ASSESSMENT OF SPATIAL AND TEMPORAL VARIATION IN SOIL FLUORIDE AND ORGANIC MATTER RELATIONSHIP: ITS IMPLICATIONS FOR CARBON SEQUESTRATION POTENTIAL

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ABSTRACT: Being the biggest and most imperative sink for various kinds of pollutants, soil plays a vital role in protecting the environment around the globe. The capacity of soil to sequester pollutants varies as per its own quality as well as that of the overall environment. Thus, determination of various controlling factors (such as soil characteristics, along with their internal associations) and their spatio-temporal variations, for sequestration of pollutant(s) especially carbon, is quite an important area of research for tackling the issue of global warming, as the capacity of soils to function at an optimum level is regulated by these factors. One of the key indicators of soil quality from carbon sequestration perspective is the presence (and protection mechanism) of soil organic matter. Among various other parameters, the soil minerals (such as fluoride) impact the retention of soil organic matter and thus consequently affect the potential of soil to sequester carbon. The present research focuses upon the impact of spatial and temporal variation on the relationship of two of the soil properties, i.e., the soil organic matter and the soil fluoride content. The study has been conducted in the twin cities of Pakistan, i.e., Islamabad Capital Territory and Rawalpindi. For the current research 204 soil samples were collected from the said areas. The samples were tested through standard protocols, i.e., loss on ignition method for analysing soil organic matter and Ion selective electrode for the determination of soil fluoride and then correlation analyses of the samples were carried out by using MS Excel. The results of the research show that during winter season the correlation coefficient for the two parameters lies within the range of 0.19–0.34 (weak positive correlation) but during summer season the correlation coefficient lies within the range of 0.35–0.89 (weak to strong positive correlation). The highest concentrations of both the organic matter and the fluoride content for both the seasons were observed in the forest area. In accordance with the results of the present study, it can be projected that a positive correlation exists between the soil fluoride and the soil organic matter. However, this correlation is not consistent with increasing levels of soil fluoride as indicated by the correlation coefficient (0.19 during winter and 0.43 during summer season) calculated for the Margalla hills forest area (even though the concentrations of both the soil parameters were comparatively higher for this land use type). So, conclusively, the present study demonstrated the existence of a positive correlation between the two parameters but the degree of this relationship may get affected by a number of other governing factors. Therefore, present study further identifies the research gaps that must be kept under consideration and given priority in the future research in the same research area.

Keywords: Carbon sequestration; Carbon sink; Correlation analysis; Effects of fluoride on soil; Seasonal variations; Soil fluoride; Soil organic matter.

INTRODUCTION

Soil is one of the most dynamic systems of our environment. From functional aspect, it not only serves as the holding ground for the primary producers but also acts as a sink for processing most of the pollutants alongside the non-polluting by-

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products of our natural and anthropogenic processes. Minerals and organic matter are the essential components of soil and they are found in the solid phase of this dynamic system. The relationship between the organic matter and the mineral substances acts as a deciding factor for determining the quality of the soil and in turn the quality of environment.[1] During 1800s, scientists began to identify the soils as unique natural bodies having exclusive size, shape, and history. These natural bodies serve some key functions that could be acknowledged as more profound than the sum of the services provided by their components.[2] The best example in this regard is that of the soil organic matter. The soil organic matter plays a key role in reaping good yields when considered from an agricultural point of view. But on a larger scale, it can be used as an indicator of the soil quality and precisely an important indicator in assessing the potential of a soil to sequester carbon.[3]

Soil bodies may vary in their structure and functions as per the variation in their spatial and temporal degrees.[2] They may show gradual to sharp changes depending upon some key factors. The processes that govern the transformation of soils over a period of time include physical weathering, i.e., breakdown of mineral content in the parent rock due to climatic factors (temperature, pressure, and precipitation) or earth movements (tectonic activity and volcanic eruption), leaching of nutrients, changes in flora and fauna, and also anthropogenic intrusion, e.g., dumping of waste. So, the key components that could be studied in order to predict any type of soil changes include the parent material, land use, topography, organisms, time, and climatic conditions.[2,4] Soils serve the ecosystems in myriads of ways. They store, process and recycle water, nutrients, and waste products. They absorb and release gaseous (e.g., greenhouse gases) and mineral (e.g., fluoride) pollutants.[5]

