

PHYSICOCHEMICAL CHARACTERISTICS, MICROELEMENTS AND BACTERIAL POPULATION DENSITY IN THE SOILS OF WADI AL-ADAIREY, HAIL, SAUDI ARABIA

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Abstract

The present study determined the physicochemical characteristics, minerals, and coliform bacteria in the soil samples of Wadi Al-Adairey, Hail region. Sandy texture (90 %) dominated the soil samples containing a very low concentration of microelements. The concentrations of copper (Cu), nickel (Ni), lead (Pb), chromium (Cr), zinc (Zn), and cadmium (Cd) were noted to be 0.5, 0.37, 0.24, 0.24, 0.71, 0.008 mg/L, respectively. The microbiological analysis revealed the absence of coliform bacteria in most of the soil samples except the samples from section (5) at a site depth of 25-55 cm. These samples contained high numbers of coliforms (9.3×10^2), faecal coliforms (4.3×10^2), and *E.coli* (5.5×10^2), which respectively decreased to 4.6×10^2 , 2.3×10^2 , and 3.2×10^2 .

Key words: Physicochemical characteristics; Microbiological analysis; Faecal coliforms.

Introduction

The physical and chemical soil properties vary with the location and type of the soil. The features such as the horizon, color, texture, composition, consistency, and bulk density represent the soil's physical properties. The chemical properties of the soil are based on the cation exchange capacity, interaction, and pH of the soil. Soil physicochemical properties are crucial for soil fertility, which determines agricultural sustainability and crop productivity (Liu *et al.*, 2019). Agricultural products contain significantly large amounts of carbon (C), phosphorus (P), nitrogen (N), and potassium (K). These elements enhance the total organic carbon and its fractions, enzymes (urease, phosphatase, invertase, and catalase), and several other physicochemical properties of the soil (Zhao *et al.*, 2019; Wang *et al.*, 2019; Dai *et al.*, 2019; Akhtar *et al.*, 2018; Duval *et al.*, 2016). Similarly, soil microorganisms actively play a key role in various functions of the terrestrial ecosystem such as litter decomposition, primary production, nutrient cycling, and climate regulation (Thakur *et al.*, 2020; Rillig *et al.*, 2019).

The stream of Wadi Al-Adairey is located in the middle of the Hail region parallel to the Aja mountain range from the southwest to the northeast and passes through the city of Hail and extends to a distance of about 60 km (The regional plan of the Municipality of Hail, 2005 AD) (Fig. 1). The field visits revealed that the stream of Wadi Al-Adaira is exposed to sewage pollution through (1) underwater leakage from house tanks (wells), and (2) wastewater discharged from a sewage treatment plant near the valley stream. The inefficiency of the wastewater treatment plant in reducing pollutants has been reported as it could not effectively remove phosphates, color, nitrates, and heavy metals (selenium, mercury, cadmium, lead, and copper) from the wastewater. The inefficiency of the wastewater treatment plant was more pronounced during the rainy season (Godfrey *et al.*, 2020). The rock fragments constitute the Wadi Al-Adairey soil and

weathering process in the surrounding rocks over thousands of years formed these soils and transported them to the valley through torrential water and sedimentation.

Pedologists characterize novel soil properties of distinctive soil types into various soil orders. Soil, a complex biomaterial, possesses an expansive scope for natural microbial habitats (Young & Crawford, 2004). These microbial habitats might only have a size of micrometers but they can contain significantly different relative extravagance, abundance, action, and uniformity of microbial taxa (Fierer, 2017). Articulated microbial differences have been reported between the soil samples, which are only a couple of centimeters apart (O'Brien *et al.*, 2016).

Soil ecological functions are significantly affected by rapid urbanization. Microbes are well known for their direct participation in key soil processes to maintain important soil functions (Weiwei *et al.*, 2021). However, the underlying mechanisms related to the presence and development of microbial communities, and the interactions among microbial taxa in Wadi Al-Adairey soils are still unexplored. The present study investigates the soil samples from Wadi Al-Adairey in the Hail region to elaborate on the physicochemical characteristics, minerals, and coliform bacteria.

