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Tracing environmental effects of microfabric in leaves of *Cupressus dupreziana* plant and soil surrounding it given the rise in COVID19

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Abstract

Background: The high death rate from COVID-19, which may contribute to increased mineral pollution in cemetery soils and necessitates future cemetery projects that are able to counteract these environmental effects, highlights the significance of studying metal concentrations in cemetery soils. The research analyzed the quantities of minerals in the cemetery soil in Najaf Governorate, Iraq, taking into account the elevated mortality during the sampling period in order to identify future projects for cemeteries targeted at mitigating environmental consequences.

Methods: Using energy dispersive X-ray spectroscopy (EDX) and scanning electron microscopy (SEM), the researchers investigated the leaf features of the soils in three cemeteries. Soil samples were taken three times from a depth of 10 to 30 cm *Cupressus dupreziana* plants with triplicate analysis, mean deviation, and used in the autoregressive integrated moving averages model, with 50% traceability to bioavailability to reduce contamination risk.

Results: The findings revealed elevated amounts of iron and lead (Pb) (Fe), tomb soil analysis. Take into account the rise in fatalities and the associated funerals. According to COVID-19, the estimated daily mortality toll is between (20-50); however, metal pollution may be more severe. In order to lessen the effects on the environment, the facility was expressed through gas and effluent processing at 80% in soil of the proportion of the curve in the spatial analysis for mineral contamination.

Conclusion: The SEM and EDX serve as reliable indicators, while the morphometric approach offers a holistic understanding of the soil and its associated plant life by analyzing elemental compositions. Employing these methods allows for quantitative assessment and comparison against the control, aiding in the thorough characterization of *Cupressus dupreziana*. Furthermore, these analyses shed light on the various organism clusters found within the study area's soil.

Introduction

The Wadi al-Salam cemetery is located in Najaf, one of Iraq's governorates. It is delimited to the south by the mausoleum of Imam Ali bin Abi Talib, on the east by the Najaf road leading to Karbala, and on the west by the old Sea of Najaf, commonly known as the Dead Sea. Religious narratives without historical context state the cemetery's return to pre-Islamic periods. This cemetery is 700 hectares in size and contains millions of bodies. The city of Najaf is one of the largest in Iraq, with a population density of one million people, but it is the city of the dead, which contains millions of remains and corpses, because it stretches for more than ten kilometers along the valley The cemetery has two types of burials: tombs that exceed 80 cm in height and catacombs that are 5 to 8 meters underground. The cemetery has been in operation for over 1,400 years and is a UNESCO World Heritage site [1].

Burials in Najaf have been documented since the Sassanid and Parthian eras, when tombs similar to them were discovered. It has been stated that 200-300 persons were buried there daily during the Iraq war, but by 2010, the number had reduced to less than 100 per day, with 500,000 burials held each year. In 2014, as part of the fight against ISIS, it was reported that the cemeteries had moved beyond the bounds of the Najaf Governorate [2]. Image analysis has proven to be the most accurate phenotypic description of environmental samples that are continuously exposed to pollution over time; thin sections were analyzed for the first time with image analyzers previously by [3].

According to, an image analysis technique was created to assess structure by quantification in all aspects such as pore size, pore shape, distribution, irregularity, orientation, and continuity on thin sections[4]. This morphometric technique has the advantage of combining pore space measurement and characterization with visual appreciation of the type and distribution of pores at a specific point in their dynamic evolution, and it has improved methods for direct quantification of pores and determining the type and level of damage. Image analyzers use a lightweight electronic analysis to examine images taken by a light or electronic microscope. The SEM may be used to study the thin sections at various magnifications. For example, if the image analyzer analysis starts at x500 magnification and the pixel size is 0.2 m, pores in the range of storage pores 0.5-50 m can be detected from the back-scattered electron scanning images [5]. Pore patterns analysis allows for the characterization and prediction of flow processes by determining the form, width, and length of these pores. Several studies have revealed variations in pore size distribution at water content when soil is saturated with water after rain and splits close as a result of saturation changes in pore size distribution, according to [6].

The extra metal in the soil is caused by anthropogenic sources such as human waste. Similarly, while human body decomposition is a natural process, it is linked to the emission of greenhouse gases into the atmosphere as well as the entry of metallic pollutants into cemetery soil [7]. Due to the natural decomposition of multiple bodies in a small region, metals have been demonstrated to contaminate communities within a 400-500 m radius of such spots , according to [7,8,9] Metals accumulate by remains during the natural process of decomposition and take the form of necroslurry, which contributes to physical contamination that can contaminate underground water supplies as well as soil, creating worries about cemetery contamination[10]. As a result, there exists an increasing need for studies on the environmental impacts of cemeteries in cities around the world in order to recommend public policies that safeguard soil and population health through increased types of necroslurry treatment 2020 and deaths 25,356 last update 10-22-2022.Each 70 kg cadaver emits roughly 30 L of necroslurry into the environment during the decomposition process[11,12,13]. The COVID-19 epidemic has dramatically increased mortality statistics [1], leading to the notion that there is a considerable increase in the content of metals in cemetery soil. The pandemic also raised overall mortality by 1.3% due to high rates of burial .

