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Are Clay Minerals in Jordanian Soils Antibacterial?

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Abstract

Clay separates have been recently evaluated for their antibacterial potential. The current research is conducted to investigate the antibacterial effects of clay fractions from Jordanian soils and their relation to the physical and chemical properties of the soils. Thirty three soil samples were collected from different sites in the country with a high clay content (mainly from northern and middle governorates). The physical and chemical properties of bulk soil and clay fractions were characterized. Thereafter, the clay fractions were screened for their antibacterial properties *in vitro* against common bacterial pathogens and clinical isolates known for their multidrug resistance.

Seven clay fractions showed antibacterial properties against one or more of the tested pathogens in liquid culture. The X-ray diffraction (XRD) and X-ray Florescence (XRF) results of the antibacterial clay were correlated with the bacterial growth inhibition to understand the antibacterial mechanisms.

Correlation analyses using Pearson's product moments and backward elimination stepwise regressions indicated that the tested microbial strains are being significantly affected by different physical and chemical properties of the soil (e.g. EC, CEC, Available water), soil solution ionic concentrations (e.g. HCO_3^- and SO_4^{2-}, Na^+, and Ca^{2+}), clay fraction composition (Clay-Fe-oxide, Clay-$CaCO_3$), and clay type (smectites, quartz, and englishite). On the other hand, Fe_2O_3, Al_2O_3, and MgO clay compositions were found to be the most effective factors governing the antimicrobial activity. Thus, each microbial strain has a specific inhibitor control that interacts with its antimicrobial mechanism. As a conclusion, this study indicates the bactericidal effect of some clay mineral fractions from Jordanian soils, which still need to be clinically tested before use in medical applications.

Keywords: Soil texture, clay fractions, chemical properties, microbial activity, antibacterial clay, XRD, XRF.

1. Introduction

Natural clays have been used in ancient and modern medicine (Carretero et. al., 2002, 2006; Wilson 2003). Applications of clay minerals ranged from geophagy (Wilson, 2003; Ferrel, 2008) to apical applications (Carretero et. al. 2006 Gomes et. al., 2007). Geophagy, is the practice of eating earth materials containing clay minerals to physically sooth an infected and inflamed gastrointestinal lining (Dory-Lefaix et al., 2006). Alternatively, clays have been used topically in mud spas (pleotherapy) to adsorb toxins from skin, and to provide heat to stimulate circulation for rheumatism treatment (Carretero et. al., 2006).

Clay minerals are ubiquitous in natural soils. They have a small particle size of less than 2.0 μm in diameter and bulk density of 2.65 g/ cm^3 as defined by stock's low (Moore et. al., 1997). This provides a high specific surface area for a cation exchange capacity, and high absorptive and adsorptive capabilities. For that, they have been used in a variety of cosmetics and pharmaceutical formulations. For example, the extremely fine particles of smectites (expandable clay minerals) and kaolin group minerals are used to remove oils, secretions, toxins and contaminants from skin by absorbing and adsorbing moisture and impurities from the skin. Clay also serves to cleans and refresh the skin surface, and aids in the healing of topical blemishes in many cosmetics (Williams and Haydel, 2010).

Multidrug resistant organisms (MDROs) are microorganisms, predominantly bacteria, that are resistant to one or more classes of the commonly used antimicrobial agents (Barie, 2012). The widespread use of antibiotics, misuse and\ or overuse of antibiotics, and the ability of microorganism to develop drug resistance contribute to the emergence of new multidrug resistant bacteria (Freber, 2002; Barie, 2012; Orsi et al., 2011; Siegel et al., 2006).

Common MDROs include, but not limited to, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Escherichia coli,* and *Clostridium difficile* which are common causes of human infections worldwide (Siegel et al., 2006).

As the rise of bacterial antibiotic resistance continues to risk human health, this elevates the need to properly detect, prevent and effectively treat these infections. The overuse and misuse of common antibiotics in recent decades stimulates the need to identify new antimicrobial compounds. Therefore,

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natural products including clays that display antibacterial properties are of special interest (Bush, 2004).

Recent scientific attention was drawn toward clay minerals in the published literature. Two French green clays were reported to WHO in 2002 by a French humanitarian Line Brunet de Courssou during her work in the Ivory cost of West Africa. The French clay was used to heal Buruli skin ulcer, a necrotizing disease, caused by *Mycobacterium ulcerans* (Williams et al., 2004). Since then, natural clay minerals have been identified to have the ability to kill a variety of pathogenic bacteria (Haydel et al., 2008; Ma'or et al., 2006; Park et al., 2009; Williams et al., 2004).

Locally, the historical use of red soil in Jordan is still practiced by some communities to treat skin infections such as infant diaper rash. The idea of this study was established from the observation of traditional use of red soil to treat diaper rash among infants in Jordan (Abuidhail, 2011). This triggered the initiation of scientific investigation of the antimicrobial properties of clay minerals found in Jordan's soil. In the Jordanian culture, it was known that mothers brought red soil, cleaned it, filtered it, then took the fleecy material and put it on the nappy rash after applying some olive oil. Using red soil was known to be dangerous, because the soil may be contaminated. It may increase the severity of skin problems as nappy rash changing it to a severe skin infection (Abuidhail, 2008). However, the identification of new inhibitory agents other than antibiotics can open a wide door for the potential of producing an inexpensive alternative that is a property of Jordan and to present such finding for utilization in the Jordanian Pharmaceutical industry.

