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Original article

Heavy metal content and microbial population in the soil of Riyadh Region, Saudi Arabia

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ABSTRACT

Heavy metal content, soil moisture, organic matter, pH, electrical conductivity, and the population of bacteria and fungi in the soil were all investigated. In the Riyadh region of Saudi Arabia, soil samples were collected from Al Muzahmiyya, Al-Moqbel, Wadi Hanifa, the Sadus Dam area, Al Uyaynah, and Bawdah. The amount of Fe, Zn, Ni, Cr, Cu, Co, Pb, Cd in soil samples was measured using ICP-MS. The bacteria and fungi population was determined using the soil plate count method. The correlations between the variables were evaluated using principal component analysis, aggregative hierarchical clustering, and Pearson's correlation. The data reveals that the soil pH, electrical conductivity, moisture content, and organic matter ranged from 8 to 8.9, 1.7 to 37.4 ds/m, 3.3 to 26.7%, and 5.9 to 2.0%, respectively. The mean quantity of heavy metals was detected in the following order: Fe > Zn > Ni > Cr > Cu > Co > Pb > Cd. The quantity of all heavy metals was less than the pollution level. The population of bacteria ranged from 6.54 CFU log/g to 7.07 CFU log/g, whereas the fungi population was between 4.19 and 4.67 CFU log/g. From the soil samples, Aspergillus, Penicillium, Mucor, Fusarium, Alternaria, Trichoderma, Rhizopus, and Botrytis were identified. The soil pH and the population of bacteria and fungus had a strong negative correlation. A negative correlation was also found between Cd and the population of bacteria and fungi. A positive correlation was noticed between fungi and Zn. The effect of soil properties on fungal diversity was also noticed. Thus, it can be concluded that the soil properties in this area vary greatly. The soil of this region is mild to very alkaline, with a moderate to high salinity. The findings demonstrate that soil factors have an impact on the region's microbiota. Although the heavy metal content in this region is not yet above the pollution threshold, a timely evaluation is suggested.

1. Introduction

Soil is a dynamic and extremely complex ecosystem with many functions. The soil composition is made up of both abiotic and biotic components. The soil generally consists of 45% mineral, 5% organic matter, 20–30% water, and 20–30% air. Geographic and climatic variations throughout time and space have caused a vast variety of soil qualities. The soil composition may alter every day due to water infiltration, weather change, and human activities (Li et al., 2021; Voltr et al., 2021). The abiotic factors of the soil in a specific area define the biotic factors of that area, which include plants, animals, and microbes. In reaction to changes in environmental conditions, soil microbes' activity and diversity fluctuate (Voget et al., 2006). In nature, minerals and organic components that make up the solid phase of soil are usually stable. Zinc (Zn), iron (Fe), copper (Cu), molybdenum (Mb), and manganese (Mn) are among the heavy metals that are essential for plants, animals, and microbes in minute quantities. In both prokaryotic and eukaryotic microorganisms, metals are commonly utilized for structural and/or catalytic purposes (Silver, 1998). However, above a certain threshold, they are all considered poisonous and harmful

Abbreviations: ICP-MS, Inductively coupled plasma mass spectrometer; CFU, Colony forming Unit; EC, Electrical Conductivity; OM, Organic Matter; MC, Moisture Content; PDA, Potato Dextrose Agar; NA, Nutrient Agar.

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to life. Mercury (Hg), cadmium (Cd), and lead (Pb) are some heavy metals that are not required by living organisms. Thus, the presence of these heavy metals may disrupt organisms' life cycles (Ali et al., 2019).

All ecosystems include microorganisms, which are integral and necessary components of them. Interaction between microbial biomass and hazardous chemicals, heavy metals, pesticides in the soil ecosystem is complex (Abdu et al., 2017). Other than pH, soil type, and salinity, the level and type chemicals may influence the soil microbial biomass (Lin et al., 2020; Silver, 1998; Yang et al., 2017). With specialized enzymes, some microorganisms including bacteria, protozoa, and fungus may breakdown heavy metal complexes and use the end product as part of their metabolism (Voget et al., 2006).