Materials and Methods

Collection of soil samples: A field survey was conducted in the areas of Wadi Al-Adairey facing Hail city. The survey covered a length of 50 km to identify the spatial differences in the valley. Sampling sites and representative sectors of the soil were also determined during the survey. Soil samples were collected at three depths (A, B, and C) of five sites in Wadi Al-Adairey, Hail. Potentially highly polluted soil sites were selected for the sample collection. Air-tight polyethylene bags were used to transport the soil samples to the laboratory. The bags were properly numbered and labeled with the date and site of collection.



Fig. 1. Nature of sediments in Wadi Al-Adairey, North of Hail city.

Determination of physicochemical properties of soil:

Soil samples were air-dried, ground, sieved (20-mesh), and placed in plastic bags until used in physical analyses. The soil color of the study area was determined in comparison to (Munsell) slides, and volume was estimated whereas the hydrometer method was followed to assess the size of the granules (Piper, 1955). Soil samples were subjected to digestion through furnace-based incineration and the weight of different samples was calculated (Page, 1982). The chemical titration methodology (Jackson, 1962) was followed to estimate the calcium carbonate content.

The cation exchange capacity (CEC) was estimated by saturating the soil samples with ammonium solution and titration of ammonium according to Jackson (1962). A soil-water mixture (1:1, W: V) was prepared to investigate the pH, electrical conductivity (EC), sodium, magnesium, and calcium cations. Sodium element was estimated using a flame photometer whereas chemical titration was carried out to evaluate calcium and magnesium elements (Richards, 1954) by applying the following equations:

$$SAR = \frac{NA}{\sqrt{\frac{Ca + Mg}{2}}}$$

$$ESP = \frac{100(-.0126 + .01475 SAR)}{1 + (-.0126 + .01475 SAR)}$$

Determination of microelements: Soil samples were analyzed to assess the presence of microelements (Zn, Cu Mn, Fe, Cd, and Pb) and major elements (K, Na, Mg, and

Ca) by dissolving in aqua regia (1:3 HNO₃: HCl). Atomic Absorption Spectrometer (FAAS), inductively coupled plasma-optical emission, and mass spectrometry (ICP-OES and ICP-MS) were carried out to detect the metals in the final solution. Standard stock solutions for all elements were either procured from Merck or prepared in the laboratory as described in Anon., (1989). Pyrex glassware was washed several times with soap, distilled water, and diluted nitric acid to remove impurities before utilizing in the experiments (Quevauviller, 1998).

Microbiological analyses

Estimation of aerobic bacteria: To study the microbiological characteristics, 1 g of soil sample was added into the medium and incubated at 170 rpm and 30°C in an orbital shaker (Kammar *et al.*, 2016). Ten-fold serial dilution of one ml soil suspension was prepared to estimate the aerobic heterotrophic bacterial counts through the standard spread-plate dilution method in triplicate (Seeley & VanDemark, 1970). Visible discrete colonies in incubated plates were counted and expressed as colony-forming units per gram (cfu/g) of soil samples.

Total coliform, faecal coliform, and *Escherichia coli*:

Total coliform, faecal coliform, and *Escherichia coli* were estimated by following a reference method based on the most probable number (MPN). Pre-enrichment was carried out in LST broth (37°C for 48 h) whereas confirmation tests were conducted in BGLB broth for total coliform (37°C for 48 h), and in EC broth for faecal coliform (44°C for 24 h). *Escherichia coli* were confirmed by testing indole production. The results were expressed as CFU/g.

Results and Discussion

Figures 2, 3 and 4 present the physical properties of the soil samples collected from the valley (Wadi Al-Adairey). The results revealed that soil samples from all sectors exhibited a sandy texture (90% sand) except sample no. (1) that was collected from sector no. (1). This might be due to the collection of samples from the surface soil of the sector that mainly consisted of silt. This area (sector no. 1) is comparatively lower than the surroundings. Therefore, it receives torrential waters that remain there for a longer period depositing preferably large particles followed by the smaller particles.

