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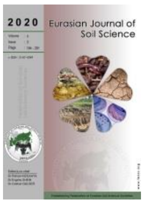

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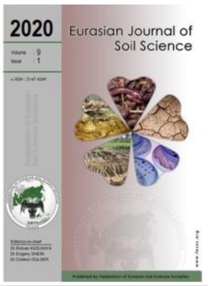

Assessment of potential ecological risk index based on heavy metal elements for organic farming in micro catchments under humid ecological condition

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Abstract

Soil pollution, influenced by both the natural and anthropogenic factors, significantly reduces environmental quality. This research was carried out in some micro catchments located on Ordu province of Black Sea Region-Turkey in order to determine potential ecological risk index based on heavy metal elements (HMs) for organic farming. For this purpose, 166 soil samples (0-20 cm) were taken from the study area and some physical and chemical and HM concentrations (Cd, Cu, Cr, Ni, Pb and Zn) analysis were done. In this study, it was determined; i-) some physical and chemical properties of catchments' soils, ii-) HM contents and the correlation relation between physico-chemical properties of soil and HM concentrations and iii-) potential ecological risk index (PERI). PERI was calculated using the data obtained to evaluate the environmental risks of HMs in the region. The results showed that Cu concentration in 3%, Cr in 0.6% and Ni in 4.8% of the soil samples exceeded the threshold levels whereas, the concentrations of other HMs were lower than the critical values. Statistically, it was also found significantly positive relationships between sand content and Cu of soils whereas, it was determined significantly negative relationships between EC and Cu. In addition, according to the obtained PERI results, while 54% of total soil samples were low while, 42% of them were moderate class. Only 4% of them were classified as significant ecological risk level. Moreover, according to mean potential ecological risk index of these HMs, it can be ordered as Cd>Pb>Cu>Ni> Zn>Cr.

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Keywords: Heavy metal, potential ecological risk, micro catchment, humid ecological condition, Black Sea Region.

Introduction

Nowadays, contamination of heavy metal elements has become an environmental issue in both developed and developing countries throughout the World (Tam and Wong, 2000; Sun et al., 2010). Natural toxicities of heavy metals, having a large number of sources, not being biodegraded and being able to survive for a long time; It has increased the importance of ecological and environmental studies on metal accumulation (Gao et al., 2016). Rapid urbanization, industrialization, agricultural practices and the use of fossil fuels are the main causes of heavy metal accumulation in soil, especially in water resources, street dust, sediment, aquatic organisms consumed by humans and in the food chain (El Nemr, 2011; Chaudhari et al., 2012; Yang et al., 2014; Cheng et al., 2015). Under certain environmental conditions, heavy metals might accumulate up to toxic concentration levels, and cause ecological damage (Bai et al., 2011; El Nemr et al., 2012).

An important problem is how to assess heavy metal deposits and how to distinguish man-made contribution from natural concentrations (Bing et al., 2013). Many indexes have been developed to evaluate the accumulation of heavy metals and the environmental problems they create. For example, in order to

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determine the accumulations of anthropogenic origin and to identify possible ecological risks; Enrichment factor, contamination factor, geo-accumulation index, potential ecological risk index are frequently used indices (Rashed, 2010; Yang et al., 2011; Bing et al., 2013; Islam et al., 2015; Kükrer et al., 2015).

Structural and textural properties of the soil, cation exchange capacity (CEC), pH and amount of organic matter have an effective role in the retention of heavy metals in the soil. Especially due to the high CEC of clay, soils hold heavy metals more than other soils with higher clay and organic matter content hold difficult metals and form strong soluble compounds. Variation of these elements or compounds deposited in the soil along the soil profile, it depends on the characteristics of the soils and the intensity of agricultural activities, and the distribution of metals in the soil profile is indicative of air pollution, soil genesis and anthropogenic pollution (Wilcke et al., 2000; Cemek and Kızılkaya, 2006).

The objectives of this present study was (i) to determine some physico-chemical soil properties of some micro catchment in Ordu province, (ii) to determine the correlation between heavy metal (HM) contents and physico-chemical properties of soil, (iii) to assess the environmental risks of heavy metals in the micro catchments.

Material and Methods

Study area

The study area chosen as Ordu, Turkey's eastern Black Sea region, is located between 40º 18'-41º 08' north parallel 36º 52'-38º 12' eastern meridians (Figure 1). Ordu is surrounded by the Black Sea from the north, Giresun from the east, Sivas and Tokat from the south, and Samsun from the west. The total area of Ordu is approximately 6141 km^2. About 10.2% of the province consists of meadow and pasture areas, while about 28.7% of it has forest areas. In addition, the area where have been used agricultural activities are carried out covers approximately 52.0% (DOKAP, 2018).