Therefore, this research is aimed at mineralogically characterizing clays in the Jordanian soil, in order to identify their antibacterial effectiveness. It will provide theoretical guidance to promote the production of antibacterial products from natural clay minerals for complementary therapies.

2. Materials and Methods

2.1. Study Area and Soil Sample Collection

A soil map of Jordan (Hunting Technical Services and Soil Survey and Land Research Centre, 1994) was implemented to allocate the potential sites for clays in Jordan. This was achieved based on field surveying in the northern regions of Jordan. Thirty-three surface (0-20cm) sampling sites were selected. At each site, three sub-samples were taken that are 30m apart. Two surface soil samples were obtained from each location; one undisturbed soil sample using core method (6 cm in diameter), and one disturbed sample using auger method. the samples were labeled and brought to the laboratory for physical and chemical analyses. The spatial location of the sampling sites was recorded using handheld GPS with 3m accuracy (Figure 1).

2.2. Clay Physical and Chemical Analyses

Disturbed soil samples were air dried, ground, then sieved through a 2-mm sieve. The percentage of coarse fractions (> 2 mm in diameter) in each soil sample was collected and estimated. All soil samples were analyzed for the major soil physical and chemical properties (Table 1) following ISO and SSSA procedures. Core samples were used to determine the soil physical properties while maintaining the exact field soil structure.

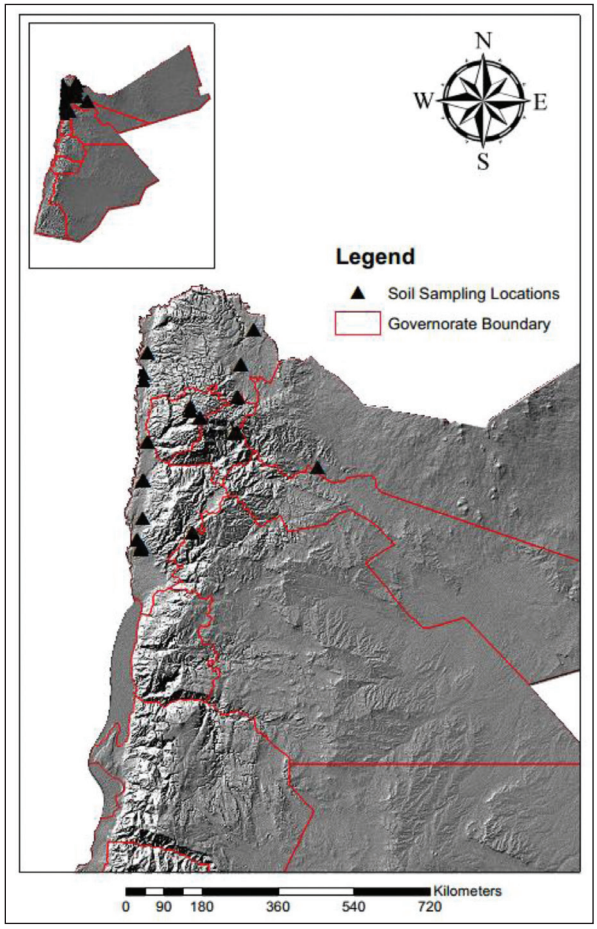

Figure 1. Soil sampling points across the northern parts of the country.

2.3. Clay Fractionation and Diffraction

Based on several trials to search for the best fractionation procedure, clay separation was achieved using the centrifuge technique. Although the gravitational decantation method is much easier, however it is not acceptable since the sedimentation takes a long time (at least 8 hours per sample), and Brownian motion interferes with settling for particles finer than 0.5 micrometers (Poppe et al., 2001). The centrifuge separation technique was achieved based on use of only 10 grams of soil per run and ending by only 0.5 to 1.0 g clay. Thus, this step had taken about a year to finish.

Clay separates were analyzed using the same analytical procedures stated above (Table 1) only the pH and ECe were done in a 1:5 clay:water suspension using a pH meter and EC meter, respectively. Seven samples which showed active microbiological properties were qualitative mineralogicals analyzed as oriented mounts on glass slides from 3u to 60u two-theta. X-ray diffraction and XRF analyses were prepared and analyzed according to the established analytic procedures at the "Water, Environment and Arid Region Research Center" in Al-Bait University using X-ray diffraction (XRD) and XRF systems. The XRD was from PANanalytical B.V. LR 39487C made in Netherlands. And the XRF was from PHILIPS Analatical X-Ray B.V. LR 39487 C made in Holland.

Table 1. Soil Physical and Chemical Analyses procedure following SSSA and ISO.