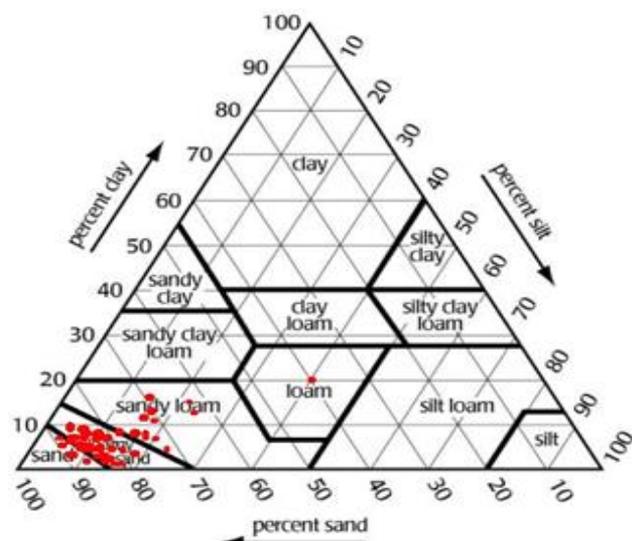


Fig. 2. Texture triangle of soil samples collected from various locations of the Wadi Al-Adairey.

Cation exchange capacity (CEC) (Fig. 5) values were noted to be very low for all the samples ranging between 1.02-4.26 mEq/100 gm of the soil. This coefficient is an indirect measure of soil fertility that directly reflects the soil texture. Adsorbed Sodium Ratio (SAR) and Exchanged Sodium Ratio (ESP) of all the samples indicated a coarse sandy texture. Adsorbed Sodium Ratio (SAR) ranged from 1.08-2.97 whereas a low exchanged sodium ratio (ESP) was observed ranging between 0.33-3.08. The highest percentages of adsorbed sodium and exchanged sodium were noted in sector no. (4), and convergence of these parameters was observed.

All soil samples exhibited a low concentration of calcium carbonate (less than 0.5%), which might be attributed to the nature of the sandy sediments in the valley. Granite rich in silicon dioxide (sand) predominates the rocks from where the soil of the valley was derived (igneous and metamorphic rocks). Contrarily, the soil samples were poor in calcium carbonate (0.01-0.36%) content. The results also revealed a significantly lower percentage of organic carbon (0.01-0.13%) that might be caused by the intense heat and severe drought leading to the scarce vegetation cover in the valley and subsequently lack of organic matter.

Microelements: The results demonstrated very low concentrations of microelements, Cadmium (Cd) concentration did not exceed 0.008 mg/liter in all the samples whereas the chromium (Cr) concentration remained at 0.24 mg/L (sector no. 3), and the maximum

Copper (Cu) concentration of 0.5 mg/L (sector no. 5) was noted. The highest concentrations of nickel (Ni) (0.37 mg/L), lead (Pb) (0.24 mg/L), and zinc (Zn) (0.71 mg/L) were noted in sector no. (1). However, a slight increase in the manganese (Mn) concentration of soil samples was noticed that reached a maximum value of 4.6 mg/L (sector no. 1) (Table 1).

Interestingly, a substantial increase in iron elements was observed in almost all the soil samples with the highest and lowest values of 185 mg/liter in sector no. 1, and 36 mg/liter in sector no. 5, respectively. The presence of rock sources (Alkaline granite rich in mica minerals, soda pyroxene, and soda amphibole) on iron oxides and iron-rich rocks in the region could be the reason behind this phenomenon. The results of the study revealed that the soil of Wadi Al-Adairey is unpolluted and the existence of minor elements in the valley soil could be attributed to the rock erosion in the region.