Fluoride affects the quality of soil in multiple ways. Increased levels of fluoride in soil may damage the vegetation, disrupt the microbial population and in turn may adversely affect the soil ecology and soil functions.[6] Besides, it can also impact the properties of soil to sequester the atmospheric carbon as it affects the organic matter content of the soil.[7] Fluoride as a soil pollutant has mostly been ignored in comparison to the other soil pollutants such as heavy metals and the organic pollutants, despite the fact that the presence of this particular mineral in soil not only affects the quality of soil but also impacts other components of the environment, such as underground and surface water bodies, the vegetation, and the microbial population of the soil.[8] Fluoride addition to soil can be attributed to a number of different sources. The most common are fertilizers, pesticides, atmospheric deposition, weathering of rocks, and through industrial emissions.[9] For drinking water, the permissible limits of fluoride content according to World Health Organization is 0.6 to 1.5 mg/L. However, for soils, no such limits are available.[10]

The present research aimed to assess the nature of the relationship that exists between the soil organic matter and the soil fluoride and the impact of spatial and temporal variations on this relationship. The implications of this association in terms of carbon sequestration potential of the soil will further be addressed.

MATERIALS AND METHODS

Study area:

For present research, 204 soil samples were collected from Islamabad and Rawalpindi. Islamabad Capital Territory (ICT) is the Capital city of Pakistan. It lies on the north-eastern margin of the Pothwar plateau and it is located approximately 14 km to the northeast of city Rawalpindi. Geographically, the city lies at the Northern latitudes 33º 49' and longitudes 72º 24' east of Greenwich. The city's altitude ranges from 457 to 610 metres. Its total area is 906.50 km^2. An additional area of 3626 km^2, which lies adjacent to Islamabad Capital Territory covers the Margalla Hills. ICT features a climate that can be considered as an atypical form of a humid subtropical climate. It has hot and humid summer season accompanied by a monsoon season which ultimately changes into cool winters. The summers (April to September) temperature ranges from 24.4ºC to 42ºC and the winters (October-March) temperature ranges from 3.4ºC to 16.6ºC. The yearly average temperature ranges are 14.1ºC to 28.5ºC. The city receives an average rainfall of 1143 mm annually while the average humidity is around 55%.[11]

Rawalpindi, also famous as Pindi, is located in Punjab, Pakistan. It remained the interim capital of Pakistan from 1959 to 1967. Currently, it is the fourth largest city of the country. As it lies adjacent to the federal capital of Pakistan so along with ICT, the twin cities make the fourth largest metropolitan area of the country.[12] Rawalpindi covers an area of 69.4 km^2. Its total population is 1.4 million. The record high temperature for the city is 45.0ºC while the average high precipitation is 20.6 mm.[13]

The CO_2 emissions from the city of Rawalpindi are 1,251,330 t. The per capita CO_2 emissions are 0.89 t and the CO_2 emission intensity is 18,032 t/km^2, while the CO_2 emissions data for Pakistan are in Table 1.

Table 1. CO_2 emissions data of Pakistan[14,15]

Location	CO_2 emissions (t)	CO_2 emissions per capita (t)	CO_2 emissions intensity (t/km^2)
Pakistan	151,878,152	0.82	190.6

Study design:

As the research aimed to assess the dynamics of the relationship between soil organic matter and soil fluoride in both spatial and temporal extent, 204 soil samples (two samples from 102 sampling spots) were collected from the three different land use types of the twin cities, in two different seasons, i.e., winter (December) and summer (June) in 2019. For the first land use type, i.e., the agricultural areas of Islamabad and Rawalpindi, the samples were collected from fields located in Rawat, Wah G.T Road and Shah Allah Ditta Village, respectively. For the second land use type, i.e., the urban area, the samples were collected from Ayub Park, Nawaz Sharif

Park and the Fatima Jinnah Park-F9. For the third land use type the samples were collected from the forest area of Margalla Hills.

The soil samples were collected from a depth of 0–30 cm. Polythene zip lock bags were used for sample collection and their safe transportation to the laboratory. For laboratory analysis, the samples were air dried, ground, and sieved through 2.0 mm sieve. They were then subjected to the physico-chemical analysis through standard testing protocols. Soil organic matter was assessed by means of loss on ignition method[16,17] and fluoride content of the soil was assessed by means of ion selective electrode.[18]

The inferences drawn from the laboratory analysis of the soil parameters were then subjected to statistical analysis. In order to assess the relationship between the two parameters, correlation analysis was carried out in MS Excel.