The Riyadh was founded on the alluvial plain of Wadi Batha, a tributary of Wadi Hanifah. The land's overall slope is to the southeast. Riyadh's weather is marked by constant hot winds and low precipitation. Temperatures in the summer range from 45 to 50 °C. Temperatures can vary by 15 °C on a daily basis. Temperatures in the winter range from 20 to 5 °C. About 30 percent of the city is covered by Quaternary Wadi deposits of fluvial or Aeolian origin. This includes clay, silt, sand, and gravel covering 30% of the area (Masoud and Aal, 2019).

Current research investigates the soil physio-chemical properties, heavy metal levels, bacterial and fungal populations in Riyadh region as well as correlation between them.

2. Materials and methods

2.1. Soil sample collection

There were six separate places selected in the Riyadh region from where soil samples were taken: from an agriculture field, natural vegetation, natural grassland, and a dam-side position (Table 1 & Fig. 1). Soil samples were collected from a depth of 10–15 cm after 3 cm of top soil was removed. Soil was collected at random from 5 sampling points of a location and mixed into one soil sample (2 kg) to represent one location. Sterilized tools were used for sampling, which was then transferred into sterile plastic ziplocked packets labeled with the collection date and location. The samples were kept at a low temperature at the sampling site and then at 4 °C in the laboratory until the analysis was performed.

2.2. Determination of physical and chemical soil properties

A pH meter was used to measure the soil pH. A soil suspension of 1:2.5 (soil: water) was used for measuring pH after shaking for an hour (Rhoades, 1986). Meanwhile, the soil moisture content (MC) was determined by oven drying at 103 °C for a total of 12 h. Electric conductivity meter was used to estimate the electrical conductivity (EC) in the soil saturated paste extract of sample. Loss of ignition method was used to estimate the organic matter (OM) (Nelson and Sommers, 2018).

Table 1
Soil sample collection site.

Code	Location	Coordinates	Type of Land
RS 1	Al-Muzahmiyya	24°26′58.7″N 46°00′43.0″E	Natural Grass land
RS 2	Al Moqbel Palaces	24°31′02.0″N 46°19′38.4″E	Agriculture farm
RS 3	Wadi Hanifa, Diriyah	24°42′28.7″N 46°34′34.0″E	Natural vegetation
RS 4	Sadus Dam	25°01′52.8″N 46°08′45.8″E	Near the Dam
RS 5	Bawdah	24°54′05.9″N 46°17′37.6″E	Natural Grass land
RS 6	Al Uyaynah	24°54′06.2″N 46°23′07.5″E	Agriculture farm

2.3. Determination of heavy metal content by ICP -MS

Using an inductively coupled plasma mass spectrometer (ICP-MS) (Agilent 7800, CA, USA), soil concentrations of Cu, Zn, Cr, Cd, Pb, Fe, and Ni were determined after extracting with HCl-HNO_3-HF-$HClO_4$ extraction. 0.1 g of soil sample was digested in 3 mL 37% HCl, 1 mL 65% HNO_3, 6 mL 65% HF, and 0.5 mL 65% $HClO_4$ during the two stages of heating. The digestion solutions were evaporated to near dryness before being dissolved in 1.0 mL of 65% HNO_3, followed by 20 mL of deionized water (Westerman, 2018). The Australian Soil Laboratory (ASL) Group in Riyadh, Saudi Arabia, performed the ICP-MS analysis.

2.4. Determination of bacterial and fungal population in soil by plate count

For the isolation of bacteria and fungi, the serial dilution technique was used, which is a classic microbiological procedure. Bacteria and fungi were isolated using Nutrient Agar (NA) and Potato Dextrose Agar (PDA). For the plate count of bacteria and fungi, soil dilutions of 10^5 and 10^3 were utilized, respectively. All isolated colonies of fungi were purified by two rounds of sub culturing on PDA in order to identify the genus of the isolated fungus. The isolated colonies of fungi were categorized into different genera according to their cultural and microscopic characteristics (Erickson, 1990; Stefanis et al., 2013). The number of samples from which a specific strain was identified was used to compute its relative abundance (%) in that location (Zhong and Cai, 2004)

2.5. Statistical analysis

Aggregative hierarchical clustering (AHC), principal component analysis (PCA), Pearson's correlation coefficient (r), ANOVA and Tukey (HSD) post hoc analyses were done through XLSTAT (version. 2021.3.1.1162).

