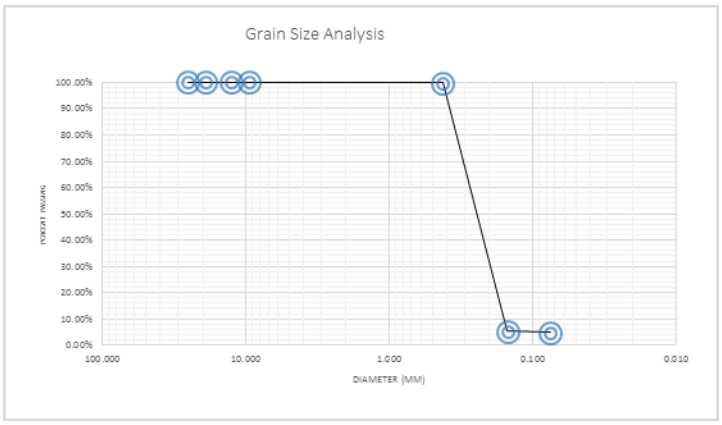

Figure 3 *Grain size analysis of Canada material*

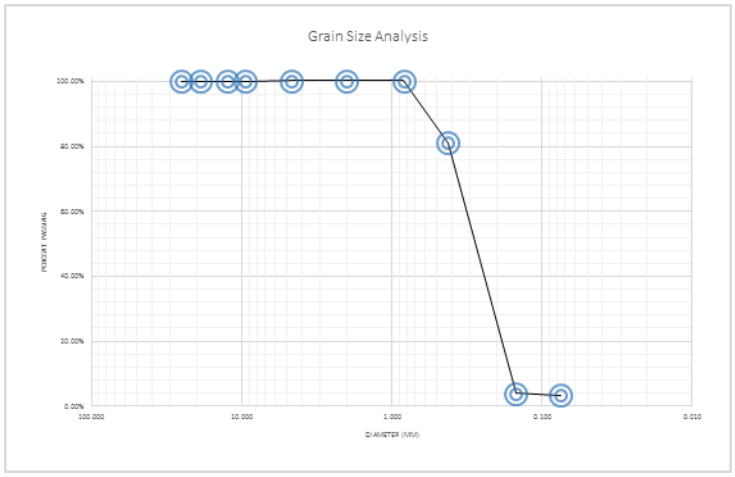

Figure 4 *Grain size analysis of Guinea-Bissau material*

Mineralogical composition. According to X Powder software matching peaks, the clayey material from Guatemala contained four identifiable minerals: montmorillonite, quartz, muscovite, and albite (Figure 5, in which the x axis is presented in degrees 2θ and the y axis is the intensity of the diffracted x-ray beam at the instrument detector). The clayey material from Canada was mainly made of quartz, muscovite, and a 7Å-clay thought to be kaolinite[25] (Figure 6). The clayey material from Guinea-Bissau contained quartz, kaolinite, dickite (a kaolinite group mineral), and montmorillonite (Figure 7). Other nine results of clayey samples are shown in supplementary documents (see Appendices).

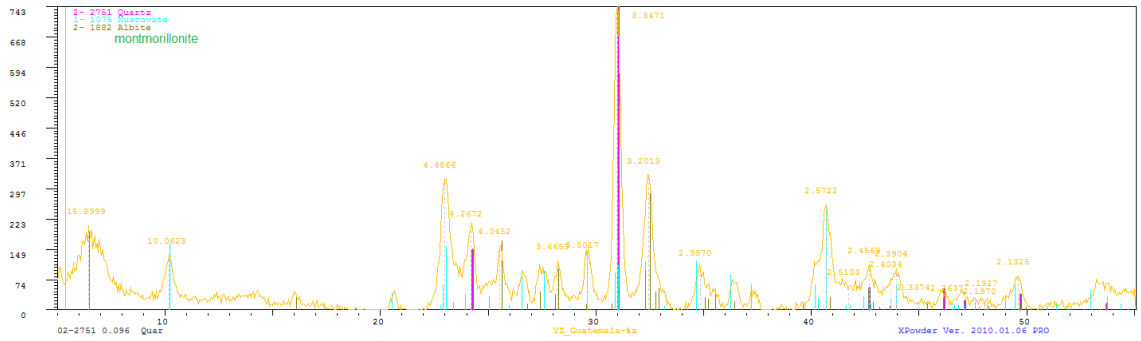

Figure 5 *XRD result of Guatemala clayey material*

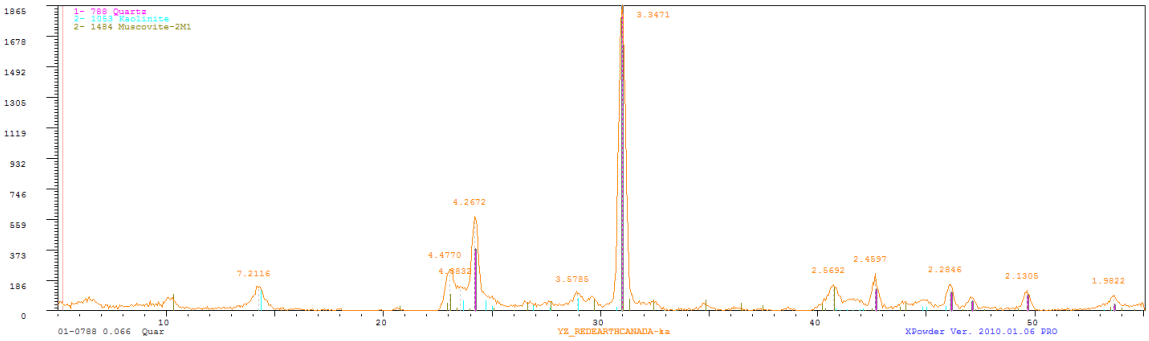

Figure 6 *XRD result of Canada clayey material*

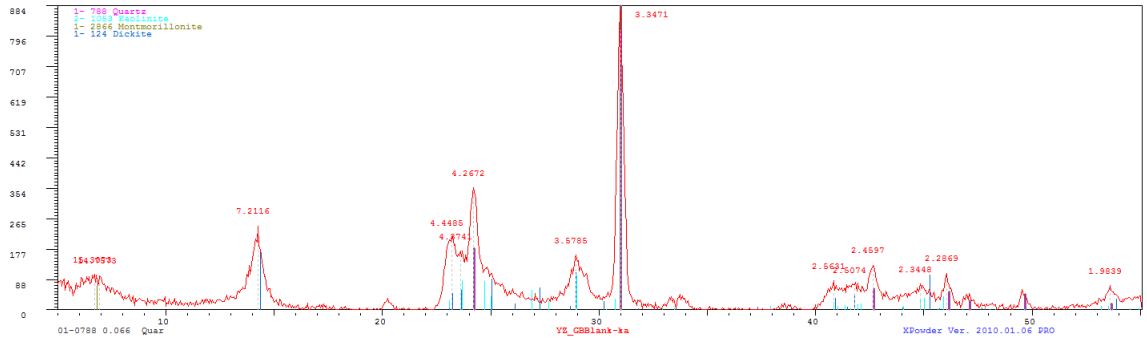

Figure 7 *XRD result of Guinea-Bissau clayey material*

Quartz, muscovite, albite, kaolinite, dickite and montmorillonite are typical minerals in clayey sand.[26] Muscovite, kaolinite, dickite and montmorillonite are common clay minerals, and quartz and albite are primary rock-forming minerals present within the analyzed sample.[27] Kaolinite ($Al_2Si_2O_5(OH)_4$) is one of the kaolinite group mineral and consists of sheets of SiO_2 tetrahedra bonded to sheets of Al_2O_3 octahedra. The structural sheets consist of 1 tetrahedron associated with 1 octahedron, and are held together by H-bonds. Dickite ($Al_2Si_2O_5(OH)_4$) belongs also to the kaolinite group and

consists of SiO_2 tetrahedral layers bonded to Al_2O_3 octahedral layers, as in kaolinite, but differs from kaolinite in terms of stacking arrangement. The most ubiquitous micaceous mineral in soils is muscovite ($KAl_3Si_3O_{10}(OH)_{1.8}F_{0.2}$), which consists structurally of one layer of alumina octahedra sandwiched between two layers of silica tetrahedra and is dioctahedral. The layers are bonded together by K^+ ions in 12-fold coordination with oxygens of the silica tetrahedra. Montmorillonite ($Na_{0.2}Ca_{0.1}Al_2Si_4O_{10}(OH)_2(H_2O)_{10}$) belongs to montmorillonite group and has interlayer water instead of K^+ ions between layers.[28]

Ceramic water filter characteristics.