The valley areas in the north of the Hail city receive sewage water that may be devoid of heavy elements except produced by the detergents. However, it does not significantly raise the heavy elements, and water drainage rather increases the organic load and microorganisms. The normal soils of the area adjacent to the disposal site of industrial waste effluents gradually become salinity-affected. Soil salinity near the pond was noted to be 80.3 dS mG1 whereas it was normal (3.2 dS mG1) at a distance of 100 m from the disposal site. The patterns of soil contamination varied with the distance from the wastewater pond. The soil was categorized as normal, medium, high, and severely contaminated within a distance of 40 m from the wastewater pond (Saleh, 2020)

Topsoil is a major source of supplements for plants and thus its properties have been investigated in several studies (Zhao *et al.*, 2019; Dai *et al.*, 2019; Wang *et al.*, 2019). However, the physicochemical properties of the subsoil also contain certain supplements that might move from the topsoil during irrigation and rainfall (Stowe *et al.*, 2010; Blanco-Canqui & Lal, 2007). The soils of cultivated agricultural lands and prairies exhibit significantly varying physiochemical properties at various depths (Li *et al.*, 2017; Peng & Wang, 2016).

Total bacterial count in soil samples: The diversity of soil bacteria contains various levels of biological organization such as genetic variability among taxa (species), relative abundance (evenness) of taxa, number (richness), and functional groups within communities (Torsvik, 2002). Soil microflora is comprised of bacteria, actinomycetes, fungi, and photosynthetic microorganisms (Jacoby *et al.*, 2017). Bacterial counts in the soil samples of different sites were generally found to be high (Table 2). The mean TBC of the soil samples ranged from 4.9×10^8 (cfu/g) to 5.6×10^5 (cfu/g). The differences in the average total bacterial counts of different sampling sites were not statistically significant. However, the highest count (4.9×10^8 (cfu/g)) was observed in the samples of section (5) at a depth of 0-25 cm whereas the lowest value of 5.6×10^5 cfu/g was observed in section (4) depth of 55-80 cm. The reduction of bacterial counts in the lower soil levels could be related to the reduced organic matter as compared to the soil surface. Moreover, the sampling sites are exposed to the washing effects of the rainwater which could lead to decreased microbial counts.