Soil Physical Property	Method	Reference
Soil Water Content	Gravimetric Method	Petersen and Calvin, 1986
Soil Dry Bulk Densities (ρB)	Coring Technique	Blake and Hartge, 1986
Soil Texture	Pipette Method	Gee and Bauder, 1986
Soil Color	Munsell Chart	Lynn and Pearson, 2000
Water Holding Capacity	Ceramic Plate Apparatus	Klute, 1986
Soil chemical property		
Cation exchange capacity (CEC)	1 M NH4OAc (pH 7.0)	Page et al., 1982
Organic carbon	Wet Oxidation method	Walkley and Black,1934
Active or amorphous iron oxide	Tamm's Reagent	Loppert and Inskeep, 2001
pHe	Paste extract (pH meter)	Sparks etal., 2001
ECe	Paste extract (EC meter)	Sparks etal., 2001
Ionic concentrations of soil solution		
Ca^{2+}, and Mg^{2+}	EDTA titration	Sparks etal., 2001
Na^{+}, and K^{+}	Flame photometer	Sparks etal., 2001
Cl^{-}	Silver nitrate titration	Sparks etal., 2001
HCO_3^{-}	Acid titration	Sparks etal., 2001
SO_4^{2-}	Turbidimetric	Sparks etal., 2001

2.4. Bacterial Strains and Growth Conditions

Staphylococcus aureus ATCC 29213, *Pseudomonas aeruginosa* ATCC 254232*, Escherichia coli* ATCC 35318, and *Klebsiella pneumoniae* ATCC 33495 obtained from the American Type Culture Collection, Methicillin-Resistant *Staphylococcus aureus* (MRSA), *Candida albicans* and *Escherichia coli* (Extended Spectrum Beta Lactamase) ESBL obtained from the Specialty Hospital Laboratories (Amman-Jordan), were used for all studies. *Staphylococcus aureus* ATCC 29213 and methicillin-resistant Staphylococcus aureus (MRSA), were grown on Blood agar , *Pseudomonas aeruginosa* ATCC 254232*, Escherichia coli* ATCC 35318, and *Klebsiella pneumoniae* ATCC 33495 were grown on Macconkey agar, and *Candida albicans was grown on sabouraud dextrose agar*. All the bacterial and Candida strains were grown at 37°C.

2.5. Antimicrobial Activity of Clay

Clay fractions of each soil sample were obtained and used to screen their antimicrobial effect against the previously described bacteria and yeast sp. by the susceptibility testing on solid medium. A fraction of 200 mg of clay minerals was measured in Eppendorf tubes and was sterilized by autoclaving for fifteen minutes at 121°C.

Eppendorf tube containing (1.0 ml of nutrient broth, 200 mg of clay minerals and 48 µl of 0.5 McFarland standard containing 1.5 $X10^8$ CFU/ml of the bacterial strain were mixed thoroughly for one minute. The tubes were then incubated at 37 °C for twenty-four hours and forty-eight hours in a shaker incubator. To count the microbial population in the clay-bacteria mixture, Petri dish plates containing 20 mL of the solidified Muller Hinton and sabouraud dextrose agar (for Candida) were inoculated with 100 µL of the prepared suspension and spread with a sterile L-shape glass rod. The plates were then incubated at 37 °C for twenty-four hours and the colony counts were then recorded. The same process was repeated after forty-eight hours of incubation.

3. Statistical Analysis

Before commencing with the modeling analysis, the types, strengths, and directions of the relationships between soil physical properties, soil chemical properties, clay specific properties, and the antimicrobial behavior were investigated by applying correlation analysis using Pearson's product moment correlation coefficient (r). Correlation analysis was useful for detecting the presence of multi-collinearity, which is a crucial characteristic in the modeling process (Howell, 1997). The strengths of the relationships were classified into three levels: weak correlation when $0 \leq |r| < 0.3$, moderate correlation when $0.3 \leq |r| < 0.7$, and strong correlation when $0.7 \leq |r| \leq 1.0$.

The backward elimination Stepwise regression (SWR) technique; as a selection technique, was used to identify the significant soil physical and chemical properties affecting the soil antimicrobial activity (Montgomery et al., 2012). The SWR represents a significant linear model subsetted at a specific significant level (usually 95 %) from the whole linear model (i.e., linear multiple regression including all of the predictors). The backward elimination technique involves deleting the predictor that contributes the least to the model, and thus the final model includes the most significant predictor. The removal of the insignificant predictors was based on Mallows' Cp criterion (Mallows, 1973) and Akaike's information criterion (AIC) (Akaike, 1974).

4. Results and Discussion

4.1. Soil Physical and Chemical Properties

Based on laboratorial results, the Northern regions of the country of Jordan are characterized by stable, well aggregated soils, with dry bulk densities ranging between 1.13 to 1.59 g/cm^3, and high porosity ranging from 40 % to 57 % with a mean of 52 % (Table 2). The water holding capacity (WHC) ranges from 233.5 to 486.0 mm/m, with a saturation percentage ranging from 37.10 to 73.71 %. These physical properties of the soil point to the effectiveness of the clay

content in enhancing the soil friability. The inspected sites' morphology indicates low soil surface crusts with visible continuous porous system and a low gravel content.