Because the COVID-19 outbreak caused high mortality rates and an increase in burials, it is critical that this study reveals metal contamination in the soil and vegetation surrounding the cemetery. The importance of this study is highlighted by the high rate of burials, as contaminants must be monitored in cemeteries in order to use the methodology described here for future global research.

Methods

Sample collection

Soil and *Cupressus dupreziana* leaf plants were collected and spaced every 100 meters from cemeteries A, B, and C. Soils were tested at two depths, from 10 to 30, and three levels of repetition between the sites, for a total of 18 samples according to protocol [14,15] on December 2021. Following each collection, the sampled material was sent to the Ministry of Commerce's Laboratory of Environmental and Instrumental Quality Control, where the procedures were as follows: analytical in triplicate with mean deviation and analytical curve with traceability percentage of pollution risk limit.

Scanning electron microscope (SEM) and EDX (energy dispersive X-Ray spectroscopy) to examine elements characteristics leaf of *Cupressus dupreziana* plants and soil near and far plants and analyses elements (Pb, Fe)
The scanning electron microscope (SEM) was the first to create images of sample surfaces based on morphology and composition using scattered electrons. It also offers a three-dimensional image system that enables measurement of individual particles to provide overall image analysis with results that are distinct and fall between (2000-5000). KX magnification with dominant fabric was chosen region in relation to the scanned surface's inspecting dimension for more precise details according to [16].

The EDX element images are then placed in a metal experimental tray, which is either white for dark specimens or black for light specimens, and then incident reflected light is used to examine the specimens. The tray may also contain a comparison grid, which is frequently treated with a sticky substance to keep the particles in place while being examined by microscopic examination, effectively allowing for the examination of specimens using transmitted polarization [16]. Lead and iron concentrations were measured in plants and soil of the study areas in leaf plants samples were collected with 3 replicates of each sample, then dried outdoors at room temperature for 3-5 days, then grinded with a mill and sifted with a 1 mm diameter sieve to be ready for analysis. Soil Samples one soil samples were collected from contamination sites with 3 replicates of sample by using cleaned polyethylene bags from 30 cm in depth and measuring concentration it by according to [17].

Results

Leaf plants and soil samples were taken in the inner area of cemeteries using the average depth pattern, and the results were obtained. The study of iron and lead revealed an increase in concentration; it has been established that exposure to too much iron in the environment might result in iron toxicity acidic soil and the presence of oxygen significantly influence soil fertility levels [14,18].

The levels of these metals found in graveyard soil and uppermost layers are quite concerning. The concentrations of iron and lead in the plant's leaves and the soil near and far from cemeteries are shown in Figures 1 and B. A quadratic movement in the behavior curve indicated a higher level of analysis sophistication. Although it indicated high metal concentrations, indicating contaminations over the WHO and CETESB-recommended limits, it may also disclose another significant factor that influenced its behavior in the investigated soil layers. Since wells provide the area's private and public water supplies, it is therefore possible to increase awareness of the extent of lead pollution in the interior region of the tombs, presuming that it can extend to the groundwater reservoirs beneath. Since there isn't yet a standard for an acceptable concentration, the high levels of Fe found inside tombs also serve as a warning , but according to [15,16], in the global COVID-19 fatal case forecast, this indicates that the environment surrounding these investigated graves may be problematic for this particular chemical element. The forecast ARIMA model is based on historical performance, this model is used as a forecasting tool to anticipate future behavior. It is employed in technical analysis to forecast the performance of an asset in the future and was only used in this investigation on COVID-19 fatal cases that have been reported internationally. Through this process, it was possible to understand the time series' behavior, especially if it had a unit root or was stationary. The series does not exhibit the steady behavior needed for modeling, the absence of stationary behavior in the series is most likely due to the aggressive spread of COVID-19. The temporal series' examined results were found to be non-stationary using the KPSS test statistic of 7.45 with p value 0.01). Given that the series possessed a unit root, the feasts R package function was utilized to look into the minimum number of deviations needed to guarantee stationary. Next, two adjustments were applied to the original series using unit root 2. In order to train the data with 95% of the entire data regular data without any gaps a weekly seasonality was found in the daily data, which was the result of another test using the feasts package to look for the presence of dead bodies with different sites[17].