Mineralogical composition. CWF are the fired equivalents of the original clayey material described above, and have been fired at temperatures up to 900ºC for longer than 3 hours in all cases. Such a heat treatment is expected to (a) collapse swelling clays in the montmorillonite (or smectite) group, collapsing the ~14Å peak in the diffractogram to a 10Å peak, and (b) destroy the 7Å peak, thus confirming detection of kaolinite group minerals.

CWF produced from Guatemalan clayey material contained three identifiable minerals: quartz, 10Å clay (likely muscovite), and albite (Figure 8), with no montmorillonite detected after firing. The Canadian CWF was mainly made of quartz, 10Å clay (likely muscovite), and hematite (Figure 9); note that the kaolinite group signal disappeared after firing. The CWF from Guinea-Bissau contained quartz and hematite (Figure 10), with no montmorillonite detected after firing. Hematite (Fe_2O_3) is the oxidized Fe-containing mineral in this assemblage.

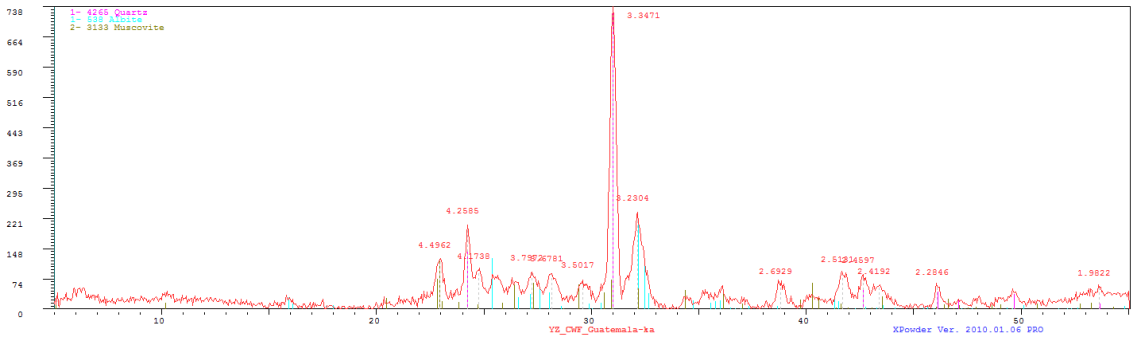

Figure 8 *XRD result of Guatemala CWF*

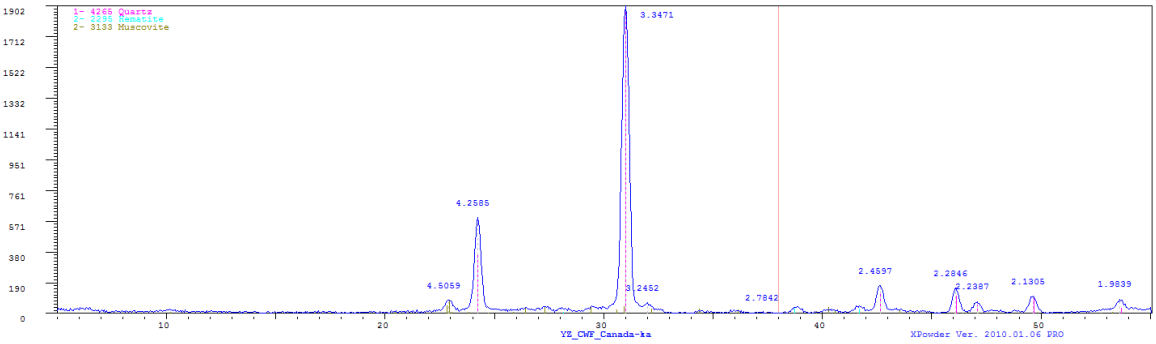

Figure 9 *XRD result of Canada CWF*

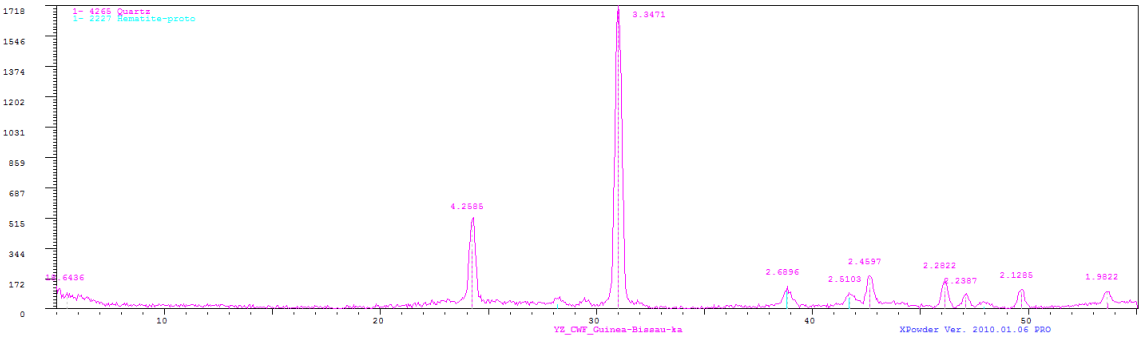

Figure 10 *XRD result of Guinea-Bissau CWF*

Sawdust ashes analysis

11different metals including Na, Mg, K, Cr, Mn, Fe, Co, Ni, Cu, Zn, As have been tested by the ICP-MS process. Ar, Se, Kr, Cd, Pb metals were not detected in

remained sawdust ashes after the firing process. The detected metals' concentrations are listed in Table 4.

Table 4 *Metal content in raw sawdust*

Metals	ppm
Na	226.01
Mg	1.96
K	53.47
Cr	0.34
Mn	0.09
Fe	2.71
Co	0.01
Ni	0.10
Cu	0.12
Zn	0.31
As	0.07

Biofilm formation analysis.

Control groups. Clayey material and ceramic water filter samples presented no fluorescence in Channel 1 when stained with SYTO™ 9 green fluorescent dye without the presence of bacteria (Figure 11). Bacteria without clayey material or ceramic water filter samples presented the expected fluorescence when stained with the same dye (Figure 12).

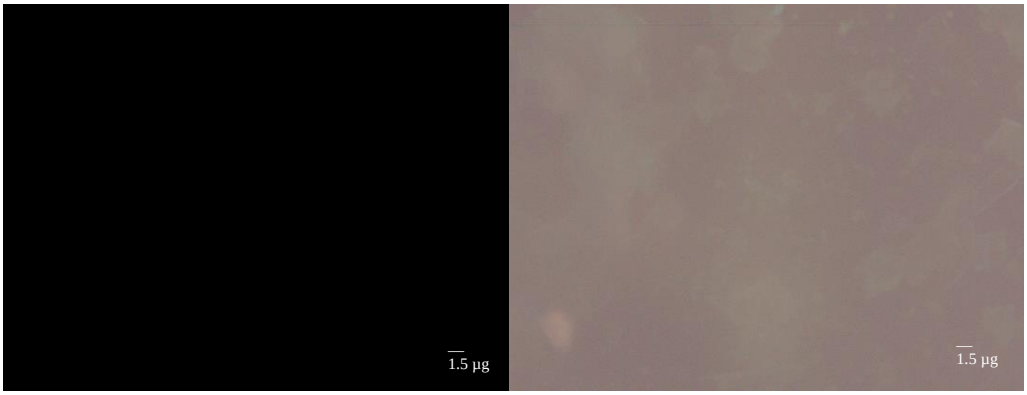

Figure 11 *soil under channel one (a), soil under channel two (b)*

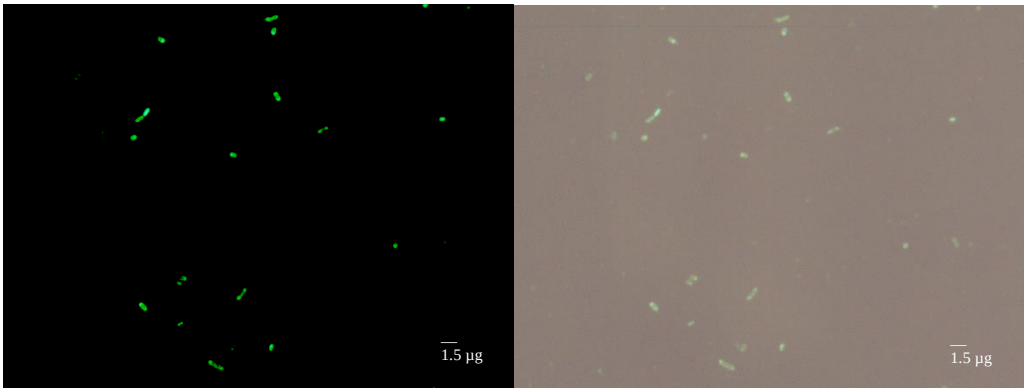

Figure 12 *Bacteria under channel one (a), bacteria under channel two (b)*

Biofilm formation on clayey material. Biofilm formation displayed green fluorescence when using SYTO™ 9 green fluorescent dye. In Figure 13a, 14a, 15a , the green areas show biofilm formation detected under Channel 1, where identifies solely bacterial biofilm formation, while Figures 13b, 14b, 15b show clayey coated surface detected under Channel 2, where present outline of materials. Biofilm formation on Guatemala clayey material was more intensive and concentrated than other two clayey materials. The average biofilm coverages for Guatemala, Canada, and Guinea-Bissau soils after calculating from 10 spots of triplicate samples were 20.02% ± 6.65%, 19.27% ± 4.59%, and 9.88% ± 5.01%, respectively (Figure 19).