3. Results

The soil samples were collected from 6 locations in the Riyadh region, ranging from the west to the north. The results of MC and OM are shown in Fig. 2. MC ranged from 3.3% to 26.7%. Maximum MC was recorded from Al-Muzahmiyya (RS1) and minimum from Al Uyaynah (RS6). The maximum level of OM was found in Al-Muzahmiyya (5.9%) and the minimum was found in Al Uyaynah (2.0%), there was no significant difference ($P < 0.05$) in the OM of other soils. In terms of chemical properties, the pH of the soil ranged from 8.0 to 8.9. While the soils of Al-Moqbel (RS 2) had the lowest pH, the soils of Wadi Hanifa (RS3) had the highest pH value. The EC values ranges from 2.5 to 37.4 dS/mm (Fig. 3). Sadus Dam area soil had the lowest EC, whereas soil of Wadi Hanifa (RS 3) had the highest (RS 4).

To determine the concentration of heavy metals in the soil samples, ICP-MS was performed. The results showed that all of the heavy metals studied were present in varying levels in the samples (Table 2). The mean concentration of different heavy metals showed that Fe was present in highest amount followed by Zn > Ni > Cr > Cu > Co > Pb > Cd. The metal content of Fe, Cu, Co, Cr, and Ni were highest in the Sadus dam area (17800 mg/kg, 20 mg/kg, 10 mg/kg, 38 mg/kg, and 48 mg/kg, respectively), whereas the maximum concentration of Pb was in Al Uyaynah (9 mg/kg) and Zn was highest in Al-Muzahmiyya (58 mg/kg). All

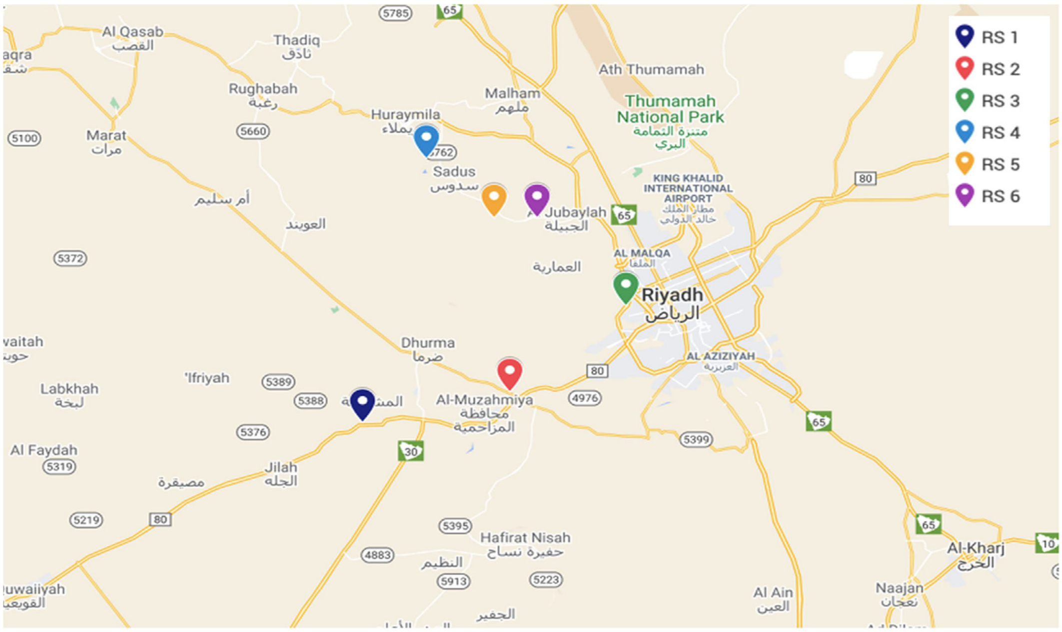

Fig. 1. Location of soil sample collection site, Riyadh, Saudi Arabia.