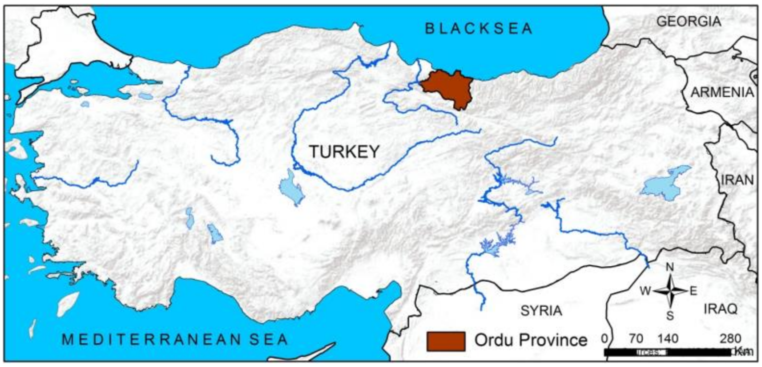

Figure 1. Location map of the study area

Ordu has a typical Black Sea climate which means that winters are mild and cool summers and shows humid ecological conditions. According to long-term climate data (1959-2018) the average annual rainfall and annual average temperature are 1038.4 mm, 14.4°C, respectively (TMS, 2015). An insight into climatic conditions of the Black Sea Region can be found in the study (Mihálikova et al., 2016). These steep slopes play an important role in growing hazelnut and tea. The study area is located at an altitude of 0-3082 m above sea level. In the majority of the northern part of the region has a mountainous and rugged topography with steep and very steep slopes (>50% slope) (Figure 2).

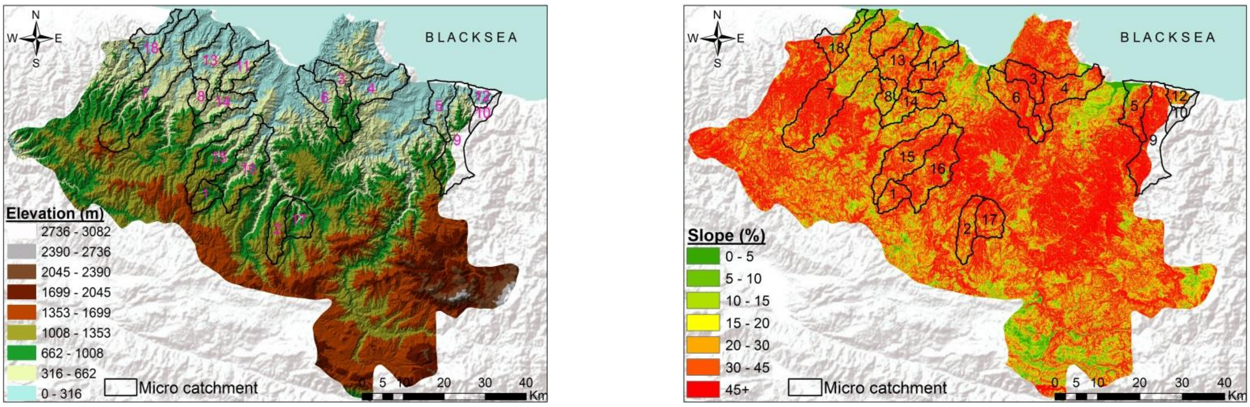

Figure 2. Elevation and slope maps of the Ordu Province

Soil Sampling and analysis

Field study was conducted in 2017. Soil samples which were classified as gray brown podzolic, brown forest, Non-calcerious brown forest, redish yellow podzolic, alluvial, high mountain meadow and colluvial great soil group according to old American soil classification system (GDRS, 1984) were taken from 166 soil in total agricultural lands (Figure 3). Sampling was carried out after autumn harvest and before the crop growing season. In addition, the coordinates were recorded using global positioning system (GPS) device.