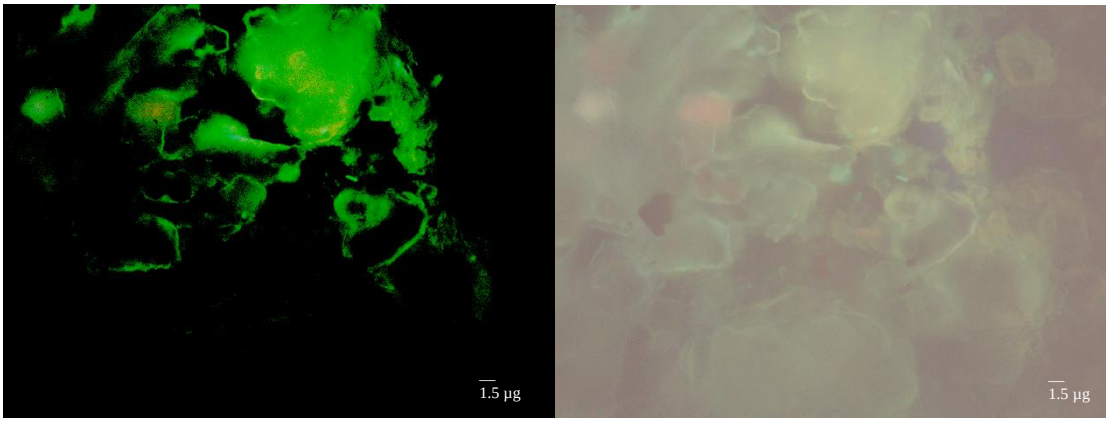

Figure 13 *Guatemala clayey material under channel one (a), Guatemala clayey material under channel two (b)*

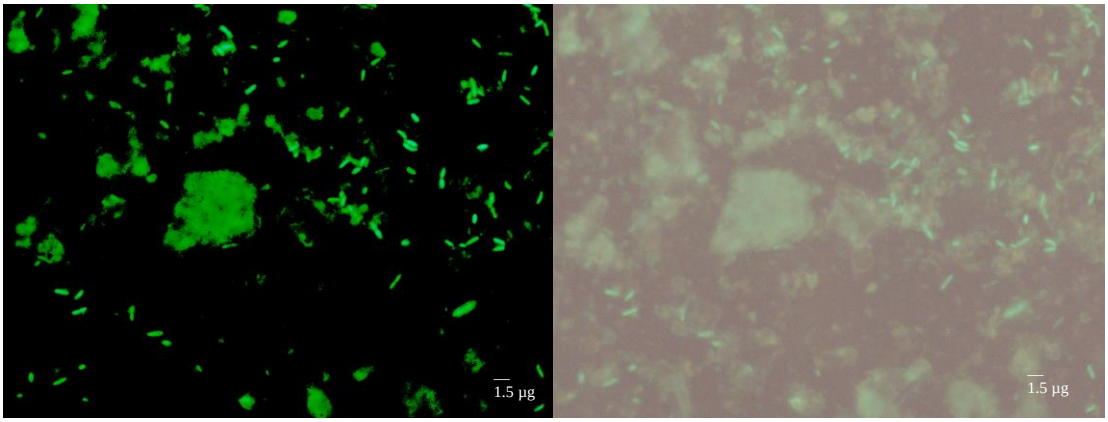

Figure 14 *Canada clayey material under channel one (a), Canada clayey material under channel two (b)*

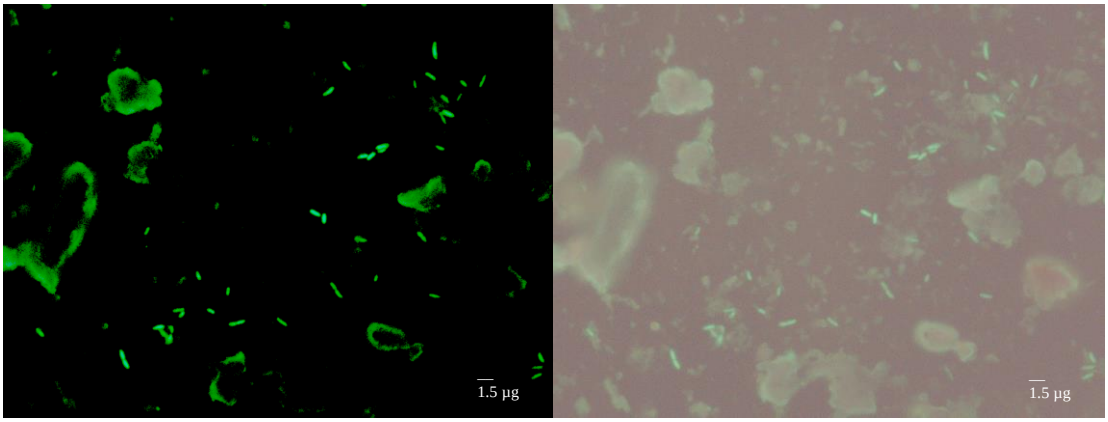

Figure 15 *Guinea-Bissau clayey material under channel one (a), Guinea-Bissau clayey material under channel two (b)*

Biofilm formation on ceramic water filters surfaces. In Figure 16a, 17a, 18a, the green areas show biofilm measured by Channel 1, while Figure 16b, 17b, 18b show ceramic water filter detected by Channel 2. The average biofilm coverage for Guatemala, Canada, and Guinea-Bissau CWFs after calculating from 10 spots of triplicate samples were 13.08% ± 4.12%, 10.39% ± 5.05%, and 8.50% ± 5.35%, respectively (Figure 20).

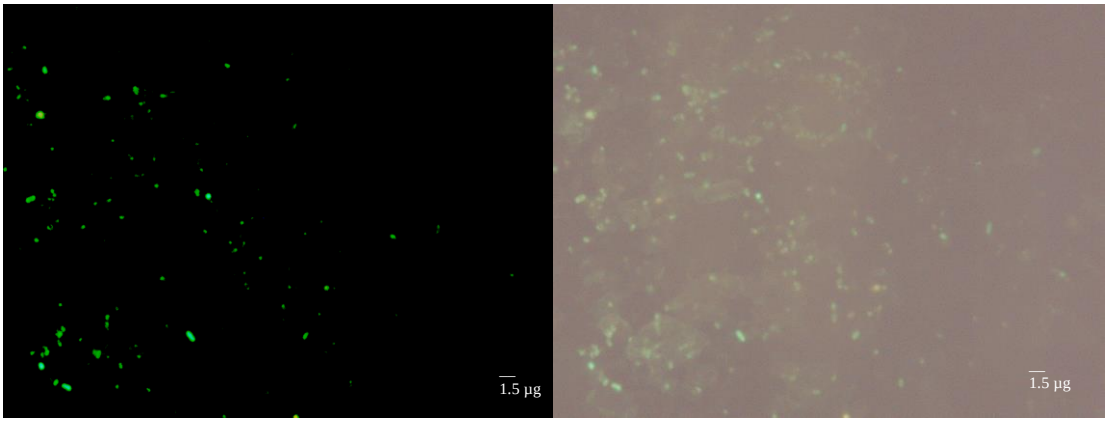

Figure 16 *Guatemala CWF under channel one (a), Guatemala CWF under channel two (b)*