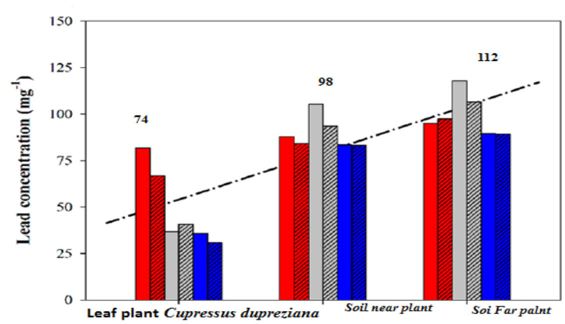

Figure 1a: Lead concentration in leaf *Cupressus dupreziana* plants and soil surrounding it.

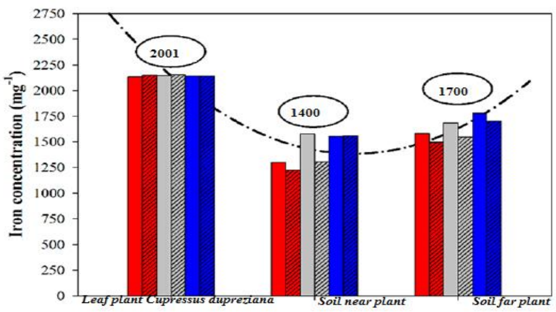

Figure 1b: Iron concentration in leaf *Cupressus dupreziana* plants and soil surrounding it.

The method in the fable package fits the series using maximum likelihood estimation and the corrected information criterion , which is the best lowest information criterion. An ARIMA analyses getting been (1, 2, 3) sites (0,1,1)MPE in plants three sites , model was chosen as the model with the best fit, the following were the test set's primary accuracy measures: 0.21490 and 0.32463; 0.24106 MPA in three sites soil are frequently scale-free methods for point forecast, cumulative fatal cases caused by COVID-19 from January 22, 2020, to July 6, 2021, according to [18,12] . Source: Unprocessed data obtained from the Center for Systems Science and Engineering (CSSE) at Johns Hopkins University's COVID-19 data repository. It was determined when and how much plant morphology was impacted by various elements, and different methods of magnifying and microscopically examining leaves were contrasted. With the high energy of a charged particle from the electrons within the sample in discrete an energy levels number, it enables us to ascertain the amount of pollutant accumulation caused by soil samples surrounding these plants in each study area as a preliminary investigation crucial for any damage, resulting in anticipated risks that adversely affect the environment according to [19,20]. As a result, *Cupressus dupreziana* leaf emission has been stimulated. The relationship between a material's behavior and its relationships to more data about its natural properties is tracked by its elemental composition. It served as the starting point for a microscope examination of samples from all study areas. We were given a clear picture of the data showing a significant correlation for any sample in total contents and relative abundances as well as important weather pollutants from the effect source sample in natural area used as a control examination. An overall observation image analysis of the cemetery was performed using magnifications between (2000, 5000) KX.

Figures (2 and 3) showed most accurate images of the thicker fibrous material were obtained using a scanning electron microscope (2000 and 5000 x Mg), and they showed that the surface was covered by additional layers. The total number of fabrics was as follows: D Fabric 51.5 m and A Fabric 31.4 m in (2000 KX at 5 m) (5000 in 10m). With 5% fibric present in this plant, the percentage composition serves as a reliable pollution indicator and serves as a key sign of accumulations with high filament percentages that cause the outer layer [21].

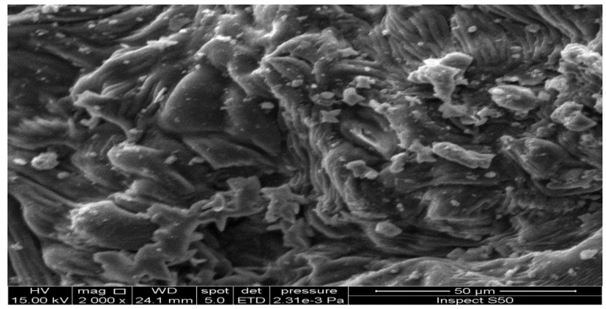

Figure 2: SEM Images of *Cupressus dupreziana* 2000KX Magnifications.