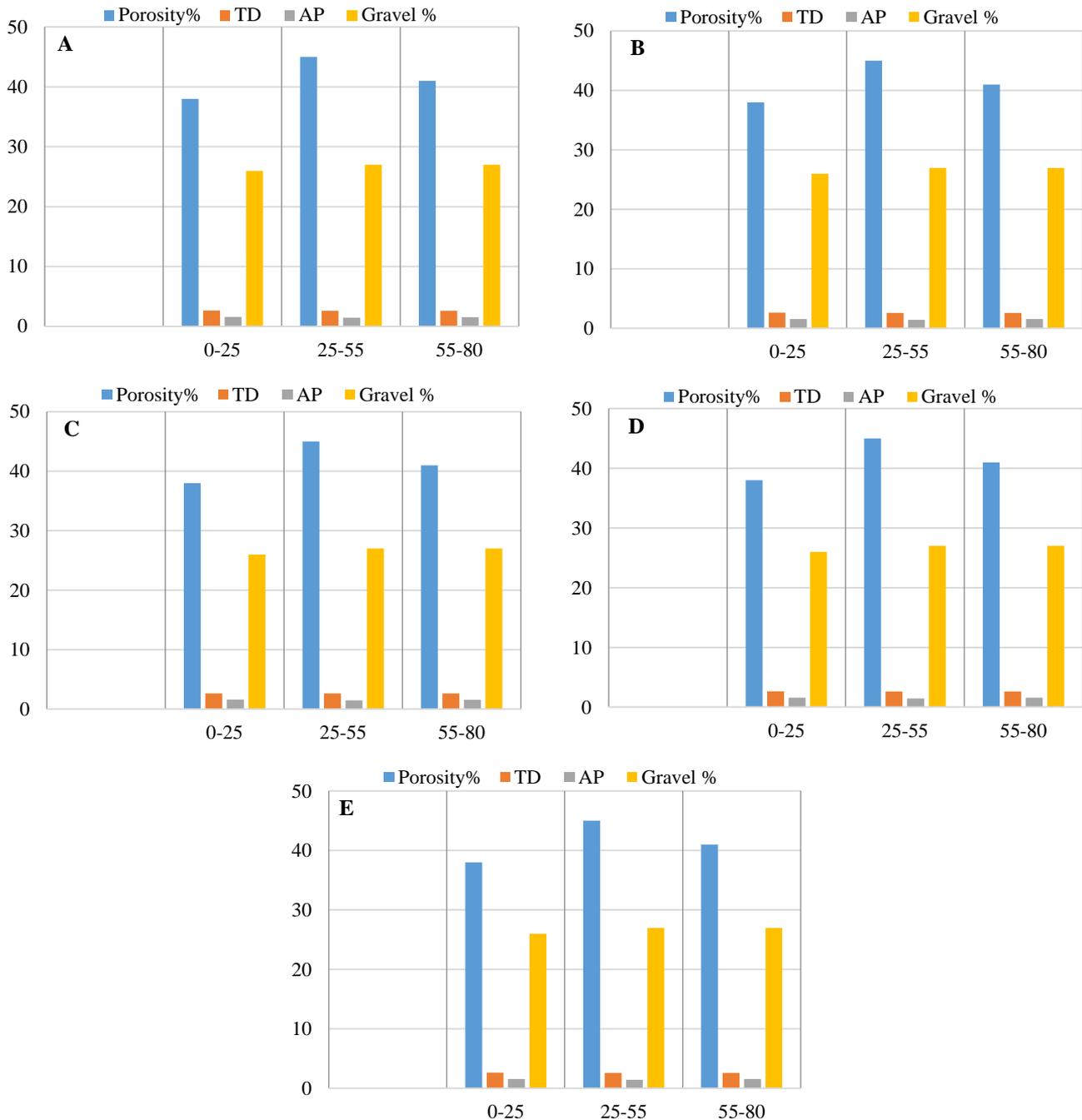


Fig. 3. Physical properties of soil from sections 1 (A); 2 (B); 3 (C); 4 (D); 5 (E).

These results are in line with the previous findings (Okoh *et al.*, 1999). Based on DNA-DNA re-association, 4000 different bacterial “genomic units” have been reported in 1 g of the soil sample and almost 5000 bacterial species have been explored (Bassey, 2008; Radha *et al.*, 2007). Dry conditions, pungency, acidity, absence of organic matter, and soil compaction debilitate microbe populations. The induction of bacterial populations through inoculation is incredibly difficult. Soil properties such as pH and nutrient (nitrogen and organic carbon) availability are known to strongly influence the underground microbial communities (Trivedi *et al.*, 2019), and soil texture (Xia *et al.*, 2020). The impact of agricultural activities on the bacterial communities at various depths of different soils is still not properly understood (Peipei *et al.*, 2020).

Bacterial activity and numbers are generally high in the surface layers of cultivated and virgin soils. Furthermore, the soils of orchards, grasses, and forests contain the maximum number of bacteria near the plant roots. The number of bacteria significantly decreases with the soil depth, but in some cases (organic soils) the highest bacterial populations have been observed at a depth of more than 160 cm. Generally, the maximum bacterial activity is found at a depth of 15 cm from the soil surface and decreases with the increasing soil depth. Soil bacterial communities represent the soil quality in agricultural systems (Ashworth *et al.*, 2017) and are crucial for soil ecological aspects including nutrient cycling, soil carbon, and greenhouse gas discharge (Hobara *et al.*, 2014; Tellez-Rio *et al.*, 2015; Thompson *et al.*, 2017). The soil microorganisms of the deep layers

have also been reported to significantly affect the ecological processes, soil formation, and maintaining groundwater quality (Li *et al.*, 2014). However, bacterial abundance and community composition change with the soil depth (van Leeuwen *et al.*, 2017; Fierer *et al.*, 2003).

Total coliform, faecal coliform, and *Escherichia coli*:

Table (2) indicates that all the samples were devoid of coliform bacteria except soil samples from section (5) at a depth of 25-55 cm, which contained high numbers of coliforms (9.3×10^2), faecal coliforms (4.3×10^2), and *E. coli* (5.5×10^2). However, these values slightly decreased to 4.6×10^2 , 2.3×10^2 and 3.2×10^2 for coliforms, faecal coliforms, and *E. coli*, respectively. This sampling area in Aledareh valley is exposed to contamination with sewage water drainage and other pollutants. The high microbial build-up in this section can be connected to organic matter that is transferred with water and facilitates the development of soil microbial communities. High organic matter and a high number of viable bacterial counts could possess diverse bacterial groups including coliforms, faecal coliforms, and *Escherichia coli* (Pommepuy *et al.*, 1992; Girdwood *et al.*, 1985).