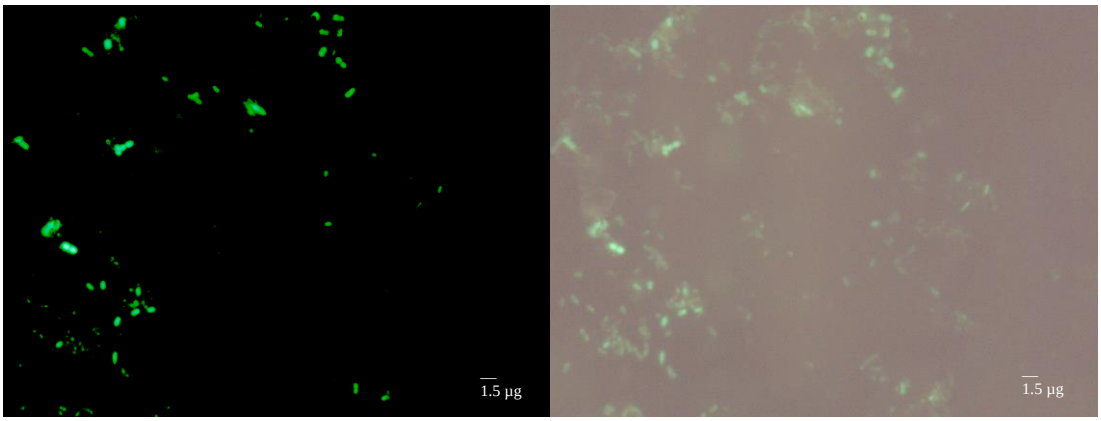

Figure 17 *Canada CWF under channel one (a), Canada CWF under channel two (b)*

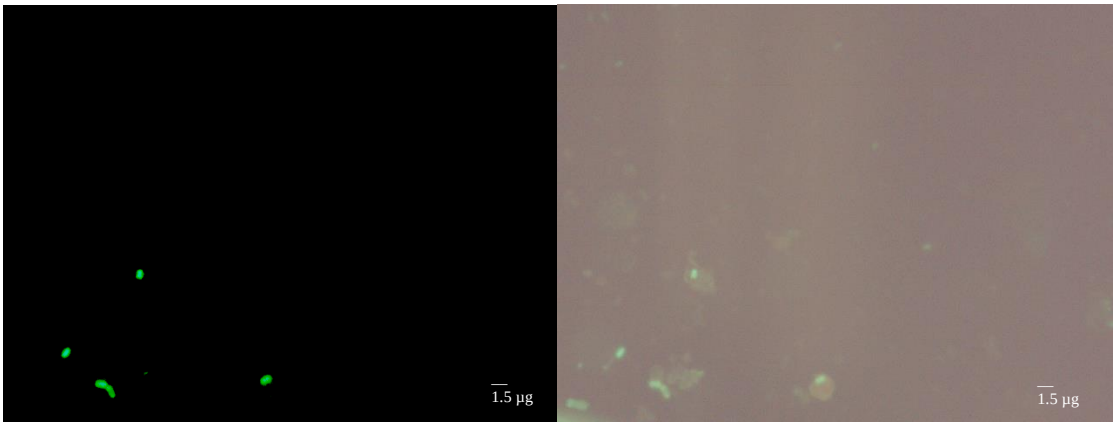

Figure 18 *Guinea-Bissau CWF under channel one (a), Guinea-Bissau CWF under channel two (b)*

Student t-test was used to determine statistical differences among each clayey material and each CWFs using a significant level of 5%. For each clayey and ceramic water filters sample among its own triplicate samples, no significant differences were shown by using paired samples for means, which indicated there were no sampling errors

among triplicate analysis. However, when comparing sample to sample data for Guatemala clay material show no significant difference from Canada, but showed a significant difference from Guinea-Bissau when comparing the t statistic value and t critical value. Data for Canada clayey material showed a significant difference from Guinea-Bissau comparing t statistic value 3.42, which was greater than t critical value 2.09 (Figure 19). Three ceramic water filters' data showed significant differences among each other (Figure 20).

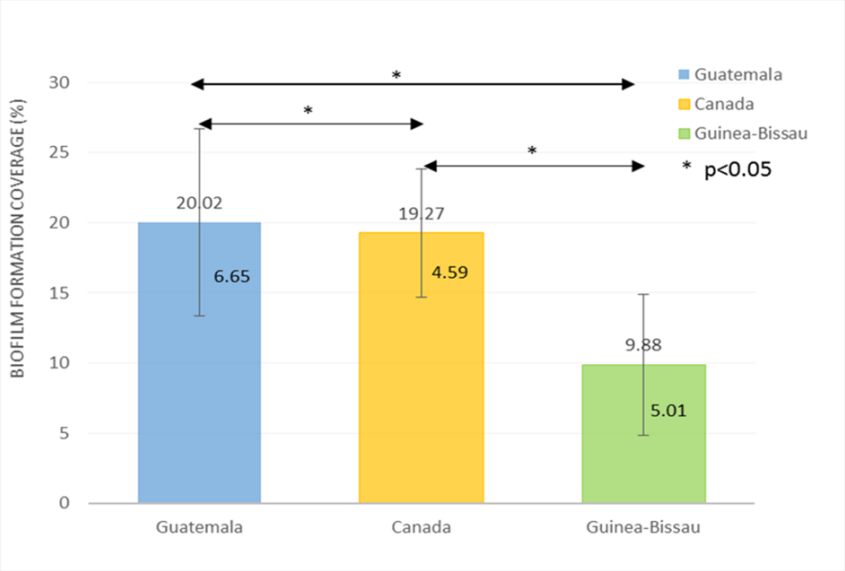

Figure 19 *Biofilm formation on clayey materials*

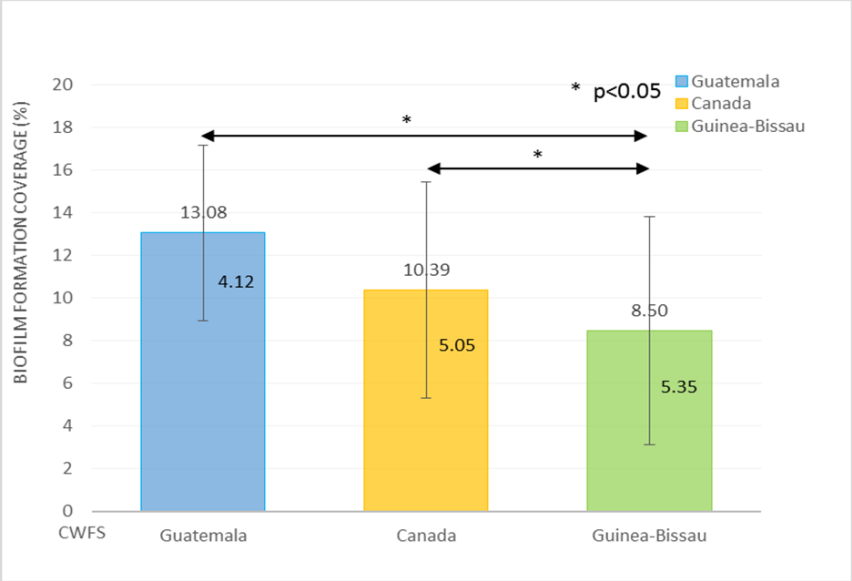

Figure 20 *Biofilm formation on CWFs*

Discussion

Guatemalan, Canadian, and Guinea-Bissau clayey material had low plastic soil (less than 7%) and medium plastic soil (7% to 17%) indexes.[29] Guatemalan clayey material would be ideal for brickmaking because of its coarser grain, and non-warping, cracking, or high rates of shrinkage characteristics, while Canadian and Guinea-Bissau clayey materials are appropriate for making CWFs because of intermediate plastic indexes. There is no brittle fracturing or breaking in firing process for ceramic water filters, indicating that the sand content in soils is suitable.[30]