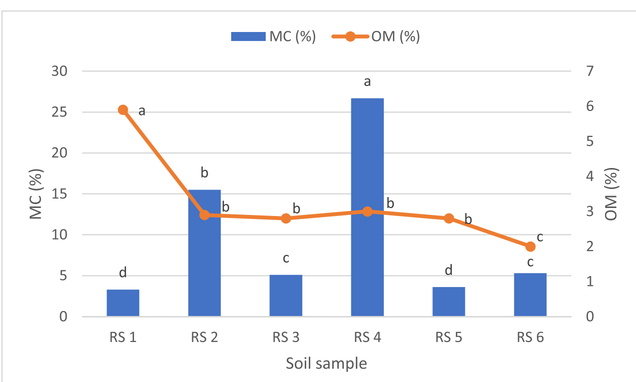

Fig. 2. Moisture content (MC) and organic matter (OM) of the soil of the Riyadh region.

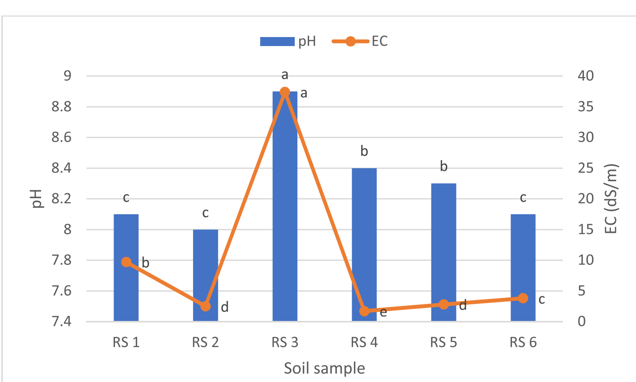

Fig. 3. pH and Electrical conductivity (EC) of the soil of the Riyadh region.

soil samples had a Cd value of<1 mg/kg, with the exception of the Sadus dam area and Bawdah, where the Cd content was 1 mg/kg.

In all analyzed soils, the bacterial population was higher than the fungus population (Fig. 4). The bacteria population ranged from 6.54 CFU log/g to 7.07 CFU log/g, with the Al Maqbel area showing the highest bacterial population. The fungi population ranges from 4.19 CFU log/g to 4.67 CFU log/g, with highest in Al Mazzamiyah (Fig. 4). The variation in the fungal diversity at the genus level was also observed (Fig. 5). Most prevalent genera identified were *Aspergillus* (20%) and *Penicillium* (20%) followed by *Fusarium* (13%) , *Mucor* (12%), *Alternaria* (10%), *Trichoderma* (5%), *Rhizopus* (6%) and *Botrytis* (4%).

In order to better understand the correlations between variables, the data was statistically analyzed using principal component analysis (PCA). F2 and F1 PCA account for 77.45 % of total variance (Fig. 6). It accounts for 18.13 % of variance and has a positive correlation with fungus and bacteria, while F1 accounts for 59.32 % and has a strong positive correlation with heavy metals. The biplot indicates that there is a strong relationship between RS4 and RS5, while RS1 is clustered with RS6 and RS2, and RS3 is similar to RS2. Furthermore, aggregative hierarchical clustering (AHC) verifies PCA findings. Two major clusters, A and B, are shown in the dendrogram. RS 4 and RS 5 are grouped in Cluster A, while the rest of the soils (RS 3, RS 1, RS 2 and RS 6) are in Cluster B (Fig. 7).

Pearson's correlation analysis was done to understand the correlation between pH, EC, MC, OM, heavy metals, bacteria and fungi (Table 3). A high negative correlation has been noticed between pH and bacterial ($r = -0.915$) and fungal populations ($r = -0.880$). Whereas, all heavy metals except Cd had a mild to weak negative correlation with pH. A strong negative correlation was found between EC and bacteria population ($r = 0.856$), Cr ($r = -0.834$), and Ni ($r = -0.812$), while other variables except OM exhibited a moderate to weak negative correlation. MC had a weak positive correlation with bacteria, Zn, Ni, Fe, Cu, Co, Cr, Cd, but a weak negative correlation with fungi, OM, Pb. There was a moderate positive correlation between OM and fungus population ($r = 0.554$) and Zn ($r = 0.575$), but only a weak correlation with Cu, Ni, Co, Cr, and Fe. In contrast, there was a weak negative correlation between OM and Cd, Pb, and the bacteria population. Cd demonstrated a positive correlation with all heavy metals, but a negative correlation with the population of fungi and bacteria in soil. With Ni ($r = 0.994$) and Fe ($r = 0.991$), Cu ($r = 0.981$), Co ($r = 0.968$), and Zn (0.871).