RESULTS

Concentration of fluoride in soils of Islamabad and Rawalpindi:

The concentration of fluoride in the soils of Rawalpindi and Islamabad ranged from 0.48 mg/L to 5.59 mg/L in winter and 0.19 mg/L to 3.88 mg/L in summer. The lowest amount of fluoride was observed in summer season. The detailed results of the fluoride analysis are given in Figure 1.

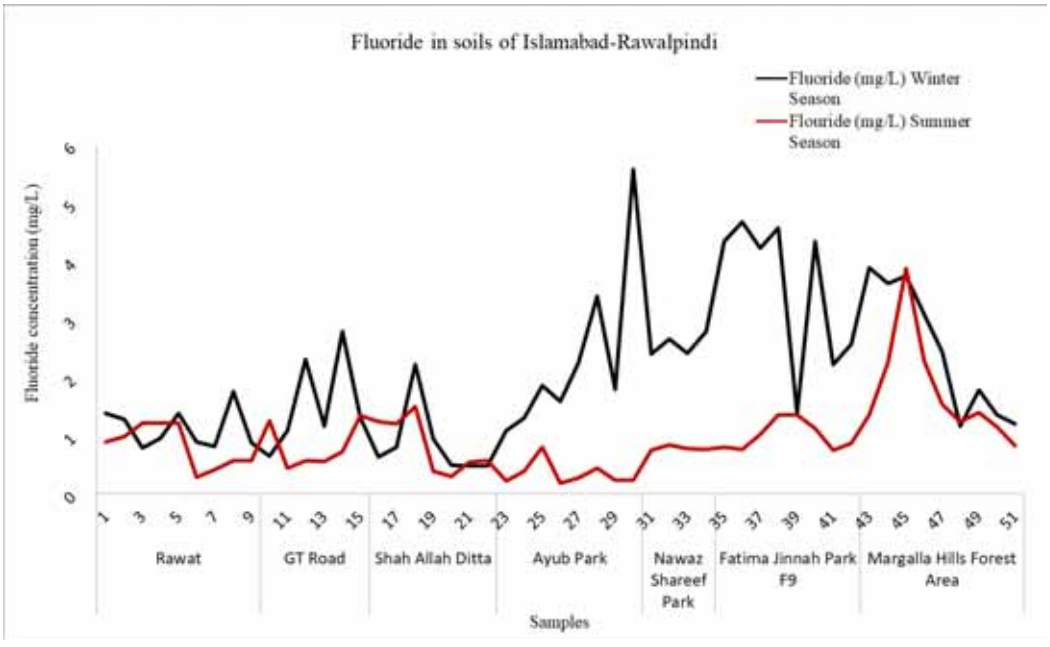

Figure 1: Fluoride concentrations in soil samples of Islamabad-Rawalpindi.

Percentages of organic matter in soils of Islamabad and Rawalpindi:

The percentage of organic matter in the soils of Islamabad and Rawalpindi ranged from 0.08 to 1.16 during the winter season and from 0.15 to 2.64 in summer as shown in Figure 2.