Regarding biofilm formation, our study was in partial agreement with previous studies.[28-32] Studies using pure minerals and similar biofilm formation procedures showed that pure montmorillonite can reduce bacterial biofilm onto clay-sized particles, while kaolinite promoted bacterial attachment when *E. coli*, *P. putida*, *A. tumefacient*, and *B. subtilis* were used.[23] Weaker *P. putida* attachment on montmorillonite, when compared to kaolinite, can be explained by different layer

charges on these two clay mineral types, and/or the role of dangling OH- groups on kaolinite layer edges. Although smectite group including montmorillonite has a greater layer charge per formula unit than kaolinite[31], it is difficult to find the aggregates of bacterial cells with montmorillonite and it appears that montmorillonite was weakly aggregated with bacterial cells, according to previous study.[23] However, *P. putida* adhered predominantly to the edge surfaces of the kaolinite rather than to the basal surfaces, which can be explained by dangling OH- groups on kaolinite layer edges.[23,32] *P. putida* and *P. fluorescens* belong to the same group and both organisms have negative surface charges, therefore, similar results were expected.[33] Biofilm formation on Canadian clayey material containing kaolinite was higher than on Guinea-Bissau clayey material which contained montmorillonite. Yet, Guinea-Bissau soil has both kaolinite and montmorillonite mineral but the lowest biofilm formation compared with the other clayey materials. The effect of kaolinite (promote attachment[23,32,34]) was probably offset by montmorillonite (decrease attachment[23,32,34]). Another plausible explanation is that the properties of kaolinite and montmorillonite in soils might also determine which mineral controls biofilm formation. If montmorillonite dominates kaolinite group minerals in the Guinea-Bissau sample, and if kaolinite dominates the Canadian sample, it is reasonable that the Canadian clayey materials has more bacteria biofilm formation, as they are facilitated by the kaolinite component. In Guatemalan clayey material, albite contained Na^+ and Na^+ on the albite surface can be exchanged with H^+ under pH 6.5 condition, thus extra Na^+ stays in the medium.[34] Na^+ and Mg^{2+} have been reported to increase bacterial attachment in previous studies.[35] However, biofilm formation on Guatemalan clayey material containing montmorillonite was

higher than on Canadian clayey material without montmorillonite, which indicates montmorillonite does not play a decisive role in both samples. By having a high aluminum content and a platey structure, muscovite promotes bacterial adhesion, thus promoting biofilm formation in Guatemalan and Canadian clayey materials.[36]

After firing process, kaolinite and dickite transform to hematite and excess silica, and montmorillonite disappeared according to previous studies, while quartz, albite, and (putative) muscovite survived because of their high melting points.[37-40] Hematite induced biofilm formation[41] which shows in Canadian CWF, compared to clayey material from Canada, and is in agreement with previous studies.[41-42] However, the emergence of hematite (a mineral taken to increase attachment) in the Guinea-Bissau CWF is accompanied by the loss of montmorillonite (a mineral taken to decrease attachment), thus controls on biofilm coverage are not clear. In Guatemalan CWF, montmorillonite disappeared after firing process, while biofilm formation reduced, which cannot be explained by changes of mineralogical compositions. Our data also suggest that the presence of muscovite in the CWF material causes significant differences in biofilm coverage, as shown by pairwise analysis of (1) Guatemalan (+ muscovite) and Guinea-Bissau (- muscovite) CWFs and (2) Canadian (+muscovite, +hematite) and Guinea-Bissau (- muscovite, + hematite) CWFs.

Metal content in ash may also provide an explanation for the differences in biofilm formation coverage between clayey minerals and ceramic water filters. Cr, Ni, Cu, Zn metals have shown, in previous studies, to reduce biofilm formation, which was consistent with the lower biofilm formation coverage of ceramic water filters in all three samples compared with the respective clayey samples.[43,44] However, previous

studies have shown that Na, Mg, and Fe can accelerate biofilm formation which contradicts our results.[35,41] In addition, K, Mn, Co, and As have not been studied yet about the influences of biofilm formation in literature.

Considering both metal loading due to incorporation of an ash component and the role of hematite in the CWF, some difference in biofilm formation can be addressed. In Guatemalan samples, the lower biofilm formation on CWF when compared to original clayey material can be explained by both heavy metal enrichment and loss of montmorillonite in the fired sample. The effect of heavy metal enrichment (decrease attachment[42]) was probably more dominant than the loss of montmorillonite effect, causing the lower biofilm formation on CWF. In Canadian samples, both heavy metal enrichment and co-occurrence of a mica family mineral (i.e., muscovite or similar) and hematite in CWF can change biofilm formation, compared to original clayey material. Small differences of total area of biofilm formation between clayey material and CWF of Guinea-Bissau cannot be explained by heavy metal enrichment and loss of the clay minerals kaolinite and montmorillonite; the initial, slightly lower biofilm coverage on clayey material when compared to the related CWF must be due to another variable.

One of the limitations of this study lies in that all samples are natural soils and X-Powder database cannot identify and match all peaks corresponding to minerals in soils, because some minerals are of low crystallinity. XRD also does not detect minerals that are volumetrically small. Minerals not identified by X-Powder database might also impact on biofilm formation. Biofilm formation has only been tested on coverslips by using a small amount of clay suspension, and coverslips are not fully representative of ceramic water filters in the field. Future studies could emphasize

testing the biofilm formation in whole ceramic water filter disks using a continuous bacterial solution feeding. Moreover, the quantity of each mineral in soils cannot be determined based on qualitative XRD analysis. Rietveld Quantitative Analysis can be adopted by later studies in order to quantify each mineral in soils.[45]

Additionally, high sand content in Guatemalan clayey material might cause filters to become brittle and break during the firing process, decreasing overall utility of this clay resource. Adding bentonite (a montmorillonite group clay mineral) might improve plasticity and avoid brittle breakage, making this Guatemalan clay more appropriate for potters. Canadian and Guinea-Bissau-sourced clayey materials are more promising for ceramic water filters: both had low biofilm coverage.

Conclusion

By using well-established geosciences, environmental and geotechnical engineering methodologies, our study is the first to assess systematically how mineral compositions impact the microbiological removal on clayey materials and CWFs mineral composition have an impact on the performance and life span of CWFs. Compared to previous studies, our study showed similar trends when *P. fluorescens* were used on diverse materials; biofilm formation on Canadian clayey material containing kaolinite was higher than on Guinea-Bissau clayey material, which contained montmorillonite. Moreover, in Guatemalan clayey material, albite contained Na^+, which can be exchanged with H^+ in the culture medium to increase bacterial attachment on the positively charged mineral surface. Muscovite has a high bacterial adhesion, which promotes biofilm formation in Guatemalan and Canadian clayey materials. Montmorillonite decreases biofilm formation in Guinea-Bissau clayey

material, but does not play a decisive role in Guatemalan clayey material. Heavy metals in sawdust ashes have the potential to lower biofilm formation on CWFs in general, when compared to initial materials—this is supported at least for Guatemalan and Canadian samples. Our data suggest that the presence of muscovite in the CWF material causes significant differences in biofilm coverage, as shown by pairwise analysis of (1) Guatemalan (+ muscovite) and Guinea-Bissau (- muscovite) CWFs and (2) Canadian (+muscovite, +hematite) and Guinea-Bissau (- muscovite, + hematite) CWFs. However, the small observed differences of biofilm formation between clayey material and CWF of Guinea-Bissau cannot be fully explained by heavy metal loading through firing with ash incorporation or montmorillonite loss coupled to hematite ingrowth, due to initially lower biofilm coverage on related clayey material. In summary, our study has laid groundwork for a standardization process for clayey material selection criteria for ceramic filter factories around the world.

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APPENDICES

Supplement

Clayey material characteristics.

Physical properties. According to USCS soil classification: grain size analysis, liquid limit, plastic limit, and plastic index analysis can determine soil category together. The liquid limit, plastic limit and plastic index of other nine clayey materials are represented in Table 5. The grain size anlysis results are presented in Table 6. Each grain size analysis is presented below as well in Figure 21-30.