Table 2
Heavy metal content in the soil of the Riyadh region, analyzed by ICP-MS.

Code	Heavy metals							
	Cadmium mg/kg	Chromium	Cobalt	Copper	Iron	Lead	Nickel	Zinc
RS 1	<1	30	8	17	13,200	7	38	58
RS 2	<1	25	5	11	9880	<5	30	51
RS 3	<1	11	<2	<5	4790	5	12	37
RS 4	1	38	10	20	17,800	8	48	57
RS 5	1	33	10	17	14,900	8	45	55
RS 6	<1	24	6	11	10,600	9	29	40
Mean	<1	26.83	6.82	13.48	11861.67	6.98	33.67	49.67
SD	0.052	9.326	3.156	5.535	4512.509	1.698	13.095	9.026
FAO/WHO	3.0	100	50	100	NG	100	75	300

SD- Standard deviation (n = 6); NG- No guidelines.

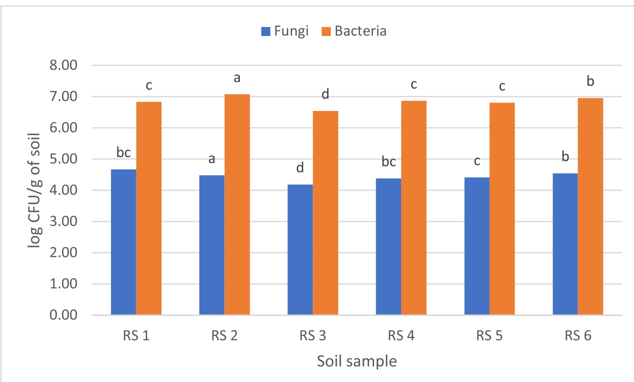

Fig. 4. Bacteria and fungi population in the soil of the Riyadh region.

Pb, fungus, and bacteria showed moderately positive correlations with Cr. Co had a strong positive correlation with Ni (r = 0.986), Fe (r = 0.977), Cu (r = 0.971), and Zn (r = 0.828), but a weak correlation with Pb, fungi, and bacteria population. Cu correlated strongly with Fe (r = 0.984), Ni (r = 0.980), and Zn (r = 0.902), and moderately with Pb, fungi, and bacteria. Fe had a strong positive correlation with Ni (r = 0.987) and Zn (r = 0.835), as well as a weak to moderate relationship with Pb, fungi, and the total CFU of Bacteria. Pb and Ni, Zn, as well as Fungi and Bacteria population had a moderate to weak positive correlation. Ni had a high positive correlation with Zn (r = 0.874) and a moderate positive correlation with the population of fungi (r = 0.443) and bacteria (r = 0.429). Zn exhibited a moderately positive relationship with the population of fungi (r = 0.494) and bacteria (r = 0.358). Between the populations of bacteria and fungi, there was a positive correlation (r = 0.645).

4. Discussion

The current investigation demonstrates that soil properties vary widely in this region. The current results show that the soil in Riyadh region is mild to extremely alkaline, whereas EC values indicate that the soil is moderate to highly saline. The current findings differ from previous reports. Masoud and Aal (2019) found an average pH of 7.64 in the Riyadh region, however their sample sites were different from the present study. While, a study reported mean pH and EC values of 7.7 and 1.03 dS/m, respectively, from the Riyadh region (AL Barakah et al., 2020). Another study recorded pH range from 7.58 to 7.76 and EC 22.5–32.0 from the second industrial city in Riyadh (Siham, 2007). Irrigation water and dissolving soil minerals are some sources of salts in soil. Moreover, the Riyadh region is rich in weathered limestone and the climate is exceedingly arid, therefore evapotranspiration exceeds precipitation. These factors may have caused rise in pH and alkalinity (Masoud and Aal, 2019; Oumenskou et al., 2019). The organic matter content of the soil at the six sites varied less. The maximum OM