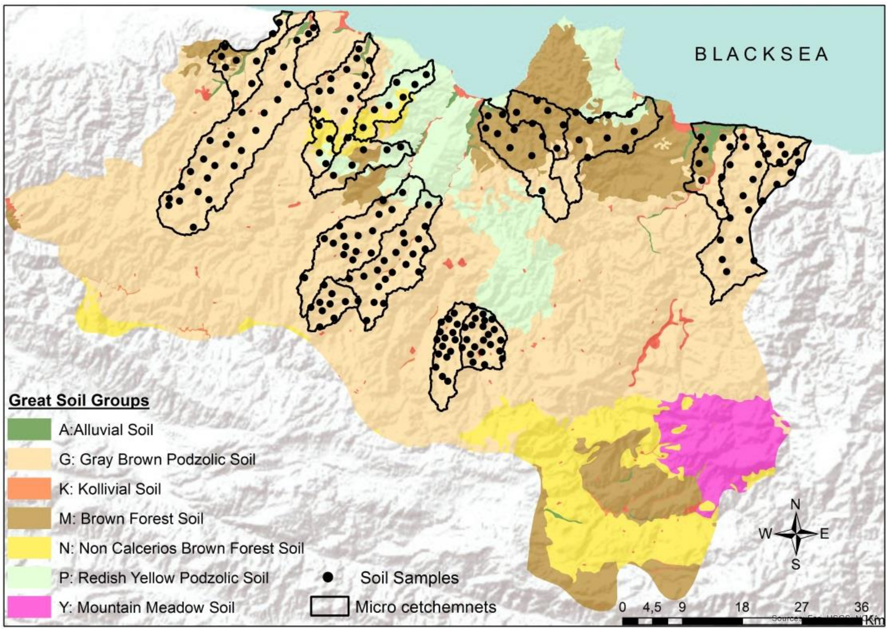

Figure 3. Soil samples pattern in great soil group map of the study area

Soil samples taken from the field were air dried and sieved from a screen with 2 mm opening size to prepare for analysis. Sand, silt, clay contents of the soil samples were determined by Hydrometer method (Bouyoucos, 1962). Soil pH values were measured in soil suspension (1:1, w:v) by glass electrode pH meter and EC values were determined in the same soil suspension (1:1, w:v) by EC meter (Rowell, 1996). Soil organic matter (OM) contents were determined by modified Walkley-Black method (Walkley and Black, 1934). Lime ($CaCO_3$) contents of soils were determined by Scheibler calcimeter (Nelson, 1982). Total heavy metal (Cu, Cd, Cr, Pb, Ni, Zn) contents were determined by EPA 3051 using ICP-OES (Kloke, 1980)

Potential ecological risk assessment of heavy metals referred to Hakanson (1980) method with the following formula:

$$CF = \frac{C_s^i}{C_r^i} \tag{1}$$

$$\text{PER} = \text{TRF} \times \text{CF} \tag{2}$$

$$\text{PERI} = \sum \text{PER} \tag{3}$$

Where; C_s^i: is the measured concentration of heavy metal in each soil sample; C_r^i: is reference value, here the background value of each heavy metal in soil is used (Taylor, 1964; Hasan et al., 2013) , CF is the pollution of a single element factor (contamination factor); PER is the potential ecological risk index of a single element; PERI is a comprehensive potential ecological risk index; and TRF is the toxic response factor of a single element (Kloke, 1980), which is determined for Zn = 1, Cr = 2, Cu = Pb = Ni= 5 and Cd = 30. The terminology used to describe the risk PERI was suggested by (Kloke, 1980; Long et al., 1995), where: PERI < 95 indicates a low potential ecological risk; 95 < PERI < 190 is a moderate ecological risk; 190 < PERI < 380 is a considerable ecological risk and PERI >380 is a very high ecological risk.

Results and Discussion

Soil physical and chemical properties and heavy metals

Some researchers indicated that some chemical such as pH, CEC, organic matter contents and physical such as clay content are the essential soil characteristics that designate the capacity to hold heavy metal pollutants (Omran, 2016; Gabrielyan et al., 2018). The descriptive statistical parameters (mean, maximum, minimum, and coefficients of variation) of the some physical, chemical properties and heavy metals related to 166 soil samples taken from surface (0-20 cm) of the crop lands in 18 micro catchments of the Ordu province were presented in Table 1. The average clay content of the micro catchments' soils was 23.31%, and 55% are slightly coarse soils. The pH values of soil samples varied between 68% strong-medium-light acid, while soil samples; It has 99% salt-free, 88% lime-free, 38% medium and 32% high organic matter content. When the heavy metal contents in the research area are examined, it is seen that Cu, Cr and Ni elements are above the limit values in the surface soil in some micro catchments (Table 1). The statistics results showed that the mean value of soil Cu, Cd, Cr, Pb, Ni and Zn concentrations was 39.99, 0.54, 15.36, 11.83, 14.86 and 30.46 mg kg^{-1}, respectively. Especially Ni and Cu were determined in micro catchments coded as 7 in Akkuş and Çaybaşı district boundaries and micro catchments coded as 2 and as 17 in Aybastı, Gölköy and Kabataş district boundaries. Grouping of heavy metal concentrations was detected in all catchments except the southern parts of the Çarşıbaşı district. Although not dense, Cu, Cr and Ni may be caused by the increase in solubility of soils due to acidification process, and Cu may be caused by pesticides containing copper element in the active substance especially in areas where intensive hazelnut cultivation is carried out. There was high variations and great skewness for Cr (117.26% and 4.76). Coefficients of variation (CV) % values show the mean variation of each sampling site in the population. In this current study, it was found that the order of the CV % for heavy metal element from high to low was Cr > Ni > Pb > Cd > Cu > Zn. In some researches, it has been shown that high Ni level is caused by parent material and Pb content is caused by particle from surrounding lead acid battery factory (Pesantes et al., 2019). In addition, mean value of PERI was found 90.91 which indicate as low potential ecological risk in general for all soil samples. However, it should be evaluated each soil samples taken from each individual micro catchment.