Although the effect of organic compounds on aggregate formation is much more stable than clay cementation, however, the measured soil dry bulk densities indicate that even at a low organic matter situation like in Jordan (i.e. average OM% less than 2 %), clay has a distinctive cementing behavior in providing higher porosity and thus reducing the soil bulk density to an average of 1.27 g/cm^3 which allows for a complete interaction with atmospheric air and the diffusion of air from and to the atmosphere. In such a condition, a microbial growth is favored especially if water exists at an optimum condition.

In regard to the soil color inspection using Munsell Chart, the Northern regions inspected in this study are characterized by having a yellowish red color with various hue magnitudes ranging from 5 to 10, and variable value and chroma. The majority of the soil samples range between 5YR to 7.5YR 4/4 indicating a strong yellowish red color attributed to the solid matrix formation (clay minerals and oxides).

According to the soil samples' texture analyses, the upper north regions of Jordan are classified as having a good potential for a high clay separate content. As indicated by the texture triangle (Figure 2), the soil texture ranged from Clayey to Silty clay to Silty Clay loam classes with a high clay content ranging from 24 % to 68 % by weight with an average content of 46 % and a standard deviation of 12 % (Table 2).

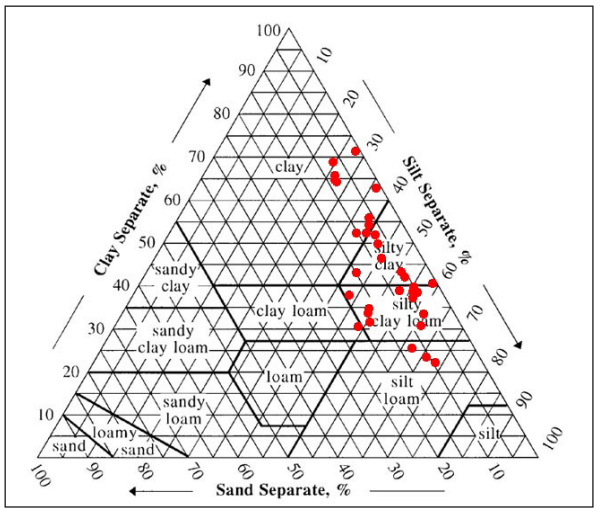

Figure 2. Soil textural class distribution of the soil samples.

Table 2. Soil physical and chemical properties of the inspected sites.

	Maximum	Minimum	Mean	Std Dev
Soil Physical Properties				
Soil bulk density, ρb (g/cm^3)	1.59	1.13	1.27	0.09
Soil Air Dry Water Content, θg (g/g)	0.075	0.043	0.043	0.018
Saturation Percentage, θs (%)	73.71	37.10	57.05	11.25
Available Water, AW (m/m)	0.486	0.233	0.366	0.064
Water Holding Capacity, WHC (mm/m)	486.0	233.5	366.5	64.48
Sand Content (%)	21.8	1.8	9.7	5.0
Silt Content (%)	61.6	23.9	45.2	9.4
Clay Content (%)	67.9	23.9	46.3	12.0
Soil Bulk Chemical Properties				
Potential of Hydrogen, pH	8.41	6.80	7.96	0.34
Electrical Conductivity, EC (µS/ cm)	1834.00	327.39	682.74	331.82
Cation Exchange Capacity, CEC (cmolc/kg)	86.99	2.60	34.08	20.08
Organic Matter Content, O.M (%)	1.60	0.55	0.98	0.37
Nitrogen-N, (ppm)	0.224	0.034	0.108	0.046
Phosphorus, P (ppm)	22.00	1.20	11.58	5.91
Ionic concentrations of the Soil Solution				
Chloride, Cl^- (meq/L)	7.25	0.50	2.60	1.70
Bicarbonate, HCO_3^- (meq/L)	13.50	1.50	5.48	2.60
Sulfate, SO_4^{2-} (meq/L)	4.75	0.69	2.27	1.15
Sodium, Na^+ (meq/L)	42.13	1.52	4.98	6.89
Potassium, K^+ (meq/L)	3.92	0.05	0.63	0.87
Calcium, Ca^{2+} (meq/L)	15.50	3.00	6.33	3.10
Magnesium, Mg^{2+} (meq/L)	17.00	1.00	4.99	3.12
Clay Fractions' Chemical Properties				
Calcium carbonate content, $CaCO_3$ (%)	39.04	21.27	29.21	5.69
Amorphous Iron oxide, (mg/kg)	57.24	39.60	49.39	3.93
Cation Exchange Capacity, CEC ($cmol_c$/kg)	119.56	20.65	60.56	23.54
Organic Matter Content, O.M (%)	2.75	0.55	1.64	0.64

In terms of the soil bulk chemical analyses, the results indicate that these soils are moderately alkaline as indicated by the associated soil pH, ranging from 6.80 to 8.41. Also, the soils are characterized by low salinity with an EC ranging from 1834 to 327.39 µS/cm, where the variation in EC may be attributed to salt removal for being located within sub-humid regions (i.e. located within regions having an average annual rainfall of 300 to 400 mm).