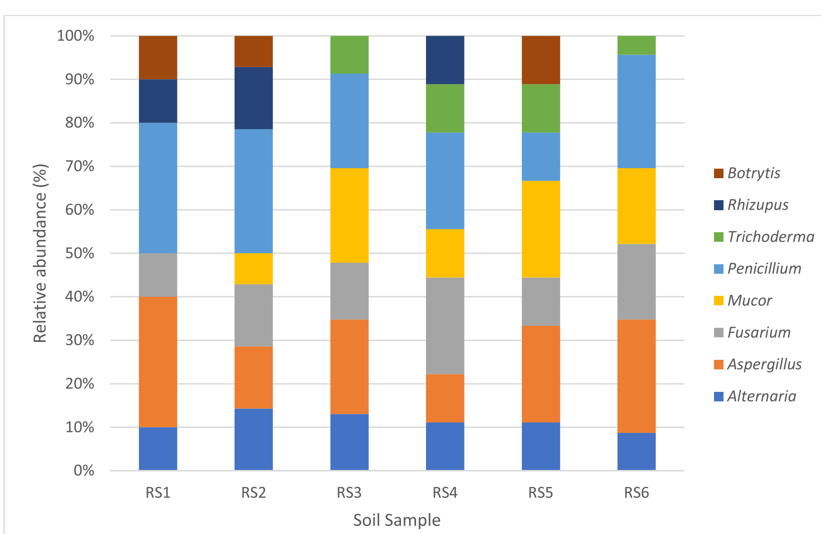

Fig. 5. Distribution of fungal genera in the soil of the Riyadh region.

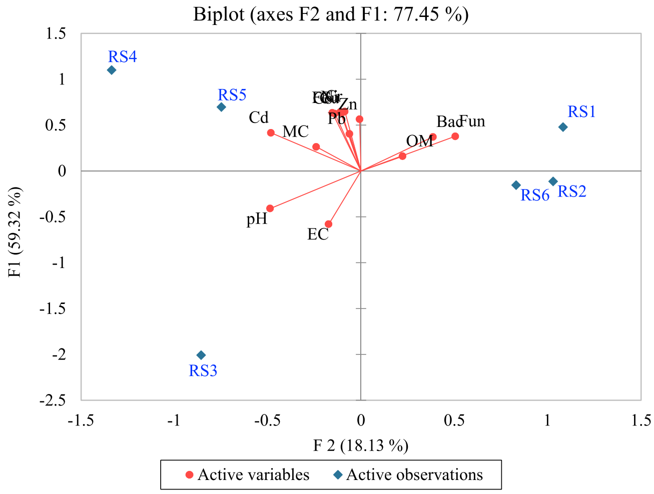

Fig. 6. PCA biplot of the soil physio chemical properties, heavy metal content, bacteria and fungi population in the soil of the Riyadh region. (P = 0.05).

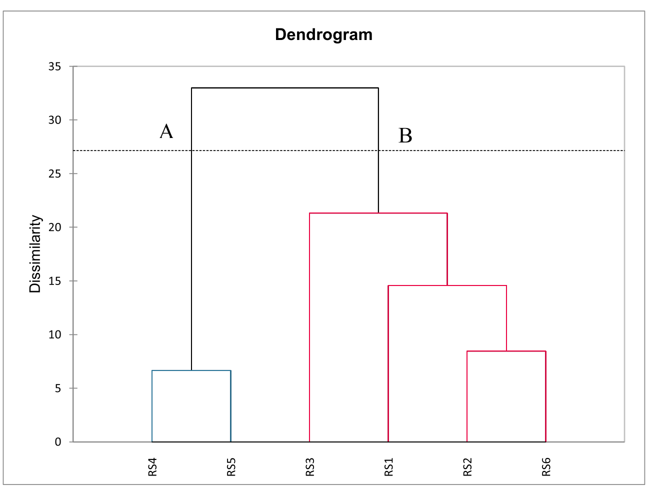

Fig. 7. Dendrogram of aggregative hierarchical clustering analysis (Ward's method).