Table 1. Descriptive statistical analysis of physical and chemical properties and heavy metal of soil samples

Parameters	Min.	Max.	Mean	SD	CV, %	Skewness	Kurtosis
Physical and chemical properties							
Sand, %	22.31	84.62	51.11	12.84	25.12	0.01	-0.47
Clay, %	5.57	56.02	23.58	10.74	45.54	0.55	-0.31
Silt, %	2.35	40.86	25.31	5.50	21.73	-0.41	1.73
pH, 1:2.5	4.24	8.10	6.02	0.92	15.28	0.25	-0.98
EC, dS m^{-1}	0.06	1.22	0.33	0.21	63.63	1.15	1.54
$CaCO_3$, %	0.00	39.25	1.50	5.48	365.33	4.72	23.38
OM, %	0.31	5.99	2.98	1.44	48.32	0.43	-0.87
Heavy metals							
Cu (0-100)$^{\#}$	8.19	164.26	39.99	24.47	61.19	1.73	4.46
Cd (0-3) #	0.00	2.42	0.54	0.39	71.46	1.46	4.67
Cr (0-100)$^{\#}$	1.20	166.04	15.36	18.01	117.26	4.76	31.87
Pb (0-100)$^{\#}$	2.20	96.24	11.83	8.85	74.78	6.34	53.78
Ni (0-50)$^{\#}$	3.54	120.13	14.86	14.76	99.33	4.10	20.88
Zn (0-300)$^{\#}$	10.52	91.23	30.46	12.77	41.91	1.65	3.90
PERI	6.80	373.66	90.91	2.89	3.18	1.50	4.71

SD: Standard deviation, Min.: Minimum, Max.: Maximum, n: sample number, EC: Electric conductivity, OM: Organic matter. EC: Electrical conductivity, pH: soil reaction, *CV (Coefficient of Variation), **skewness:< |∓0.5 |= Normal distribution, 0.5- 1.0 = Application of character changing for dataset, and > 1.0 → application of Logarithmic change, #: Maximum permissible concentration (mg kg^{-1})

Correlation Analysis

Correlation analysis of some physical and chemical properties and heavy metal content of micro-catchment soils were performed (Table 2). Heavy metals have a significantly relationships organic content, clay and fine silt particles because of their high CEC (Tjahjono and Suwarno, 2018). A positive correlation was determined between sand content and Cu ($p<0.01$) and Zn ($p<0.01$) whereas, a negative correlation was detected between clay content and Cu ($p<0.01$) and Zn ($p<0.01$). In addition, there was a negative relationship between the EC value and Cu content of the soils whereas, a positive correlation between lime value and Cd content as well as a positive relationship between OM value and Cd content. Moreover, it was found that a significant negative correlation was found between the total copper content and the Cd concentrations, whereas a positive correlation was determined between the Cu content and Cr ($p<0.01$) and Zn ($p<0.01$) contents. There was a positive correlation between Cd content and Pb ($p<0.01$) content and a negative correlation with Cr content ($p<0.01$). In addition, positive relationships were found between both Cr content and Ni ($p<0.01$) concentration and between Pb content and Zn ($p<0.01$) concentration. Many researchers have obtained similar results in correlation analysis between OM, pH, lime and soil particle distribution with heavy metal content of soils (Demir et al., 2016; Özyazıcı et al, 2017; Tang et al., 2018). On the other hand, some researchers (Tume et al., 2006; Nizami and Rehman, 2018) reported that there was no relationship between pH and OM content of soils and heavy metal contents. Potential ecological risk index was calculated and evaluated both separately for each metal and to reveal the total potential impact of all metals.

Table 2. Correlation relationships between some physical and chemical properties of the soils and the heavy metals in the surface