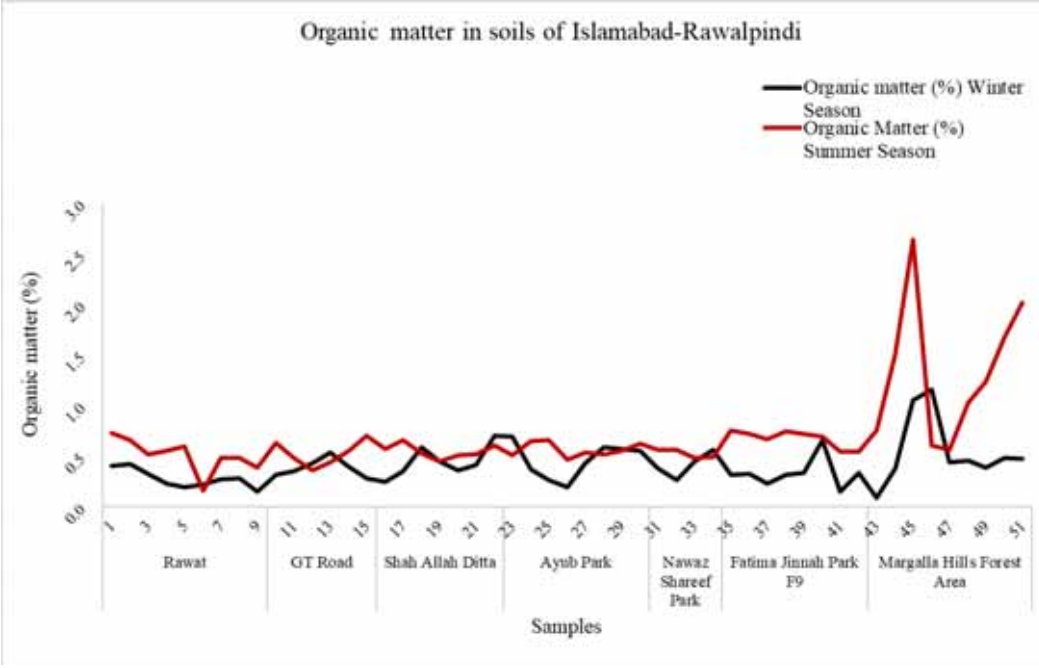

Figure 2. Organic matter percentage in soil samples of Islamabad-Rawalpindi.

Correlation between soil organic matter and soil fluoride:

For winter season, weak to slightly moderate correlation values were observed that ranged from 0.19 to 0.34. For majority of the land use types the correlation coefficient lay within the range of 0.25 to 0.29 as shown in Figures 3A–3G.

3A

A: Correlation between Soil Fluoride and Soil Organic matter - Rawat (Winter)		
	Fluoride (mg/L)	*Organic Matter (%)*
Fluoride (mg/L)	1	0.28
Organic Matter (%)	0.28	1

3B

B: Correlation between Soil Fluoride and Soil Organic matter - GT Road Wah (Winter)		
	Fluoride (mg/L)	*Organic matter (%)*
Fluoride (mg/L)	1	0.25
Organic matter (%)	0.25	1

3C

C: Correlation between Soil Fluoride and Soil Organic matter - Shah Allah Ditta Village (Winter)		
	Fluoride (mg/L)	*Organic matter (%)*
Fluoride (mg/L)	1	0.34
Organic matter (%)	0.34	1

3D

C: Correlation between Soil Fluoride and Soil Organic matter - Shah Allah Ditta Village (Winter)		
	Fluoride (mg/L)	*Organic matter (%)*
Fluoride (mg/L)	1	0.34
Organic matter (%)	0.34	1

Figures 3A–3D: Correlation between soil fluoride and organic matter during winter season.

3E

E: Correlation between Soil Fluoride and Soil Organic matter - Nawaz Sharif Park (Winter)

	Fluoride (mg/L)	*Organic matter (%)*
Fluoride (mg/L)	1	0.29
Organic matter (%)	0.29	1

3F

F: Correlation between Soil Fluoride and Soil Organic matter - Fatima Jinnah Park-F9. (Winter)

	Fluoride (mg/L)	*Organic matter (%)*
Fluoride (mg/L)	1	0.29
Organic matter (%)	0.29	1

3G

G: Correlation between Soil Fluoride and Soil Organic matter - Margalla Hills Forest Area (Winter)

	Fluoride (mg/L)	*Organic matter (%)*
Fluoride (mg/L)	1	0.19
Organic matter (%)	0.19	1

Figures 3E–3G: Correlation between soil fluoride and organic matter during winter season.

For majority of the land use types the correlation coefficient for the two parameters for the summer season was found to be positive. Out of seven sampling areas the correlation coefficient for the fields of Rawat (Figure 4A) and GT road Wah (Figure 4B) area were observed to be the strongest, i.e., 0.69 and 0.89, respectively. For most of the land use types, the correlation coefficient lay in the moderate category, i.e,. 0.35 to 0.59 as shown in Figures 4C–4G..