was recorded from Al Mazzamiyah. Since the soil sample from Al Mazzamiyah site was taken from a grassland, the increased amount of organic matter is justified as dead plant materials decompose into OM. The concentration of heavy metals in the examined soils were much below the permitted threshold (FAO/WHO, 2001). The results of present findings are consistent with prior reports (Al-Hammad and Abd El-Salam, 2016; Al-Shayeb, 2003; Al-Shayeb and Seaward, 2001; Hasayan et al., 2017). However, the present results contradict the findings of Alturiqi et al., (2020) who reported greater amounts of Cd from Al Mazzamiyah and Al Uyaynah. Current study also noted higher Fe values than previously reported from this area (Al-Hammad and Abd El-Salam, 2016; Alturiqi et al., 2020). Alshayeb found that heavy metal concentrations of lead, copper, chromium, zinc and lithium differed between the soil's surface and subsurface layers as well as both urban and rural areas had different heavy metal concentrations (Al-Shayeb, 2003)

Soil biogeochemical processes rely heavily on microorganisms. Their abundance and diversity in soil, therefore, are vital. In all of the study sites, bacterial CFU was higher than fungal CFU. AL Barakah et al., has also documented a larger prevalence of bacteria than fungus in the Riyadh region (AL Barakah et al., 2020). A higher soil pH favors bacterial growth over fungal development (Rajapaksha et al., 2004). The data indicates that fungal diversity varied in studied area, that can be related to the soil physio - chemical properties. The soil parameters influence the function and microbial diversity (Zhong and Cai, 2004). The genus *Aspergillus* and *Penicillium* were the most prevalent genera in all soil samples examined. Also, *Mucor, Fusarium, Alternaria, Trichoderma, Rhizopus*, and *Botrytis* were found in the soil samples. Recently, a group of researchers reported microbial diversity and abundance in the Sabkha and desert areas of Saudi Arabia. *Fusarium, Alternaria, Chaetomium, Aspergillus, Cochliobolus*, and *Pencillium* were the most frequent fungal genera (Alotaibi et al., 2020). Whereas, *Chaetomium* sp. *Bipolaris* sp. and *Fusarium venenatum* were isolated from the soil of AlQasab, Tabuk, and Almuzahimiyah, Saudi Arabia (Mohammed et al., 2021). A study on the soil of Riyadh's Second Industrial Area found *Aspergillus, Fusarium, Mucor, Penicillium, Alternaria, Cephaliophora*, and *Eurotium*, the most common genus was *Aspergillus* (Siham, 2007). Another study reported *Aspergillus, Fusarium, Penicillium*, and *Ulocladium* as the most common genera in Riyadh's Jubail industrial area (Hashem, 1995). The fungal diversity in RS1, RS2, and RS5 was higher than in the other soils investigated. It has been observed that high pH limits the diversity of fungi in soil, whereas minerals and organic matter support fungi diversity (Abdu et al., 2017). It was noticed that the soils of R1, R2, and R5 had high OM, this may have aided in fungal diversity. Some Researchers reported strong correlations between soil fungal diversity, plant and soil attributes, and soil metrics, regardless of land use type. In both direct and indirect ways, soil and plant factors influenced fungi diversity (Yang et al., 2017). Culturing techniques have traditionally been used to study soil microbial communities. However, there is a chance of only a limited portion of the community being cultured, and other methods such as molecular, sequencing, and hybridization techniques can be used to investigate the microbial community (Rajapaksha et al., 2004; Stefanis et al., 2013).

Statistical analysis provides a much clearer overview of the data acquired. AHC analysis is a multivariate approach for identifying genuine data groups and grouping samples based on similarities. Ward's method was employed for agglomeration in AHC analysis, while Euclidean distance was used to measure similarity. Whereas the PCA was used to reduce the dataset into new variables. The PCs can address the majority of differences in the original variables. In addition, to find out the correlation between variables Pearson's correlation analysis was included. Similar studies have been carried out in different parts of the world (Abdu et al., 2017; Lin et al., 2020; Oumenskou et al., 2019; Rajapaksha et al., 2004; Yang et al., 2017), this is, however, the first study from this region.