Parameters	Sand, %	Clay, %	Silt, %	pH	EC, dS m^{-1}	$CaCO_3$, %	OM, %	tCu, mg kg^{-1}	tCd, mg kg^{-1}	tCr, mg kg^{-1}	tPb, mg kg^{-1}	tNi, mg kg^{-1}	tZn, mg kg^{-1}	PERI
Sand, %	1													
Clay, %	-0.906**	1												
Silt, %	-0.564**	0.161*	1											
pH	-0.087	0.203**	-0.195*	1										
EC, dS m^{-1}	-0.138	0.217**	-0.102	0.400**	1									
$CaCO_3$, %	-0.247**	0.255**	0.078	0.421**	0.016	1								
OM, %	-0.045	-0.022	0.148	-0.052	0.175*	0.107	1							
tCu, mg kg^{-1}	0.222**	-0.253**	-0.026	-0.042	-0.235**	-0.188*	-0.055	1						
tCd, mg kg^{-1}	-0.143	0.152	0.037	0.057	0.118	0.233**	0.244**	-0.231**	1					
tCr, mg kg^{-1}	-0.100	0.064	0.109	-0.013	-0.097	-0.057	0.102	0.209**	-0.210**	1				
tPb, mg kg^{-1}	-0.116	0.122	0.033	-0.196*	-0.033	-0.051	0.054	0.193*	0.272**	-0.011	1			
tNi, mg kg^{-1}	-0.054	0.068	-0.008	0.069	-0.017	-0.010	0.091	0.177*	-0.149	0.817**	-0.031	1		
tZn, mg kg^{-1}	0.200**	-0.245**	0.013	0.086	-0.143	-0.126	0.142	0.504**	0.006	0.100	0.230**	0.129	1	
PERI	-0.141	0.149	0.039	0.067	0.105	0.221**	0.246**	-0.174*	0.997**	-0.180*	0.336**	-0.120	0.046	1

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Assessment of PERI for soil samples

Among investigated heavy metals (Cu, Cd, Cr, Pb, Ni and Zn), only cadmium and lead significantly correlated with the PERI (Table 2). According to researcher, this case shows that the possibility of both heavy metals in the micro catchments located inducing ecological risks was quite high (Effendi et al., 2016). Distribution map of soil samples for PERI classes was given in Figure 4. It was not detected high PERI classes in the micro catchments of Ordu province. In addition, PERI results, it was detected that while 54% of total soil samples were low while, 42% of them were moderate class. According to the potential ecological risk index results, 54% of the catchment soils was low; 42% was moderate; 4% was at significant ecological risk level. Moreover, it can be seen that almost all micro catchments have moderate classes whereas some micro catchments coded 11, 13, 16, 6 and 12 have considerable class. On the other hand, two micro catchments coded 3 and 4 have no potential ecological risk.

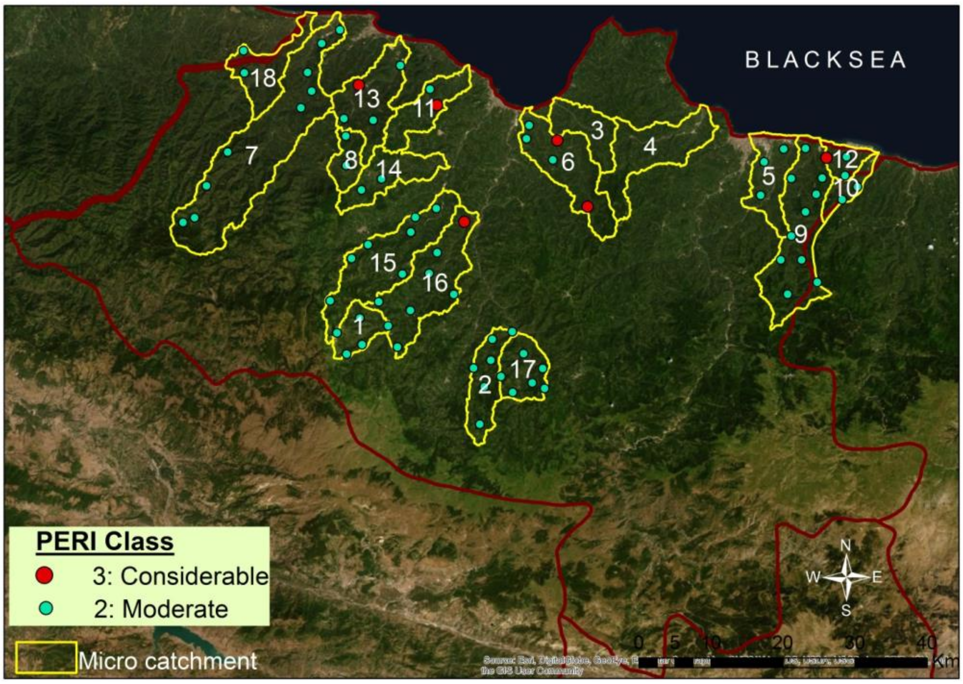

Figure 4. Distribution map of soil samples for PERI classes

In this present study, the greatest contribution to PERI comes from the Cd element. Accordingly, the ranking of heavy metals according to calculated average potential ecological risk indexes is Cd> Pb> Cu> Ni> Zn> Cr. In a study conducted by Islam et al (2015), considering the seriousness of potential ecological risks for single metal (PER), the decreasing order of pollutants Cd>As> Cu> Pb>Ni>Cr. Given the potential ecological risk (PERI), it has shown that all land soils are important for a very high potential ecological risk.