4A

A: Correlation between Soil Fluoride and Soil Organic matter - Rawat (Summer)

	Fluoride (mg/L)	*Organic Matter (%)*
Fluoride (mg/L)	1	0.69
Organic Matter (%)	0.69	1

4B

B: Correlation between Soil Fluoride and Soil Organic matter - GT Road Wah (Summer)

	Fluoride (mg/L)	*Organic Matter (%)*
Fluoride (mg/L)	1	0.89
Organic Matter (%)	0.89	1

4C

C: Correlation between Soil Fluoride and Soil Organic matter - Shah Allah Ditta Village (Summer)

	Fluoride (mg/L)	*Organic Matter (%)*
Fluoride (mg/L)	1	0.48
Organic Matter (%)	0.48	1

4D

D: Correlation between Soil Fluoride and Soil Organic matter - Ayub Park (Summer)

	Fluoride (mg/L)	*Organic Matter (%)*
Fluoride (mg/L)	1	0.59
Organic Matter (%)	0.59	1

Figures 4A–4D. Correlation between soil fluoride and organic matter during summer.

4E

E: Correlation between Soil Fluoride and Soil Organic matter - Nawaz Sharif Park (Summer)		
	Fluoride (mg/L)	*Organic Matter (%)*
Fluoride (mg/L)	1	0.35
Organic Matter (%)	0.35	1

4F

F: Correlation between Soil Fluoride and Soil Organic matter - Fatima Jinnah Park-F9. (Summer)		
	Fluoride (mg/L)	*Organic Matter (%)*
Fluoride (mg/L)	1	0.46
Organic Matter (%)	0.46	1

4G

G: Correlation between Soil Fluoride and Soil Organic matter - Margalla Hills Forest Area (Summer)		
	Fluoride (mg/L)	*Organic Matter (%)*
Fluoride (mg/L)	1	0.43
Organic Matter (%)	0.43	1

Figures 4E–4G. Correlation between soil fluoride and organic matter during summer season.

For both the seasons, the lowest correlation coefficient existed among the samples collected from the forest area, i.e., Figures 3G and 4G.

DISCUSSION

According to the results of the current study, the fluoride content in the soils of Rawalpindi and Islamabad ranged from 0.48 mg/L to 5.59 mg/L during winters and 0.19 mg/L to 3.88 mg/L during summers as shown in Figure 1. These concentration ranges of fluoride in the studied areas are backed by previous studies.[19,20] According to Khalid et al.,[19] fluoride from the brick kilns accumulates within the soil of Rawalpindi and Islamabad. As per their results, 2.57 mg/kg to 4.08 mg/kg fluoride was found in the soils of this region. Urooj and Ahmed [20] also reported the presence of soil fluorine (i.e., 0.014–3.443 ppm) originating from the brick kilns present in Rawalpindi and Islamabad.

The second parameter, i.e,. organic matter in the soils of Islamabad and Rawalpindi, ranged from 0.08 to 1.16 percent in the winter season and from 0.15 to 2.64 percent in the summer season as shown in Figure 2. The organic matter ranges are also validated by the available literature.[21-22] According to Azam et al[21] and Shaheen,[22] the lands of the Pothwar region including Islamabad and Rawalpindi are deficient in organic matter consisting of 0.52 to 1.38 percent[21] and 0.89–1.25 percent[22] soil organic matter, respectively.

Soil organic matter is considered to be an important indicator for assessing the quality of soil. Its presence as well as its association(s) with other soil parameters (especially minerals) implies the current and the future potential of the soils of an area to sequester carbon.[23] Results (Figure 2) of the present research give a picture of the current levels of OM in the soils of Islamabad and Rawalpindi region which is quite low. Cumulatively, it ranged from 0.08 to 2.64 percent. Aligning with the

outcomes mentioned within Figure 2, a former study reports that the soils of Islamabad and Rawalpindi region are deficient in soil organic matter and this deficiency could be attributed to multiple reasons. One of the most widely accepted explanation for this lower percentage of soil organic matter is the temperature range.[24] The higher summer temperatures of this arid region promotes quick losses of organic matter content.[22] This loss is also witnessed within the present study as depicted in Figure 2. Thus, prevalent high temperatures could also be attributed to impact the relationship of organic matter with the fluoride as shown in the correlation figures (4A–4G) for the summer season. In the areas where organic matter quantities were lowest, i.e., Rawat and Wah G.T Road, the correlation coefficient values were found to be highly positive, i.e., 0.69 (Figure 4A) and 0.89 (Figure 4B), respectively.