Table 5*: Classification, liquid limit, plastic limit and plastic index of other nine clayey samples*

Name	**Classification**	**Liquid limit (%)**	**Plastic limit (%)**	**Plastic Index (%)**
Indonesia	Silt sand	68.85	20.92	47.93
Tanzania	Silt sand	42.98	21.74	21.23
Nicaragua	Silt sand	32.69	0	32.69
Mozambique	poorly-graded sand with silt	42.72	20.17	22.54
Guayaquil	poorly-graded sand	51.07	34.94	33.68
Biyo Mire Black	poorly-graded sand	27.82	19.53	8.29
Biyo Mire Red	poorly-graded sand	49.47	30.49	18.98
Guinea Bissau Black	poorly-graded sand with silt	32.87	20.92	11.95
Guinea Bissau Red	silt sand	29.01	21.30	7.71
Nova Scotia #1	poorly-graded gravel	44.60	24.47	20.13

Table 6*: Grain size analysis results*

Name	**% passing No.200**	**% of C.F. passing No.4**	**C_u >6 and 1<C_c<3 ?**	**PI > 0.73(LL-20)% ?**
Indonesia	32.34	100.00	-	no
Tanzania	23.32	100.00	-	no
Nicaragua	25.37	100.00	no	no
Mozambique	9.80	100.00	no	no
Guayaquil	2.51	100.00	no	-
Biyo Mire Black	3.50	100.00	no	-
Biyo Mire Red	3.07	100.00	no	-
Guinea Bissau Black	5.05	100.00	no	no
Guinea Bissau Red	15.34	100.00	-	no
Nova Scotia #1	2.00	8.66	no	no