Conclusion

In addition to fossil fuels, chemical fertilizers, pesticides, fungicides and similar pesticide residues used in industrial applications and agricultural activities are pollutants that result from human activity. Heavy metals contaminated and accumulated in soils can cause many environmental, plant and human health problems, such as microbial activity, soil fertility, biodiversity and yield losses, and food chain poisoning. In this present study, heavy metal element contents for organic agriculture and their potential ecological risks were determined in some micro catchment soils of Ordu province. According to the Potential Ecological Risk Index (PERI), cadmium is the highest risk posing the toxic metals, whereas the other elements have low risk.

DAP, TSP and compound fertilizers consumed in agricultural soils to increase the yield, especially the Cd content is quite high and affect the many metabolic activities such as photosynthesis, respiration, ion uptake, growth and development by passing into the plant structure. That's why; the fact that Cd, which has the highest ecological risk value, is not associated with other elements suggests that it reaches the ecosystem in different ways. The anthropogenic and geogenic elements were considered for the enrichment of metals in soil. The average of ecological risk potential was classified as moderate and low risk in this current study. Moreover, the soil pH decreases with the use of acidic fertilizers and causes the activity of heavy metals to increase. We all have various responsibilities for the protection of our land and water resources. Nowadays, it is seen that there is not enough precautions for soil pollution which is a universal problem in our country and there is not enough studies about polluted soils. Therefore, chemical fertilizers should be applied with a fertilization program based on analysis results under expert control.

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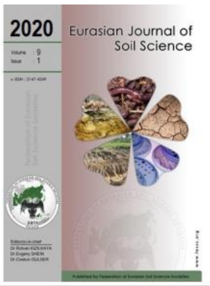

The correlation of Azerbaijan arid soils with WRB-2014

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Abstract

The geographical principles are used in grouping of the soils the highest taxonomic standards in the soviet soil scientists works concerning the classification. But the Azerbaijan soil classification is based on genetic profile. Traditionally – topographic, color, climate and so on names are used in this classification, which in the soils genetic types idea is assumed as a basis. The International classification system is identified on the basis of the diagnostic parameters in the field researches and specified as a result of the laboratory analyses. The Azerbaijan soil classification consists of eight taxonomic level and a high taxonomic level is considered- soil class, section, genetic soil type, low soil taxa - soil gender, sort, diversity, row, soil variants. Mainly used central taxon is soil type. An aim of the research is to determine correlative chances of WRB-2015 International soil classification system with the Azerbaijan soil classification. It was detected that a correlation of both soil classifications is possible. The correlation is taken place in different taxon levels. Therefore, the soils were analysed at a type and subtype standard besides high taxas when the soils were correlated. As a result of correlation, it was defined that irrigated meadow-grey soils should be correlated Anthrosols, but meadow-grey soils concerned Calcisols and Gleysols.

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Introduction

Soil classification is a means which have traditional and necessary importance in performing of the scientific and applied works in connection with the correct and expedient management of the soil productivity and registration of the soil resources. The soil classification is the form of defining soil diversities and also expanding of researches and modernization of soil reserves (Lebedeva et al., 2005; Sarmast et al., 2016). It is also an important instrument in the communication of soil research results at and international levels of soil science (Zádorová and Penížek, 2011; Rozhkov, 2012). Soil classifications are dynamic systems and their changes are closely related to the latitude of thought of soil researchers and the level of knowledge about soils. Yet soil classification principles should remain consistent with the geographical features of territory's evolution (Lebedeva and Gerasimova, 2009). There is a special soil researche program in every country some of them are fulfilled at an economical standard, some at state standard and some at national standard. Two approaches are available in soil classification establishment classification based on genetics and diagnostics (Bockheim and Gennadiyev, 2000). The first soil classification was established by the Russia soil scientists and built on genetic principles according to Dokuchaev′s soil forming theory (Gerasimova, 2015).

Azerbaijan is one of such regions by its large specter of the horizontal and vertical soil zones more diversity of the lowest taxons and soil types. The modern soil regime processes are a basis of the Azerbaijan soil classification by paying attention to soil features and soil forming factors. The Azerbaijan soils classification was prepared by a genetic-profile principle. The classification taxa diagnostics on the basis of the soil profile structural characters is given by suggesting of the soils' division on genetic profile and this approach gives a chance to pay attention to all the natural and anthropogenic changed soils in the single soil classification.