Recent research shows that within soil's inherent ecosystem, major governing factors, for organic matter retention are: 1) accessibility of microorganisms to the soil organic matter and 2) physical protection of organic matter by adsorption on mineral surfaces.[25] The processes that control the stabilization of soil carbon by means of physical protection include: occlusion (i.e., clogging up with the aggregates), adsorption (attachment onto mineral surfaces, and the substrate based biological rate limitation.[26-28]

For long-term retention of soil organic carbon, the major controlling factor is the interaction of organic matter with the mineral content.[29] Mineral associations based protection of organic matter not only involves its protection by physical means[30-31] but it also includes the chemical associations.[32-36] In this context, a number of minerals have been studied in relation to soil organic matter, like iron and aluminium, and their impact on the retention of soil organic matter has been studied in a lot of research.[37-38] However, the relationship of soil organic matter with the soil fluoride has not remained the focus of a huge amount of research.

According to a previous research, the physicochemical characteristics of the soils are one of the key deciding factors for determining how the carbon pool will be protected in both the short and the long run. The intrinsic properties of soil define the maximum limit of the soil to capture and retain the organic carbon.[39] This not only gives an indication of how the relationships among soil parameters can be used for good but also about how soils' inherent characteristics could themselves limit the maximum capacity of a certain type of soil to store organic matter with increased inputs of the organic residues. It is a complex issue of how environmental factors affect carbon sequestration.

Spatial, temporal, and geochemical variations affect the soil organic matter. So, in order to assess the storage behaviour of soil, these factors must be considered.[40] Fluoride present in soils has mostly been studied for its effects on vegetation and microbial communities. But its association with soil organic matter in terms of understanding and increasing the carbon sequestration potential has not been studied in depth.

According to the results of the present study, as illustrated in Figures 3A–3G and 4A–4G of the results section, soil organic matter is positively correlated to the fluoride content of the soil. The findings of the current research are supported by the former studies,[7,9] which state that the organic matter content of the soil is affected by

the fluoride content, as its accumulation deteriorates the microbial population and enhances the accumulation of the organic matter within the soil. The current research has been conducted on the alkaline soils of Islamabad and Rawalpindi region. As per the results of the present research (Figures 3A–3G and 4A–4G), within alkaline soils, the fluoride content, shows positive correlation to the organic matter and consequently inhibits its breakdown. This correlation can be further utilized to understand and enhance the retention of soil organic matter both in the short as well as the long run.[7]

The observed spatial variation in the concentration of fluoride within the soils of Islamabad and Rawalpindi is in an order of agricultural fields (A) <managed parks (P) <Forest area (F) during the winter season. For the summer season the order is P<A<F. The organic matter percentage for the same data set showed the order of A<P<F in both winter and summer seasons.

A positive correlation coefficient has been observed among the two parameters which gives an indication that the addition of fluoride content increases the retention of organic matter despite the seasonal variation. The lower temperature could be considered as one of the major controlling factors affecting the degree of this relationship as the correlation coefficient values for most of the sites in winter season lie within weak to moderate levels but during summers the two parameters are correlated at moderate to strongly positive levels.[41-46]

There are many other factors that could possibly affect this relationship, e.g., presence of metals. Organic matter can be mobilized when fluoride is present along with metallic species like iron and aluminium. The organic matter (OM) that is present in the metal bonded form, upon addition of fluoride-based chemicals, form mixed F-M-OM complexes, as per the following reaction (Figure 5).

$$\text{Clay-M-OM} + F^- \rightarrow \text{Clay} + \text{F-M-OM}$$

Or

$$\text{Clay-M-OM} + F^- \rightarrow \text{Clay-M-F} + \text{OM}$$

Where:

M = Metal
OM = Organic matter
F = Fluoride

Figure 5. Reaction of mineral-associated organic matter with fluoride.[43]

As suggested by former studies, other possible controlling factors that govern the soil's behaviour (in terms of carbon sequestration) when fluoride is being added to it, include pH, and the origin (type of vegetation and litter), type (constituents of organic matter) and formation mechanism (climatic and microbial influences) of organic

matter.[7] According to a previous research, fluoride-induced increases in pH of the soil is one of the important factor in this regard.[42] Another research suggested that the fluoride-induced leaching of organic matter can also be dependent on the type of humus content, e.g., moder and mor humus show higher levels of leaching than the mull.[46] Thus, fluoride content along with the above mentioned soil properties must be considered in future studies to get a clearer picture of the role of this mineral in controlling the carbon storage. As suggested by the inferences of the present study and supported by a previous study,[40] temperature also affects the relationship of the organic matter and fluoride. Therefore, to make vivid and accurate predictions about the role of this relationship in sequestration of carbon, the climatic factors and type of vegetation that controls and regulate the temperature of the soil and the atmosphere must also be kept under consideration.