Total coliform bacteria are commonly found in the environment and generally are not harmful. Faecal coliform bacteria are a sub-group of total coliform bacteria inhabiting the human and animal intestines and faeces in large numbers. Faeces of sick persons serve as a source for contaminating food and infecting healthy persons with various diseases including dysentery, cholera, gastroenteritis, and typhoid fever. Coliform bacteria are considered universal microbiological indicators of water quality (Aram *et al.*, 2021; Neill *et al.*, 2004). Faecal coliforms originate in the intestines of warm-blooded animals whereas non-faecal coliforms belong to the family Enterobacteriaceae. Furthermore, the faecal coliform bacteria indicate the presence of pathogenic microorganisms in food and water whereas non-faecal coliforms ferment lactose to produce gas (Fresno & Fresno, 2009). *Escherichia coli* are a sub-group of faecal coliforms that inhabit the intestines of warm-blooded animals and humans in enormous numbers. *Escherichia coli* are mostly innocuous, however, a few strains can cause diseases. Therefore, *Escherichia coli* presence in water is often considered as tainting of water with faeces and other human pathogens (Anon., 1985).

Table 1. The concentration of microelements in soil samples collected from Wadi Al-Adeirey (mg/L).

Section	Sample No.	Zn	Pb	Ni	Mn	Fe	Cu	Cr	Cd
1	1	0.71276	0.01>	0.37363	4.62569	185.000	0.28325	0.17279	0.01>
	2	0.24946	0.01>	0.18003	0.9787	109.300	0.07536	0.08057	0.01>
	3	0.35422	0.01>	0.21163	0.86933	136.343	0.10166	0.03603	0.01>
	4	0.32917	0.01>	0.21277	1.90012	110.988	0.22054	0.14634	0.01>
2	5	0.32014	0.01>	0.20948	2.15189	113.029	0.16341	0.17255	0.01>
	6	0.17907	0.01>	0.12301	1.08742	81.1249	0.11611	0.08169	0.01>
	7	0.32411	0.01>	0.23035	1.94676	117.888	0.17828	0.15216	0.01>
3	8	0.16301	0.01>	0.10462	1.14692	64.2691	0.10355	0.05688	0.01>
	9	0.25666	0.01>	0.20385	1.75974	94.1565	0.14091	0.24397	0.01>
	10	0.13952	0.01>	0.06811	0.86105	53.9025	0.11991	0.04827	0.01>
4	11	0.19935	0.01>	0.13042	1.21385	85.5114	0.1087	0.08232	0.01>
	12	0.17089	0.01>	0.11795	1.31062	85.2104	0.12469	0.08098	0.01>
	13	0.12744	0.01>	0.10164	0.93086	78.5267	0.06009	0.11167	0.01>
5	14	0.29984	0.01>	0.20102	2.14982	75.0579	0.17004	0.06501	0.01>
	15	0.26603	0.01>	0.18105	2.34724	82.4125	0.12773	0.1128	0.01>
	16	0.59567	0.01>	0.21933	1.98367	36.2489	0.50911	0.05425	0.01>

Table 2. Microbiological characteristics of soil samples collected from Wadi Al-Adeirey (cfu/g).