The soil organic matter (OM) is very low ranging from 0.55 % to 1.6 %, which remains within the typical range of the Jordanian environment associated with a low organic addition and a high organic decomposition being located within typical thermic temperature regime. The soils cation exchange capacity (CEC) ranges from 2.60 to 87 cmolc/kg due to the presence of 2:1 clay minerals and due to the low organic matter content. The soil with higher CEC will be capable to adsorb more cations from the soil solution.

In terms of the Ionic concentrations of the soil solution, the chemical results show that sodium is the dominant cation ranging from 1.52 to 42.13 meq/L, whereas the dominant anion is bicarbonate ranging from 1.50-13.50 meq/ L (Table 2). These results are consistent with the fact that most of the soil samples are calcareous being originated from a limestone parent material.

The chemical Properties of the clay fractions show a calcareous behavior as the $CaCO_3$ % ranged from 21.27-39.04 %. The clay fractions CEC ranges from 20.65 cmolc/kg to 119.56 cmolc/kg. The existing differences between CEC results of clay fractions are due to the differences in the mineralogical composition of these samples. On the other hand, the iron content in the clay fractions ranges from 39.6 to 57.24 mg/kg.

4.2. X-ray Diffraction

The X-ray diffraction (XRD) analyses of the soil samples showed a varying composition of clay minerals and non-clay minerals (Figure 3). The most abundant clay minerals are the Kaolinite and smectite group, while the non-clay minerals were mainly calcite and quarz. The smectite clay mineral group has high CEC, which explains the variation among the chemical composition of the soil samples. The soils rich in these minerals have a higher capability to adsorb more cations from the solution and replace them with ones in the exchange sites. On the other hand, the Kaolinite clay mineral group has a different structure and holds lower CEC. Generally, the adsorption mechanisms of clay and non-clay minerals to micro-organisms vary according to the clay structure and its chemical property. Both clay and non-clay mineral surfaces can adsorb bacteria through electrostatic and hydrophobic interactions (Yee and Daughney, 2000).

4.3. X-ray Fluorescence

The results of X-ray fluorescence (XRF) analysis of the major chemical components are shown in Figure 4. The results were in consistence with the XRD analyses, where silica and aluminum, which compose the crystal structure of Smectites and Kaoline minerals, were the predominant elements present. These elements are followed by calcium, iron and sodium. In addition, one of the samples shows higher phosphorus concentration, which is consistent with the presence of Englishite mineral, that is mainly phosphates. The presence of Ca^{2+}, Mg^{2+}, Na^{+}, and K^{+} ions are unlikely to contribute to antibacterial activity, because these ions are essential to bacterial cells (Stotzky, 1989). However, the presence of TiO_2, which is present in all samples, is known to irreversibly adsorb *E. coli* through acid-base attractions occurring between the Waal O antigens lipopolysaccharide and the metal oxide (Jucker et al., 1997).

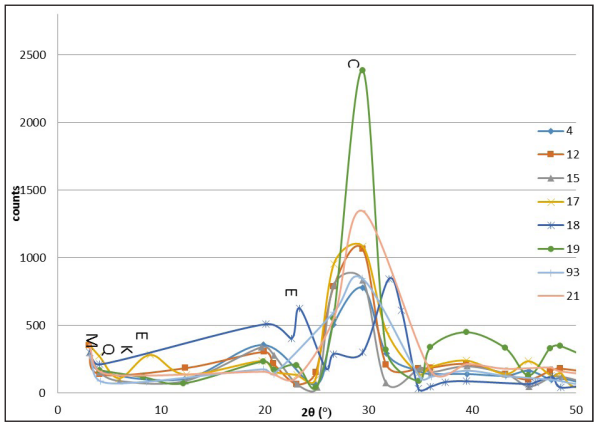

Figure 3. X-ray diffraction spectra of the selected clay separates (E= Englishite, K=Kaolinite, M= Montmorillonite, Q= Quartz, C= Calcite).

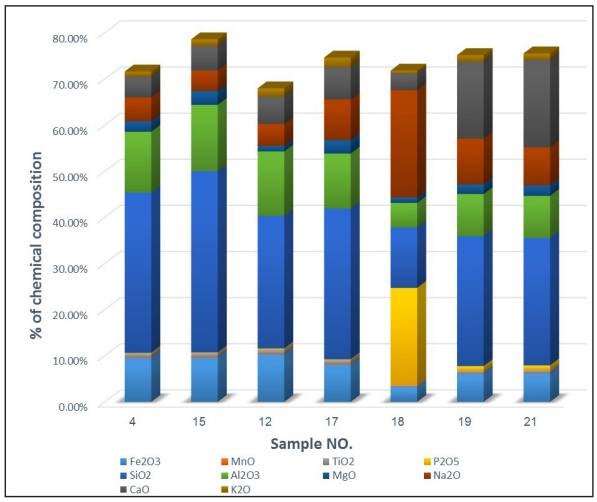

Figure 4. X-ray fluorescence (XRF) analysis of the selected clay separates.