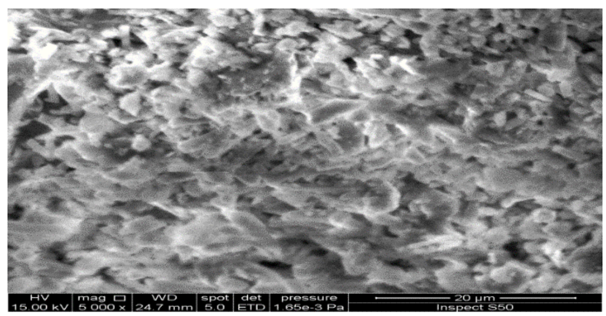

Figure 3: SEM Images of *Cupressus dupreziana* 5000KX Magnifications.

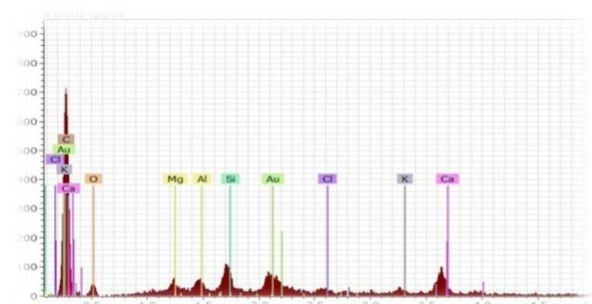

Figure 4: EDX elemental mapping images of *Cupressus dupreziana.*

Figure (4) depicted the EDX elemental mapping images of carbon, antimony, oxygen, zirconium, calcium, silicon, ruthenium, aluminum, magnesium, and potassium at the center metal oxide sintered compact, while the other wire embedded in resin was not observed because the incident electron has a very clear penetration depth, causing the sintered compact to emit characteristic X-rays [22].

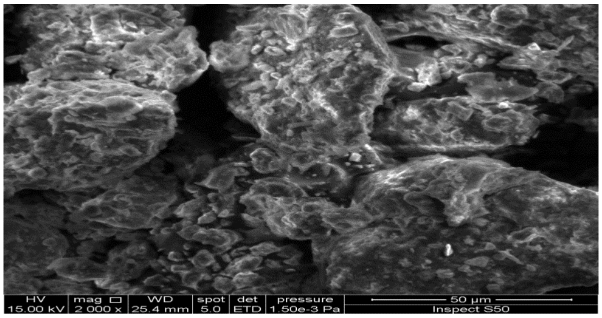

Figure 5: SEM image of Soil sample 2000KX magnifications.

The highly dense clay matrices with large inter-assemblage pore spaces and few intra-assemblage pore spaces that dominate the fabric, indicating imbalance and damage, make up the microfabric of the soil samples, as shown in Figures (5 and 6). These matrices have perturbed parallelism and very small intra-assemblage pore spaces as well as large inter-assemblage pore spaces of various shapes. In the absence and presence of 6.5% fibric at control (2000x mag), cling scanning electron microscope micrographs showed more fibric, which appeared to be covered by a thick extra layer surface based on the SEM viewing at higher 0.04 magnification .

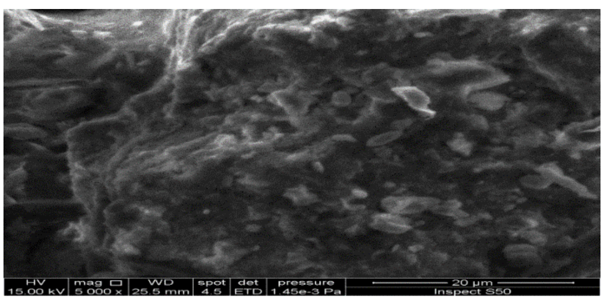

Figure 6: SEM image of Soil sample 5000KX magnifications.

The layer thickness on the surface increased by 40%, and it appeared that the composition of the elements depended on concentration where there were more layers on the surface according [22]. The fabric numbers were expressed as the mean standard deviation from determination as follows: Fabric 39.1 m in (2000 KX at 5 m), and Fabric 66.5 m (5000 in 10 m). According to leaf plant storage concentrations of metals from soil can build up over time, as a result, with the absence and presence of 6.9% fibric at cemetery, the percentage composition can be considered a reliable pollution indicator.

It became clear to us that in soils with high organic content that are subject to natural degradation, these fractions are such complex mixtures of related compounds that they exhibit a wide range of solubility and charge characteristics, particularly after degradation. Following the decomposition of the corpses, which occurred very close to the time of sample collection, Antimony, Molybdenum, Calcium, Silicon, Niobium, Aluminium, Oxygen, Carbon, Magnesium this is agree with [23,24].