Section No.	Soil depth	Total bacterial count (cfu/g)	Coliforms (MPN)	Faecal coliforms (MPN)	<i>E. coli</i> (MPN)
1	0-25	9.2×10^5	ND	ND	ND
	25-55	2.5×10^6	ND	ND	ND
	55-80	5.4×10^5	ND	ND	ND
2	0-25	6.4×10^7	ND	ND	ND
	25-55	8.5×10^4	ND	ND	ND
	55-80	5.2×10^5	ND	ND	ND
3	0-25	5.5×10^5	ND	ND	ND
	25-55	6.7×10^7	ND	ND	ND
	55-80	4.6×10^7	ND	ND	ND
4	0-25	5.7×10^4	ND	ND	ND
	55-25	6.3×10^5	ND	ND	ND
	55-80	5.4×10^4	ND	ND	ND
5	0-25	4.9×10^8	ND	ND	ND
	25-55	2.6×10^8	9.3×10^2	4.3×10^2	5.5×10^2
	55-80	5.8×10^8	4.6×10^2	2.3×10^2	3.2×10^2

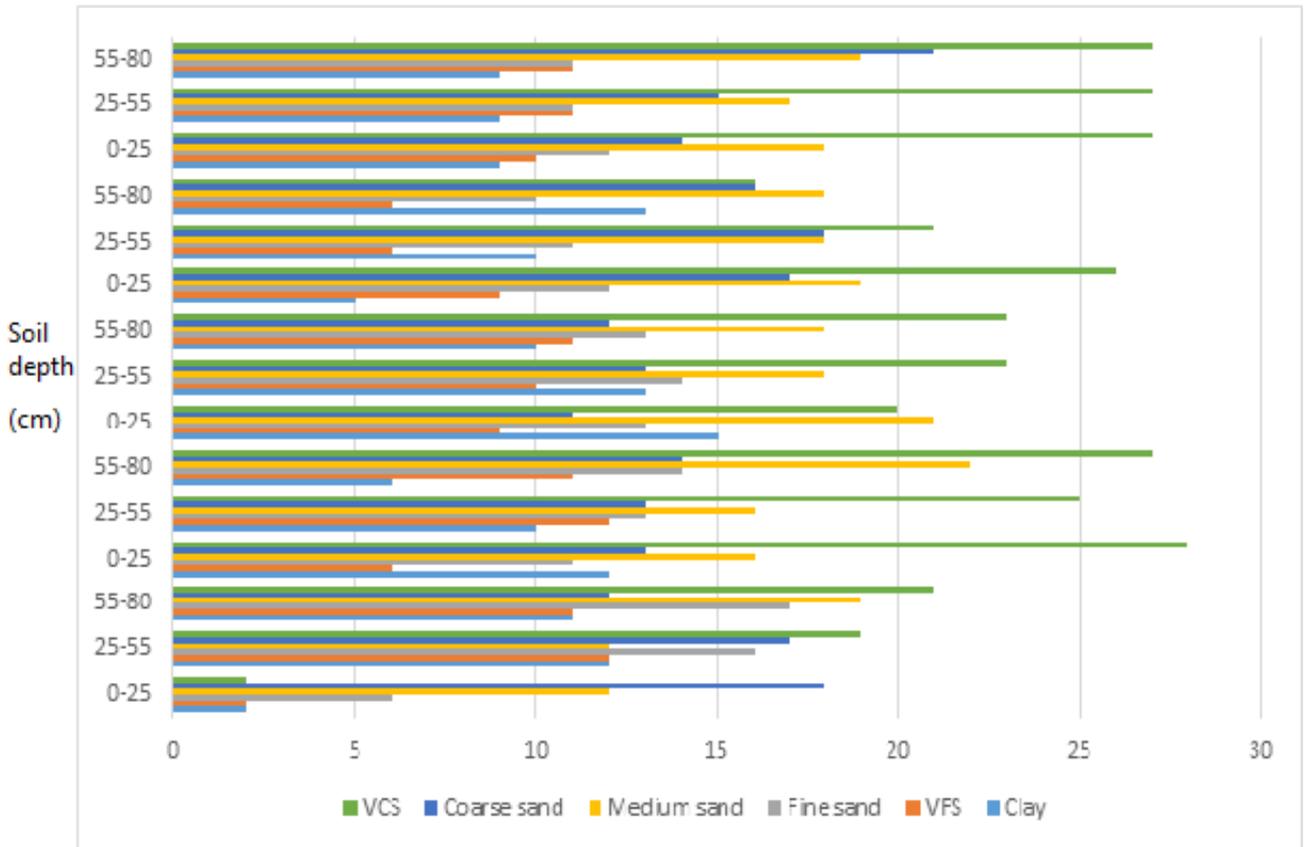


Fig. 4. Particle size distribution of soil at various depths of the study area.