4.4. Microbial Activity

Based on Well Agar Diffusion laboratorial experiment for testing the microbial activity of seven strains using nine fractioned clay samples, the counts after twenty-four hours and forty-eight hours incubations are presented in Table (3). According to the antimicrobial activity results, it seems that each fractioned clay sample has a different effect regarding the microbial strain. Some clay samples may depress the growth substantially as being exclusively effective/elective on specific strains. This indicates that the antimicrobial activity of the clay samples vary significantly according to the strain type and thus suggesting a unique inhibition mechanism of the clay minerals against each microbial strain.

For example, the *E. coli (ESBL)* is being inhibited in all the samples except for sample number twenty-one. On the other hand, the *C. albicans* microbial strain is being inhibited using only two samples number seventeen and twenty-one.

4.5. Correlation between Microbial Activity and Soil Physical and Chemical Properties, Clay Fraction Properties, and XRF Results

In order to investigate the antimicrobial effects of the physical and chemical properties of the soil (including ionic concentrations of the soil solution), the multivariate correlation analysis was achieved using the Pairwise correlation method (Table 4). According to the correlation matrix, there exist moderate and weak relations between the soil physical and chemical properties and antimicrobial activity. In general, the importance of the soil physical and chemical properties varies according to the microbial strain type. Thus, relations are variable and do not direct nor reflect a general conclusion other than that the soil behaviors vary depending on the existing environmental conditions at the field and the management pursued at the micro-scale. The potential medical effect of the clay is still vague, however it appears from these correlations that the chemistry and type of the soil clay are going to be the determinant of the effectiveness of these soils.

Similar to the previous correlation, the antimicrobial effects of the clay fraction properties were investigated using the multivariate correlations analysis (Table 5). The correlation matrix indicates that only Clay-Fe-oxide, Clay-OM, and Clay-pH have a moderate positive correlation with the antimicrobial activity. The reduction effect of Clay-Fe-oxide, Clay-OM, and Clay-pH on the microbial growth is significantly clear concerning *K. pneumoniae, C. albicans, and E. coli (ESBL)*. However, other microbial strains such as *S. aureus, P. aeruginosa,* and *E. coli* were non-significantly affected by the clay fraction properties

The bactericidal mechanism was not due to the physical attraction between clay and bacteria, thus suggesting a chemical transfer or reaction. It was found that the pH and the oxidation state, buffered by the clay minerals through the cationic exchange at the clay surfaces are key to controlling the solution chemistry and redox reactions occurring at the bacterial cell wall (Williams and Haydel, 2010). A group of researchers suggest that the healing power may be attributed to the antibiotic-producing bacteria they have found living in the soil (Falkinham et al., 2009). In contrast, other studies suggested that the acidic environment of hydrated minerals contributes to the antibacterial activity by increasing the availability and toxicity of metal ions in the solution chemistry and redox-related reactions occurring at the bacterial cell wall (Williams and Haydel, 2010; Cunningham T., *et.al*., 2010). A Study investigating different clays from Swaziland, Botswana, and South Africa also revealed antibacterial activity, and attributed that to the low pH and the availability of certain ionic species such as Cu, Al, S and Cl (Sisai *et.al*., 2010).

The correlation matrix generated between the antimicrobial activity and XRF results indicates that the clay composition is more effective in microbial inhibition (Table 6). The matrix indicates the presence of strong relations between the antimicrobial activity and different clay fraction composition, especially MnO, TiO_2, MgO, CaO, and K_2O. The strong relations are microbial strain specific and not effective for all strains. On the other hand, SiO_2 seems to have a moderate inhibition control over all microbial strains.

Results obtained in this study showed that each of the short listed clay minerals was active against one or more of the tested pathogens, and none showed an activity against all the bacteria, this was investigated in another study (Lynda Williams, 2017) and justified by the fact that bacteria are metabolically diverse, and they evolve different adaptation mechanisms to thrive in a variety of environments; some can even live in extreme environmental conditions. Therefore, antibacterial clay can be effective against a specific type or group of bacteria.

Table 3. Antimicrobial activity of seven strains using nine fractioned clay samples