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According to Salaev (1991), an ecological-genetic classification is more separated taxa system than other classification systems type- subtype-gender-sort. According to this approach the Azerbaijan soil map scaled as 1:100000 was prepared (1998) and adopted for the soil classification at a country's standard. As above mentioned, an available classification system of Azerbaijan soils is characterized regionally and its correlation to the International soil classification is necessary for participation in the integration process under the modern globalization condition. As it is known, WRB soil classification system (FAO, 2015) is rather good for a national classification systems correlation, because the international correlation chances were taken into account while establishing this system, it is used for the middle and little scaled maps classification very much (Krasilinikov, 2009). There were efforts by several researchers in a correlation direction of the national soil classification based on genetic profile for the WRB. One of the main problems is the utilization of the different analysis methods for definition of the soil diagnostic indications and information shortage causes definite mistakes during correlation (Reintam and Koster, 2006; Urushadze et al., 2016). Though the Azerbaijan Soil Resources were investigated before the XX century, since 1945 they were begun to be studied in institutional form. Factually majority of the methods used in soil analyses is ancient and isn't used for WRB classification by International Union of Soil Sciences (IUSS). Though there is enough information about Azerbaijan soil resources for the local use, but its utilization in an international scale is difficult. It makes definite difficulty in integration to the International Soil Science. The differences and connections between the World Reference Base for Soil Resources and the Azerbaijan soil classification system were first summarized by Krasilnikov et al. (2009) who also established correlation keys primarily based on field experiences and the definitions of the classification units. However, they pointed out that the classes of the two systems cannot match due to their different approach and methodology. At present, the development of international cooperation in different sciences, including soil science, poses the problem of the creation of a new soil classification system that should be based not only on the regional and national classification concepts but also linked with the internationally accepted soil classification systems (Babaev et al., 2006; Gerasimova, 2015). An increasing demand for harmonized digital soil information can be observed nowadays. The correlation of national systems with WRB has got a new priority, as it is necessary for the development of European and global databases, giving the opportunity to enrich them with more new data (Kabała et al., 2015). The former Soviet countries mainly used form the classification grounded on genetic profile (Shishov, 2004), last years the correlation process is performing for the WRB international classification system. In this direction the expert based, concept based and centroid based methods are mostly used, these methods effectively help the correlation process (Lang, 2013). The research aim is to investigate a correlation of the Azerbaijan soil classification to the WRB soil classification and to define their different and unlike characters. As a research object the meadow grey, irrigated meadow-grey soils which are dominant soil type for the Azerbaijan zone have been taken.

Material and Methods

The study area was Mugan plain, located the central part of Kur-Araks lowland between 39°43'18″ N and 39°44'40″N latitude and 47°58'14″ and 48°57'32 E longitude covering an area of 4500 km^2. It has an arid climatic condition and a quantity of the rainfalls is 180-250 mm. Evaporation is between 800-1000 mm. Annual average temperature changed between 14-15 º C. The arid sone of Azerbaijan characterized with its agrarian importance and soils mainly used under different agricultural plants such as wheat, barley, cotton, lucerne and vegetables. The main part of research area is located under sea level for this reason soils genetically have high risk to salinization. The elevation of the main part of the research area is located under sea level and changed between -37-50 m. Most of the current topography is a result of post-glacial processes during the quaternary period. The low elevation areas are mainly covered by alluvial, marine and lacustrine materials.

The WRB-2014 (2015 update) criteria which were adopted by the world soil scientists were assumed as a basis. While investigating the correlation opportunities the method (Minasny et al., 2010) based on conception and Krasilnikov et al. (2009) approaches were used. The last version of the Azerbaijan soil classification was in 2011 (Babaev et al., 2011) and the main taxa were determined.

45 soil pedons were excavated in the research area. The main soil types are meadow-grey and irrigated meadow-grey soils. On field checks, the geographical positions of representative soils for each site were determined by Global Positioning System. The soil profiles were excavated and described according to the "Guidelines for soil description" (FAO, 2006). Soil samples from different genetic horizons were taken, and samples were air dried, crushed, and passed through a 2 mm sieve. Pipette method was used for texture analysis (Gee and Bauder, 1986), Organic matter by Walkley–Black method (Walkley and Black, 1934),

calcium carbonate by calcimetric method (Loeppert and Suarez, 1996), cation exchange capacity by was defined as sum of exchangeable cations (Bower and Hatcher, 1966), pH and EC by pH-meter and EC-meter.