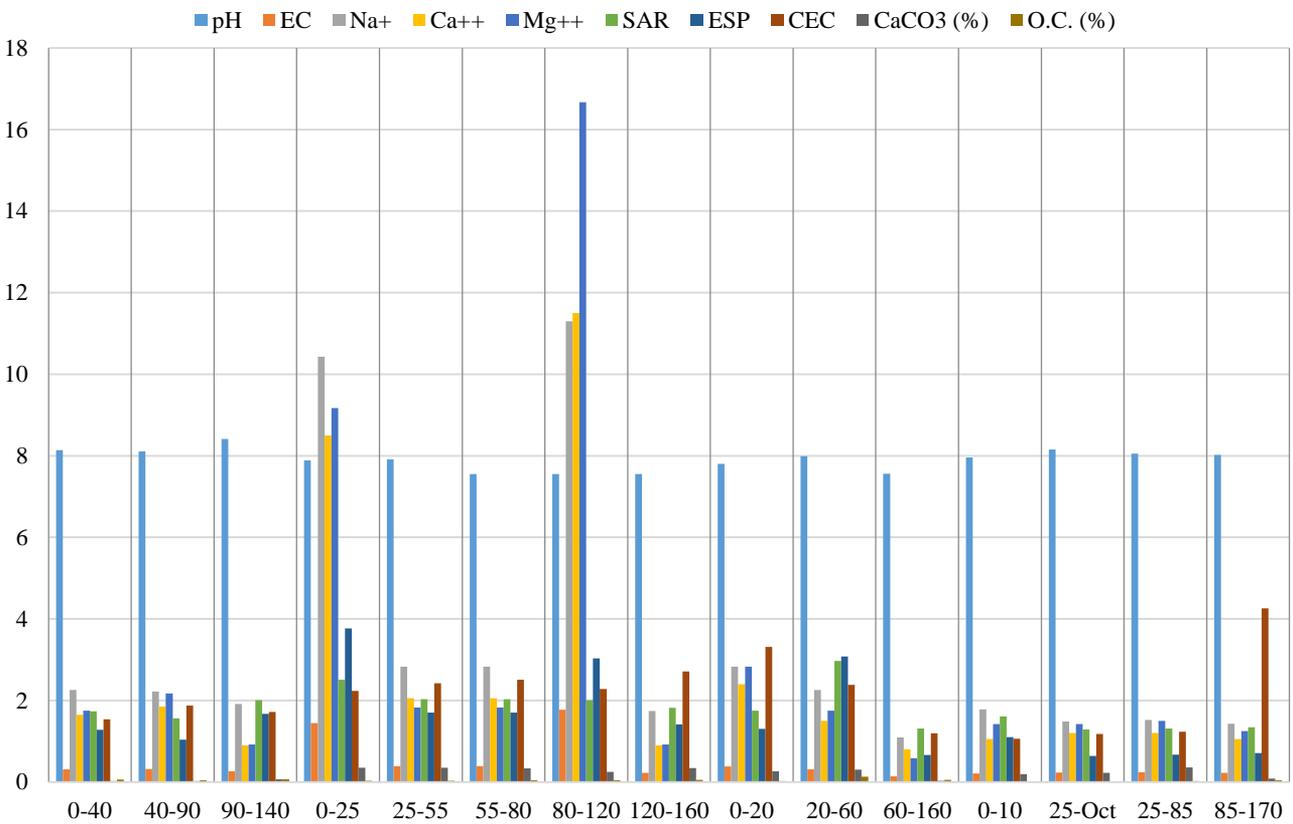


Fig. 5. Chemical properties of soil samples collected from Wadi Al-Adeirey (mg/L).
 EC = Electrical conductivity; SAR = Sodium adsorption ratio; ESP = Exchangeable Sodium Percentage; CEC = Cation exchange capacity; O.C = Soil organic carbon.

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