Sample |ID No.	S. aureus		P. aeruginosa		E. coli		K. pneumoniae		C. albicans		E. coli (ESBL)		MRSA	
McFarland standards	1.5 X10^8 CFU/ mL		1.5 X10^8 CFU/ mL		1.5 X10^8 CFU/ mL		1.5 X10^8 CFU/ mL		6.0X10^8 CFU/ mL		1.5 X10^8 CFU/ mL		1.5 X10^8 CFU/ mL	
	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs
4	616	TMTC	N.G	N.G	TMTC	TMTC	TMTC	TMTC	TMTC	TMTC	N.G	N.G	201	TMTC
12	TMTC	TMTC	N.G	N.G	TMTC	TMTC	TMTC	TMTC	TMTC	TMTC	352	TMTC	TMTC	TMTC
15	TMTC	TMTC	N.G	N.G	N.G	N.G	76	N.G	TMTC	TMTC	N.G	N.G	TMTC	TMTC
17	TMTC	TMTC	TMTC	TMTC	TMTC	TMTC	N.G	N.G	1008	N.G	N.G	N.G	TMTC	TMTC
18	83	TMTC	TMTC	TMTC	TMTC	TMTC	TMTC	TMTC	TMTC	TMTC	N.G	N.G	227	TMTC
19	488	TMTC	TMTC	TMTC	TMTC	TMTC	7	N.G	TMTC	TMTC	N.G	N.G	40	325
93	TMTC	TMTC	TMTC	TMTC	TMTC	TMTC	N.G	N.G	TMTC	TMTC	N.G	TMTC	TMTC	TMTC

TMTC: Too many to count. NG: No growth.

Table 4. Correlation between antimicrobial activity and soil physical and chemical properties including soil solution ionic concentrations

Microbial Strain Type	pH	EC	Clay	CEC	O.M	Cl^-	HCO_3^-	SO_4^{2-}	Na^+	K^+	Ca^{2+}	Mg^{2+}	θg	bulk density	Available water	θs	P	K	N
S.aureus	-0.0463	0.0466	-0.0777	-0.1162	0.1696	0.0123	0.2908	-0.0013	-0.0864	0.0279	-0.0685	0.1248	-0.1089	-0.1028	-0.3136	0.0159	-0.0204	0.1194	0.0661
P. aeruginosa	-0.0131	0.1259	0.1037	-0.0287	0.2409	-0.0365	0.2208	0.0204	-0.0804	0.1014	-0.1282	0.0376	-0.0423	-0.0850	0.0201	0.2506	0.1676	-0.1627	0.1810
E.coli	-0.0522	0.3843	-0.0244	-0.1393	0.1476	0.0796	0.2005	0.2676	0.0176	0.2722	-0.0439	0.1541	-0.0447	-0.1408	0.0616	0.2322	0.3374	-0.0297	0.1070
K.pneumoniae	0.0308	0.4810	-0.2082	-0.3906	0.3253	0.2905	0.5149	0.3809	-0.0059	0.2666	-0.0176	0.2054	-0.3093	-0.0881	-0.0378	-0.0376	0.5017	0.1272	0.3786
C. albicans	0.0308	0.4810	-0.2082	-0.3906	0.3253	0.2905	0.5149	0.3809	-0.0059	0.2666	-0.0176	0.2054	-0.3093	-0.0881	-0.0378	-0.0376	0.5017	0.1272	0.3786
E.coli (ESBL)	0.1941	0.0119	-0.1381	-0.2061	0.2915	0.0350	0.4386	0.0252	-0.0812	-0.0412	-0.1797	-0.0175	-0.3000	-0.0225	-0.2607	-0.1387	0.2494	0.1676	0.3278
MRSA	-0.2028	0.3682	-0.0781	-0.2501	0.2717	0.1581	0.3475	0.2025	-0.0902	0.2778	-0.0064	0.2828	-0.1758	-0.1367	-0.1754	0.0960	0.1069	0.0576	0.1687

The strengths of the relationships were classified into three levels: weak correlation when $0 \leq |r| < 0.3$, moderate correlation when $0.3 \leq |r| < 0.7$, and strong correlation when $0.7 \leq |r| \leq 1.0$.

Table 5. Correlation between antimicrobial activity and clay fraction properties

Microbial Strain Type	Clay-$CaCO_3$	Clay-CEC	Clay-Fe-oxide	Clay-OM	Clay-pH	Clay-EC
S.aureus	-0.0810	-0.2503	0.2929	0.1649	0.0273	0.3389
P. aeruginosa	-0.0783	-0.0408	0.1284	0.2173	-0.0242	0.0357
E.coli	0.1337	0.0599	0.2732	0.1688	0.1765	-0.0752
K.pneumoniae	0.0581	-0.1014	0.5022	0.3561	0.4011	-0.1340
C. albicans	0.0581	-0.1014	0.5022	0.3561	0.4011	-0.1340
E.coli (ESBL)	-0.1988	-0.2295	0.4753	0.3554	0.2663	0.1407
MRSA	0.0874	-0.2889	0.2696	0.3088	0.1462	0.1800

The strengths of the relationships were classified into three levels: weak correlation when $0 \leq |r| < 0.3$, moderate correlation when $0.3 \leq |r| < 0.7$, and strong correlation when $0.7 \leq |r| \leq 1.0$.