According to AHC, the soil parameters of the RS4 Sadhus Dam area soil and the RS5 Bawdah grassland soil were similar. Whereas, despite the fact that the soil samples for RS1 and RS5 were gathered from grasslands, they were categorized into separate clusters. Many factors cause soil metamorphism, a variety of geological and hydrological processes are responsible for wider range of soil properties (Al-Harazin and Abderrahman, 2003; Masoud and Aal, 2019). Furthermore, an arid and semiarid region is distinguished by a diverse set of soil properties (Al-Harazin and Abderrahman, 2003).

pH demonstrates significant correlations with other properties. Soil pH affects other soil variables and controls the soil physical, chemical, and biological properties. The mobility of heavy metals in soil is up to some extent is governed by soil pH. It has a noteworthy effect on metal solubility and metal separation at the soil–solution interface (Abdu et al., 2017). Except for Cd, all heavy metals showed a positive correlation with the bacteria and fungi population. Heavy metals can have beneficial or negative affect on the microorganisms. Some of the heavy metals are used by bacteria and fungi as electron donors or acceptors during metabolism.

Table 3
Pearson's correlation matrix of soil physio chemical properties, heavy metal content, bacteria and fungi population in the soil of the Riyadh region.

Var	pH	EC	MC	OM	Cd	Cr	Co	Cu	Fe	Pb	Ni	Zn	Fun	Bac
pH	1													
EC	0.839*	1												
MC	−0.041	−0.363	1											
OM	−0.211	0.027	−0.203	1										
Cd	0.118	−0.412	0.432	−0.191	1									
Cr	−0.516	−0.834*	0.472	0.224	0.720	1								
Co	−0.416	−0.756	0.289	0.223	0.781	0.968*	1							
Cu	−0.435	−0.732	0.396	0.372	0.702	0.981*	0.971*	1						
Fe	−0.414	−0.774	0.474	0.201	0.770	0.991*	0.977*	0.984*	1					
Pb	−0.312	−0.568	−0.011	−0.136	0.464	0.596	0.686	0.584	0.646	1				
Ni	−0.483	−0.812*	0.400	0.222	0.759	0.994*	0.986*	0.980*	0.987*	0.606	1			
Zn	−0.472	−0.636	0.348	0.575	0.544	0.871*	0.828*	0.902*	0.835*	0.207	0.874*	1		
Fun	−0.880*	−0.639	−0.179	0.554	−0.227	0.469	0.425	0.479	0.398	0.392	0.443	0.494	1	
Bac	−0.915*	−0.856*	0.346	−0.090	−0.044	0.482	0.323	0.346	0.382	0.221	0.429	0.358	0.645	1

*Correlation is significant at P = 0.05; Moisture content (MC), Organic matter (OM), Electrical conductivity (EC), Bacteria (Bac), Fungi (Fun).

Microorganisms indirectly influence the properties and bioavailability of heavy metals by altering the soil configuration and chemical properties ((Lin et al., 2020; Rajapaksha et al., 2004). A report found that heavy metals differently influence various microorganisms (Yang et al., 2017). A study reported that Zn and Cu concentrations influenced the growth of bacteria and fungi (Rajapaksha et al., 2004). Despite the fact that soil microbes use heavy metals, microorganisms have proven to be the most vulnerable to heavy metal stress of all soil organisms (Abdu et al., 2017). Fungi, in general, have a substantial survival advantage over other microbes because of their strong resistance to high temperatures, pH, food scarcity, and high metal concentration levels (Lin et al., 2020). Several microorganisms have been found to be resistant to copper. A low copper concentration promoted the growth of *A. candidus* while, high cadmium content hindered it (Hashem, 1995). Even though it's well-known that many heavy metals have an injurious effect on microbial populations and diversity, there's still no consensus on the toxic level of heavy metals. One of the challenges in setting a metal limit is the necessity to estimate the amount of metal that can be utilized by the microorganisms, rather than total soil metal concentrations (Abdu et al., 2017).

5. Conclusions

The present study ascertain that a variation in soil properties and microbial population exist in the soil of Riyadh region. The soil of this region is mild to very alkaline, with a moderate to high salinity. Heavy metal content was present in varying amounts but within allowable levels. Fungal diversity at genus level was also noted from this region. Although, the heavy metal content in the studied soils is lower than the specified pollutant level, there is cause for concern because the city's development and expansion may cause changes in the soil environment, which may impact the region's heavy metal content and microbial richness. Thus, a timely comprehensive evaluation of the soil properties and microbial diversity is suggested. The current research will help analyze environmental conditions and microbial diversity, as well as lay the groundwork for future studies.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could influence the work reported in this paper.

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