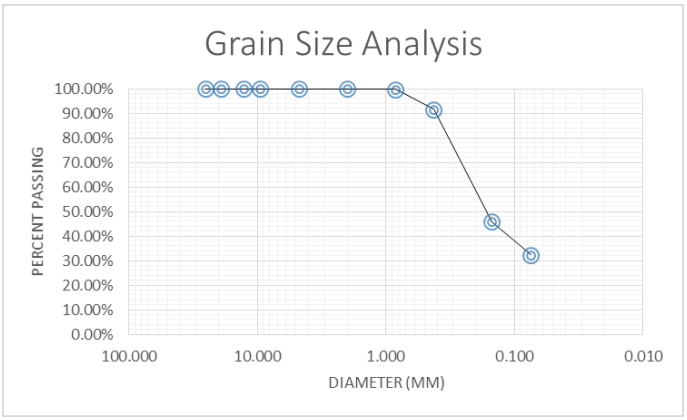

***Figure 21**: Indonesia grain size analysis*

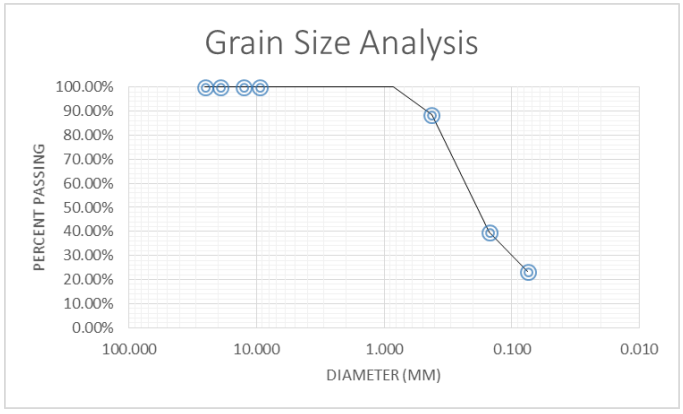

***Figure 22**: Tanzania grain size analysis*

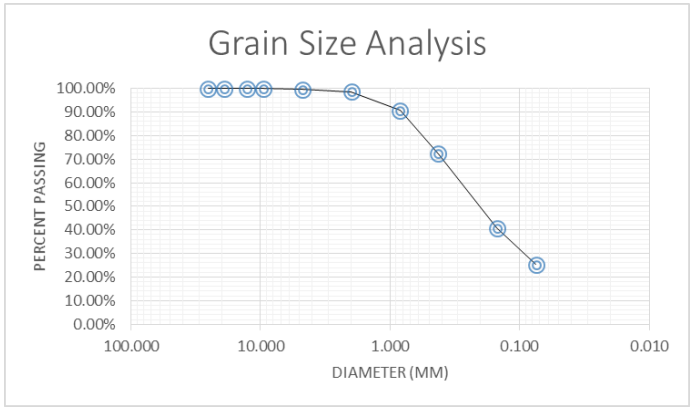

***Figure 23**: Nicaragua grain size analysis*

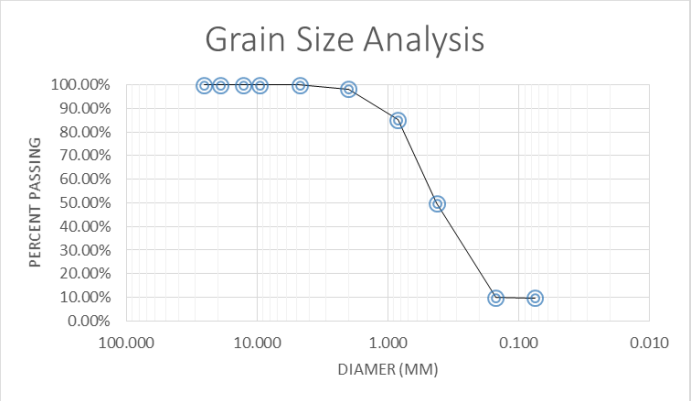

***Figure 24**: Mozambique grain size analysis*

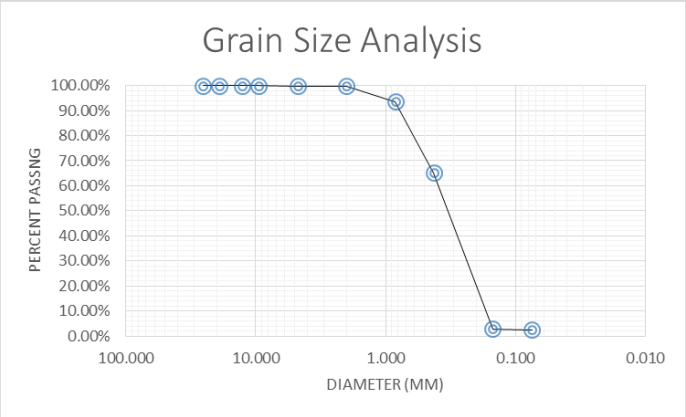

***Figure 25**: Guayaquil grain size analysis*

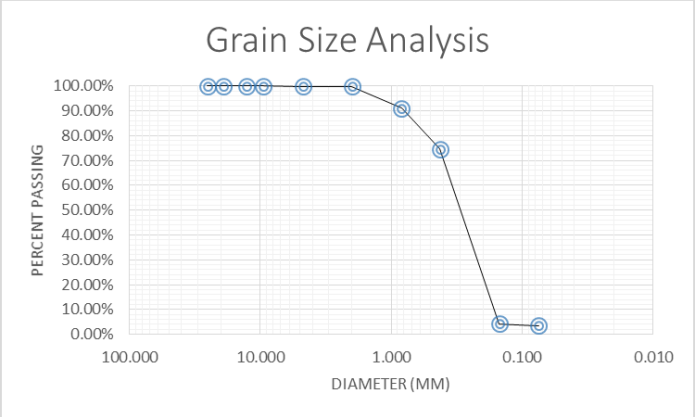

***Figure 26**: Biyo Mire Black grain size analysis*

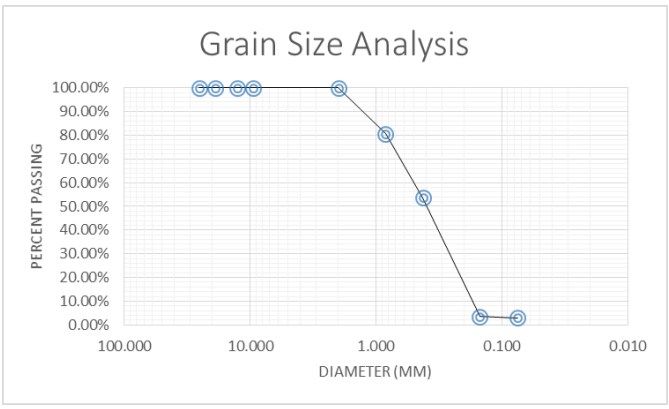

***Figure 27**: Biyo Mire Red grain size analysis*

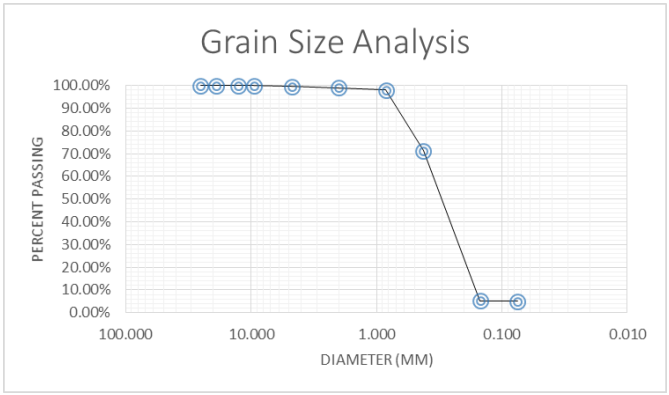

***Figure 28**: Guinea Bissau Black grain size analysis*

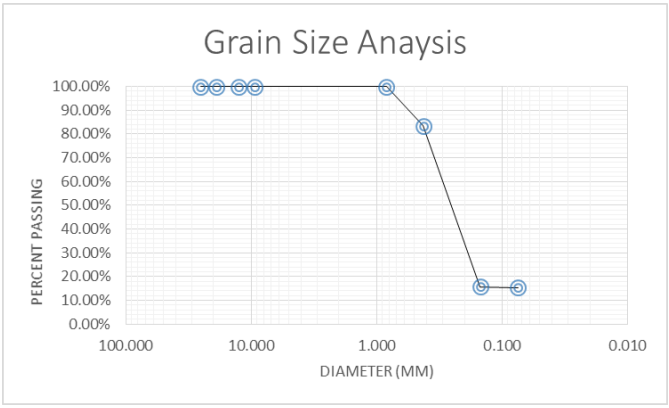

***Figure 29**: Guinea Bissau Red grain size analysis*

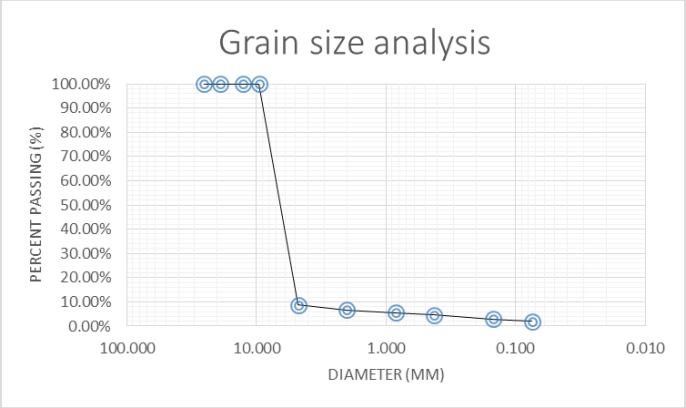

***Figure 30**: Nova Scotia #1 grain size analysis*

Mineralogical composition. In Table 7, each sample containing minerals tested by XRD has been summarized below. The Figure 31-40 show the results of XRD for other nine clayey materials.

***Table 7**: Minerals in each clayey material sample*

Name	**Minerals**
Indonesia	Fluorapophyllite, Chloritoid, Quartz, Montmorillonite, Muscovite
Tanzania	Spangolite, Kaolinite, Quartz, Vermiculite, Chloritoid
Nicaragua	Chloritoid, Quartz, Montmorillonite, Muscovite
Mozambique	Phlogopite, Chloritoid, Spangolite, Biotite, Montmorillonite, Quartz
Guayaquil	Quartz, Montmorillonite, Illite, Albite
Biyo Mire Black	Quartz, Montmorillonite, Illite, Albite, Calcite, Pyroxene
Biyo Mire Red	Quartz, Montmorillonite, Albite, Calcite, Kaolinite, Muscovite, Vermiculite, Palygorskite
Guinea Bissau Black	Quartz, Kaolinite
Guinea Bissau Red	Quartz, Kaolinite
Nova Scotia #1	Quartz, Montmorillonite, Kaolinite, Muscovite