The complexity of fluoride-organic matter relationship can further be unfolded by assessing the impact of fluoride on soil microbiota and vice versa.[47] Fluoride has mostly been reported to affect the microbial community of the soil. So, the stronger correlation coefficients in the summer season, as inferred in the present study might be attributed to the increased activity of the soil microbes.[48] This could be considered as another governing factor of this relationship. As reported in a study,[40] the retention of carbon in soil is directly impacted by the changes in the soil matrix. These changes in the soil matrix are dependent on the microbial communities of the soil and the way they interact with the nutrients. So, the fluoride-induced change within the soil's microbial profile is a possible avenue that may lead to a better understanding of how this association could help understand the potential of a specific soil type to sequester carbon.

Fluoride is earth's thirteenth most abundant element. So, the parent rock type and the soil formation processes could affect the presence of this element within the soil of a region. Research regarding presence in drinking water and brick-kilns based sources of fluoride[19-20] have been conducted in the region of Islamabad and Rawalpindi and its presence in the soils of other regions of Pakistan[48] but the element has not been studied within the soils of Islamabad and Rawalpindi region from the perspective of its association to the organic matter. So, in order to understand the complexity of relationship of fluoride with that of organic matter for this particular region a more detailed and complex array of factors must be addressed.[23]

Results of the present study reveal that the highest concentrations of fluoride are observed in the forest area of Margalla Hill as compared to the agricultural areas or managed recreational areas, i.e., public parks. However, the correlation coefficients were found to be strongest in the areas of moderate levels of fluoride concentrations. For the forest area, the correlation was found to be at weak and moderate levels for winters and summers, respectively. So, the results of the present study agree with the previous studies, i.e., the fluoride positively correlates to the soil organic matter[35] but an in-depth analysis, i.e., the trends (spatial variation) of both the soil parameters suggest that this relationship is more complex and multidimensional as the areas with highest concentration of fluoride show weak to moderate positive relationships. Therefore, a comprehensive approach based on multiple variables must be adopted to address the current research area.

CONCLUSIONS AND RECOMMENDATIONS

Soil organic matter is one of the critical indicators for assessing the potential of carbon sequestration of the soils of a particular region. The results of the research show that during winter season the correlation coefficient for the two parameters lie within the range of 0.19–0.34 (weak positive correlation) but during summer season the correlation coefficient lies within the range of 0.35–0.89 (weak to strong positive correlation). The highest concentrations of both the organic matter and the fluoride content for both the seasons were observed in the forest area. In accordance with the results of the present study, it can be projected that a positive correlation exists between the soil fluoride and the soil organic matter. However, this correlation is not consistent with increasing levels of soil fluoride as indicated by the correlation coefficient (0.19 during winter and 0.43 during summer season) calculated for the Margalla hills forest area (even though the concentrations of both the soil parameters were comparatively higher for this land use type). So, in conclusion, the present study demonstrated the existence of a positive correlation between the two parameters but the degree of this relationship may get affected by a number of other governing factors. This fundamental indicator shows positive correlations with the fluoride content of the soil. In order to fully comprehend and enhance the soils' organic matter in various land use types of a common region, it is important to address the particular behaviour of the soils of that region. Increasing soils' natural ability to retain organic matter is an important climate change mitigation strategy. Therefore, the type and levels of mineral content such as fluoride that plays a significant role in retaining the soil organic matter must be researched in depth and at a broader scale.

According to the limitations identified in the present research, following are the areas in which detailed research is required to fully make use of the fluoride-organic matter association:

• Other physicochemical and biological factors governing the fluoride-organic matter relationship must be assessed, e.g., soil pH, metal interferences, type of humus and microbial communities (which are affected by fluoride and at the same time affect the organic matter) must also be researched. Moreover, climatic factors reshaping this association must be understood.

• Anthropogenic activities, i.e., fluoride pollution and the treatment of different land use types with respect to fluoride-organic matter relationship must be monitored.

• The mineral associated organic matter is also affected by the carbon-nitrogen ratio of the soil. This ratio also impacts other soil properties along with the fluoride content. Therefore, carbon-nitrogen ratio must also be kept under consideration in the future studies addressing this research area.

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CONFLICT OF INTEREST DECLARATION

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