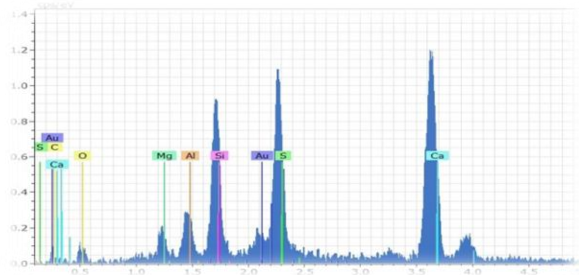

Figure 7: EDX elemental mapping images of Soil samples images.

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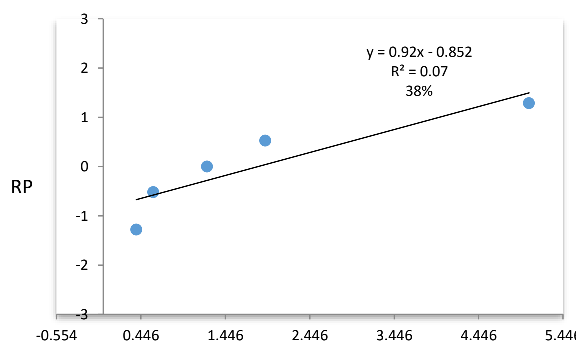

R^2 = 0.07, the percentage relative proximity values 38%.
Figure 8: The percentage of bioavailability of pollution risk limit.

The sensitivity and accuracy of factors and approximately and converging parallel these soil samples have a linear regression slope of 0.07. Figure (7 and 8), only the values over the PR then 38% of the data exist in close proximity to each other. The percentage of plant absorption is very low, indicating poor soil quality due to soil type and the highest bioavailability of pollution risk limit [25,26]. In this study, fatal COVID-19 cases were only reported by Iraqis. It was possible to understand the time series' behavior during this process, in particular the number of deaths per day if the series is stationary. The SEM and EDX can be regarded as a good indicator, and the morphometric method can produce comprehensive information about the soil and plant surrounding it through the presence of elements. These analyses will help us to know it by counting and comparing it to the control. *Cupressus dupreziana* was characterized in detail groups of organisms discovered in the soil of the study area. Random groups of organisms were discovered in the soil of the study area, the majority of which were mega fauna such as earthworms, which aerate the soil by breaking the solid bonds on its surface and transferring organic matter from the ground to the surface, which has a positive effect on maintaining the basic properties of the soil as well as developing the soil structure of the matrix of plants and other organisms. Vertebrates such as snakes, lizards, hares, mice, and scorpions also played important roles in the decomposition process. They decompose complex proteins and polypeptides, and nucleic acids in corpses, producing ammonium ions, nitrates, and nitrites, which plants then use to build tissues, a natural process in the environment.

Discussion

In this study, fatal COVID-19 cases were only reported by Iraqis. It was possible to understand the time series' behavior during this process, in particular the number of deaths per day if the series is stationary. The SEM and EDX can be regarded as a good indicator, and the morphometric method can produce comprehensive information about the soil and plant surrounding it through the presence of elements. These analyses will help us to know it by counting and comparing it to the control. *Cupressus dupreziana* was characterized in detail groups of organisms discovered in the soil of the study area. Random groups of organisms were discovered in the soil of the study area, the majority of which were mega fauna such as earthworms, which aerate the soil by breaking the solid bonds on its surface and transferring organic matter from the ground to the surface, which has a positive effect on maintaining the basic properties of the soil as well as developing the soil structure of the matrix of plants and other organisms. Vertebrates such as snakes, lizards, hares, mice, and scorpions also played important roles in the decomposition process, decompose complex proteins and polypeptides, and nucleic acids in corpses, producing ammonium ions, nitrates, and nitrites, which plants then use to build tissues, a natural process in the environment.

The SEM and EDX serve as reliable indicators, while the morphometric approach offers a holistic understanding of the soil and its associated plant life by analyzing elemental compositions. Employing these methods allows for quantitative assessment and comparison against the control, aiding in the thorough characterization of *Cupressus dupreziana*. Furthermore, these analyses shed light on the various organism clusters found within the study area's soil.

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Conflict of Interests

The authors declare no conflict of interest.

Author Contributions

Study conception and study design: Estabraq Mohammed Ati (EA) Awatif M. Abdulmajeed (AA); data collection: Basmah M. Alharbi (BA), Reyam Naji Ajmi (RA), Abdalkader Saeed Latif (AL); contributed data or analysis tools: EA, AAi; performed the analysis and interpretation of results: BA, RA, and AL; draft manuscript preparation: AL, RA, BA; Revised it critically for important intellectual content: EA and AA; Supervised the whole study and experimental work and approved for further proceeding: EA, AA, and RA; All authors reviewed the results and approved the final version of the manuscript.

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