Table 6. Correlation between antimicrobial activity and XRF results

Microbial Strain Type	Fe_2O_3	MnO	TiO_2	P_2O_5	SiO_2	Al_2O_3	MgO	Na_2O	CaO	K_2O
S.aureus	-0.4712	-0.5333	-0.5733	0.4800	-0.4383	-0.5068	-0.3679	0.5083	-0.0892	-0.7214
P. aeruginosa	0.6563	0.1833	0.5988	-0.4827	0.5159	0.6288	0.0894	-0.6799	-0.0349	0.0604
E.coli	0.0819	-0.0913	0.1740	-0.2478	0.3528	0.1563	0.4667	-0.3008	0.3604	0.0041
K.pneumoniae	-0.0518	0.1833	0.1388	-0.4496	0.4153	0.0518	0.7449	-0.2621	0.6267	0.4570
C. albicans	-0.0518	0.1833	0.1388	-0.4496	0.4153	0.0518	0.7449	-0.2621	0.6267	0.4570
E.coli (ESBL)	0.2432	0.3064	0.1859	0.1340	0.0838	0.2523	-0.0892	0.0665	-0.7128	0.0160
MRSA	-0.6432	-0.7500	-0.7047	0.3852	-0.4976	-0.6852	-0.3048	0.4613	0.4148	-0.7328

The strengths of the relationships were classified into three levels: weak correlation when $0 \leq |r| < 0.3$, moderate correlation when $0.3 \leq |r| < 0.7$, and strong correlation when $0.7 \leq |r| \leq 1.0$.

4.6. Stepwise Regression Analyses

The backward elimination stepwise regression analysis was useful to indicate the significant factors governing the antimicrobial activity based on soil physical and chemical properties, clay fraction properties, soil solution ionic concentration, and XRF results. Table (7) shows that each microbial strain is being affected by different soil factors. The positive or negative sign of each soil factor is indicative of the effect of the parameter on the antimicrobial activity as being either inhibitor or catalyst of microbial growth.

Table 7. Stepwise Multiple Regression Results for soil physical and chemical factor selection governing microbial reduction.

Microbial Strain	R^2	RMSE	P-value	Equation
S.aureus	0.1148	27.9	0.0537	(-10.864) + 0.004 * Clay-EC (µs/cm)
P. aeruginosa	0.0108	33.49	0.5656	(0.912) + 0.255 * clay content (%)
E.coli	0.9900	0.05	<.0008*	(-354.147) -57.153 * CaO (%) + 20.042 * Na^+ (meq/L) -1.442 * Ca^{2+} (meq/L) + 797.648 * AW (m/m)+ 0.143 * K (ppm)
K.pneumoniae	0.9900	0.11	0.0014*	(-55.258) -263.952 * Fe_2O_3 (%)+ 5619.897 * MgO (%) -0.845 * CEC (cmolc/kg) + -0.382 * Clay-$CaCO_3$ (%) + 3.138 * P (ppm)
C. albicans	0.9900	0.03	0.0014*	(61.185) + -65.988 * Fe_2O_3 (%) + 1404.974 * MgO (%) -0.211 CEC (cmolc/kg) -0.095 * Clay-$CaCO_3$(%) + 0.784 * P (ppm)
E.coli (ESBL)	0.0574	41.63	0.4121	42.910) -0.522 * CEC (cmolc/kg) -6.218 * K+(ppm)
MRSA2	0.5625	38.73	0.0522	(235.000) -149999.732 * MnO (%)

** Highly significant at 95% confidence level.*

Therefore, different antibacterial clays may exhibit different modes of action, some of which may react with bacteria by adsorption, or interact with soil solution ionic species. The same conclusion was made by (Williams, 2017) saying that different mechanism could apply to the bactericidal effects of the soil clay fraction type, or clay physical and chemical properties, or through specific ionic species of the soil aqueous solution.

Conclusion

Jordanian soils exhibit a high clay content that reaches up to 57 %. These soils were found to have a good potential for their inhibitory effect against some pathogenic bacteria. The correlation between the tested microbial strains and soil bulk physical and chemical properties, and soil solution ionic concentrations, indicated that some of these properties have moderate relations especially soil EC, CEC, available water, HCO_3^-, and SO_4^{2-}, P, and K. However, the effect of each soil property is variable according to the microbial strain.

Correlations with the clay-separate properties and clay composition indicated that Clay-Fe-oxide, Clay-OM, and Clay-pH are the most effective factors governing the microbial activity positively of the *K. pneumoniae, C. albicans, E.coli (ESBL)*, and *MRSA* bacterial species. On the other hand, the Clay-EC is found to be positively effective in inhibiting the *S. aureus* growth.

The X-ray diffraction indicated that these soils are in majority composed of Kaolinite, Smectite, Englishite, Calcite and Quarz. The correlations with clay-fraction composition revealed that MgO is a strong inhibitor of the *K. pneumoniae and C. albicans* growth and a moderate inhibitor of the *E.coli* growth. On other hand, Fe_2O_3 *and* TiO_2 were found to be moderate to strong inhibitors of the *P. aeruginosa* growth, while Na_2O and P_2O_5 were found to be moderately effective in inhibiting *S. aureus and MRSA.*

The backward elimination stepwise regressions were able to identify the most common significant factors governing each microbial growth. These factors vary by the microbial strain suggesting the presence of different antibacterial mechanism (e.g. absorption, adsorption, toxicity, etc). Therefore, further

specific investigations are encouraged to understand the mechanisms of the microbial inhibition**.**

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