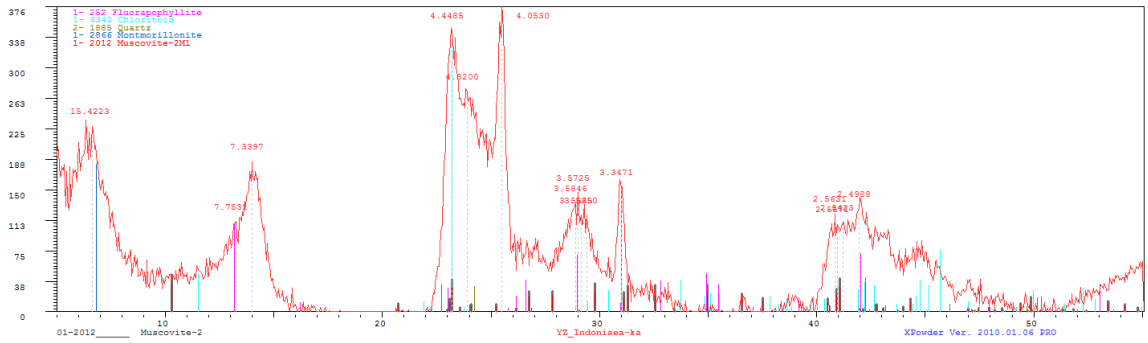

***Figure 31**: XRD result of Indonesia material*

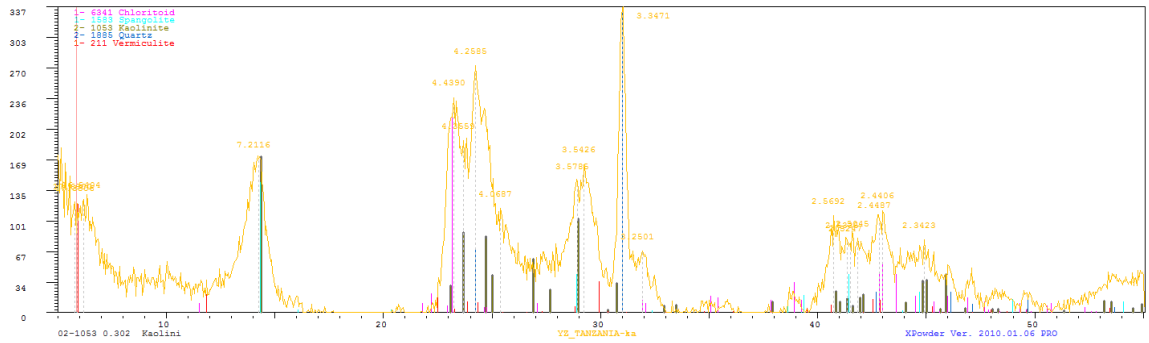

***Figure 32**: XRD result of Tanzania material*

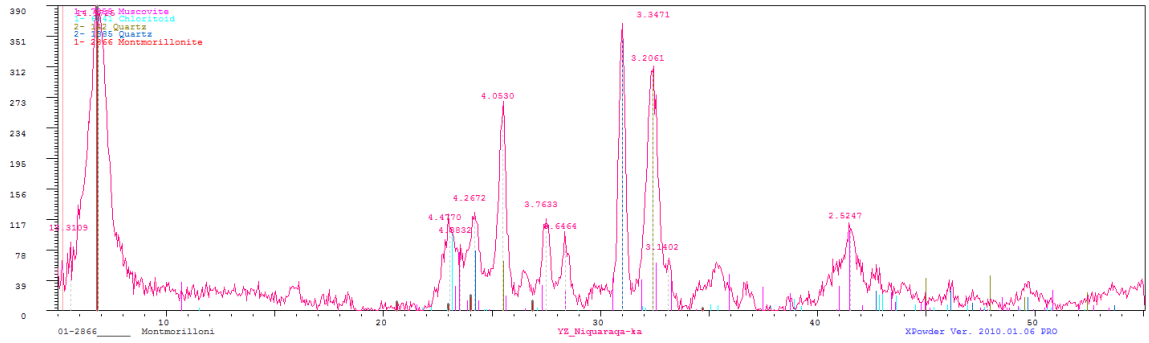

***Figure 33**: XRD result of Nicaragua material*

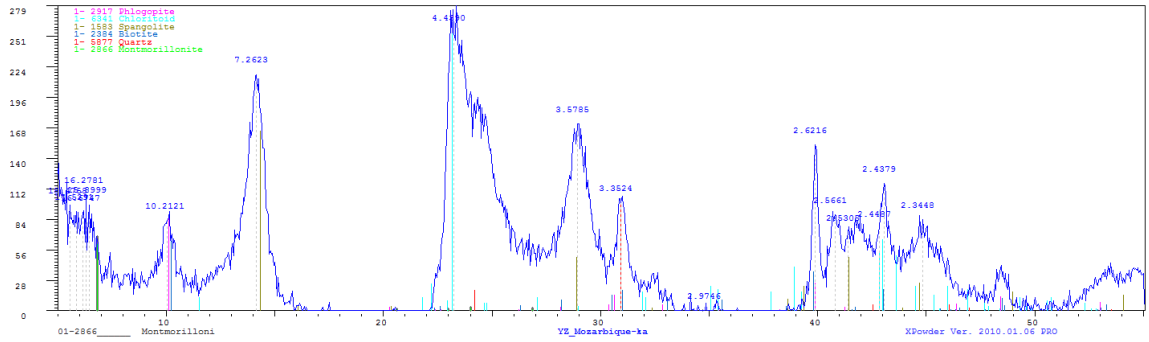

***Figure 34**: XRD result of Mozambique material*

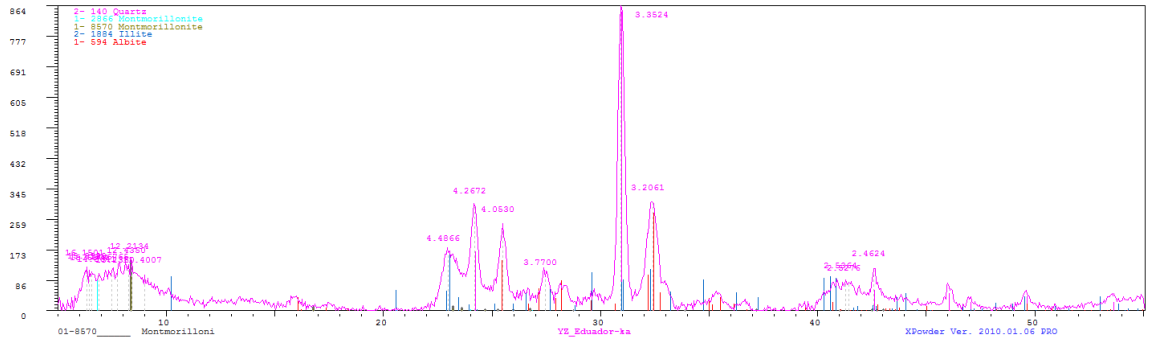

***Figure 35**: XRD result of Guayaquil material*

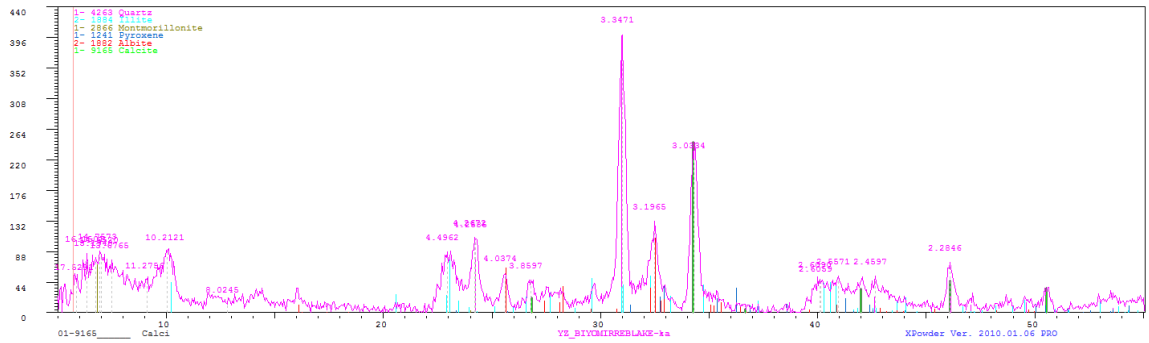

***Figure 36**: XRD result of Biyo Mire Black material*

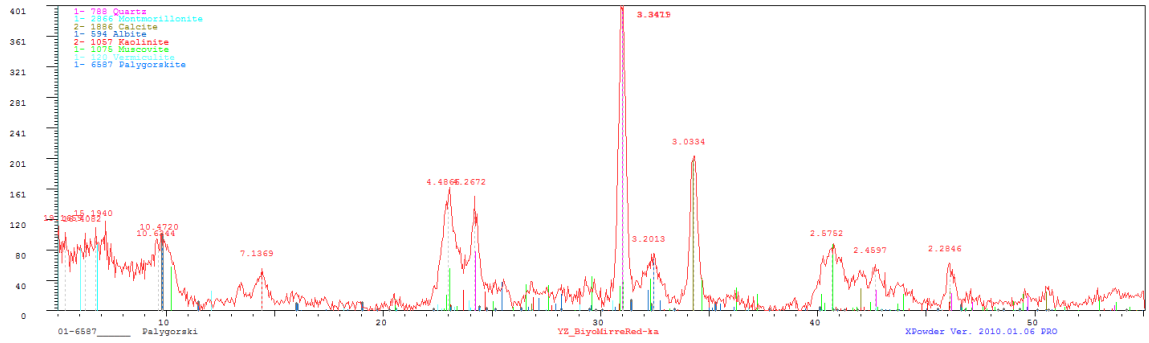

***Figure 37**: XRD result of Biyo Mire Red material*

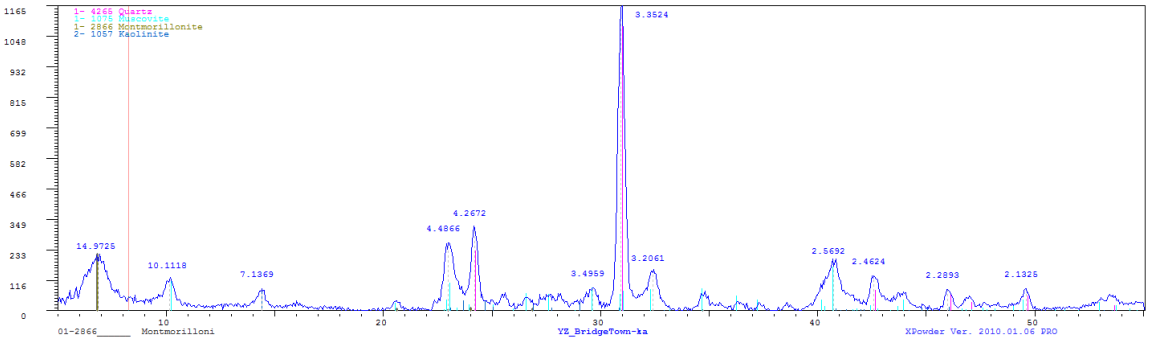

***Figure 38**: XRD result of Nova Scotia #1 material*

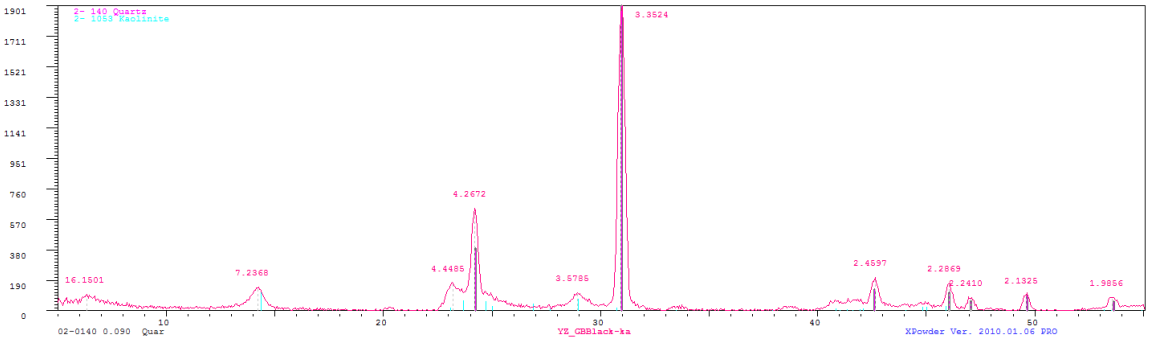

***Figure 39**: XRD result of Guinea Bissau Black material*

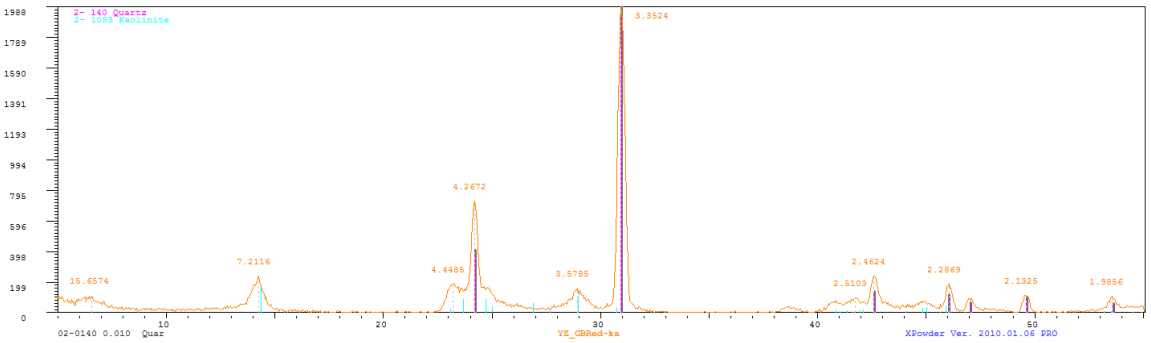

***Figure 40**: XRD result of Guinea Bissau Red material*