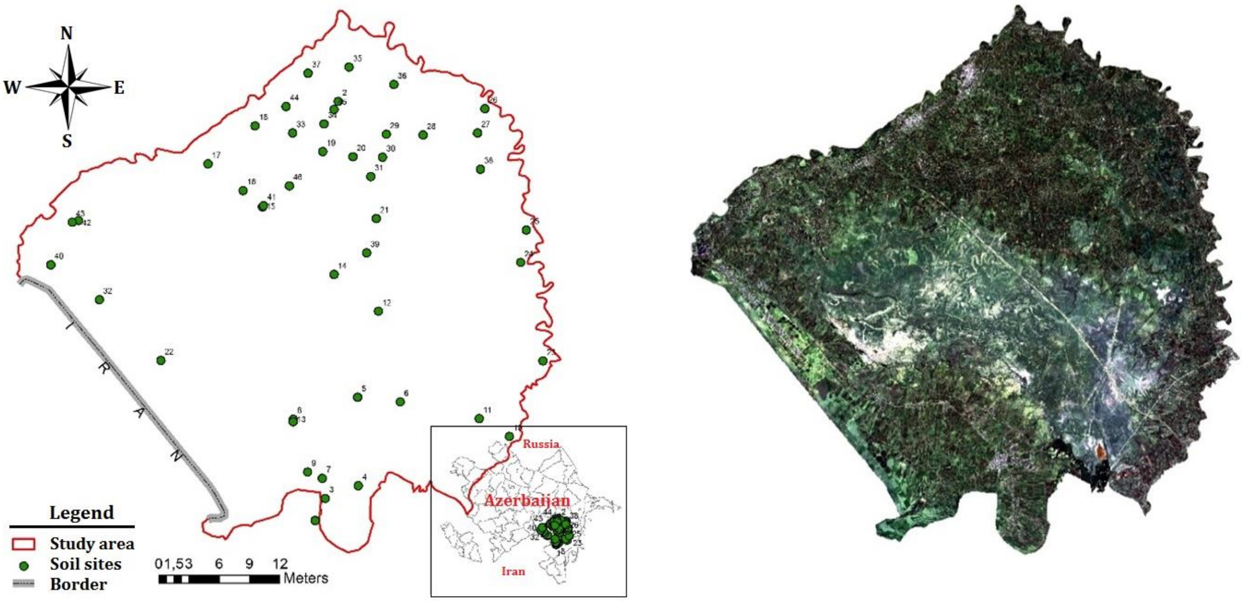

Figure 1. Study area (soil pedon distribution and natural color composition)

A last version of the Azerbaijan soil classification was printed in 2011 (Babaev et al., 2011). The Azerbaijan soil classification consists of 3 classes, 15 sections, 37 types, 90 subtypes, 146 genders, (species); 388 sorts, 140 diversities, 157 rows, 47 variants. This variety forms large information about the soils and simplifies correlation opportunities. The soil passports (over the types) prepared according to the Azerbaijan soil classification rules and Azerbaijan Soil Map with the scale of 1:100000 were used. Soils were classified according to the WRB (FAO, 2015) soil classification systems. Finally, the soil units of WRB were compared with Azerbaijan soil classification based on genetic properties.

Results and Discussion

The irrigated meadow-grey, meadow-grey soil types which are dominant for the Azerbaijan arid soil zone were taken in order to investigate the correlation opportunities to the WRB soil classification. Azerbaijan soil classification were correlated the at a type level and determined the principal soil types paying attention to the main soil forming character concerning the WRB 2006 version (Babaev, 2018). This process has not realized in the low taxa. However, one soil type concerns 2 Soil Reference Group (RSG) in the WRB in some cases. On the other hand, the small taxa which are taken as a diagnostic indication in the Azerbaijan soil classification aren't characteristic for the WRB RSG in some cases. Therefore, using of more information is rather important while performing the WRB correlation process. The Azerbaijan soil classification is hierarchic structural. The greatest taxon is considered soil class, the smallest taxon is considered a variant. In general the Azerbaijan classification involves two units system. A high taxonomic level-soil class, section, genetic soil type. The low soil taxons-soil gender, sort, diversity, row, soil variants paying attention to the soil fruitfulness ability. Prior to elaboration of the new Azerbaijan soil classification system a complete systematized list of soils was compiled on the basis of the explication to the soil map of Azerbaijan on a scale of 1:100000, which characterizes in detail the spatial distribution of different genetic soil groups (Babayev et al., 2006). Correlation of the meadow-grey and irrigated meadow-grey soils to the WRB was identified as Calcisols in the first initiative performed according to the archive materials. A main diagnostic indication in definition of these soils as an independent soil type is a sign of the oxidation-reduction process which is formed by subsoil water in the location depth and soil profile. Meadow-grey soil type developed under the winter pastures in the plain zones. The soil profile thickness is >120 cm. There are two subtypes. The ground waters in the meadow-grey subtype are situated below 200 cm and oxidation- reduction processes mainly occur B and C horizons (Babayev, 1984).

Gleying is observed beginning of B layer in the profile as a result of the ground waterseasonal fluctuation in the meadow-grey soils. The soil profile is calcareous and an average quantity of carbonates is 10,8%. As a result of the high evaporation $CaCO_3$ or gypsum accumulation on the upper layers is characterized by the ground waters. The quantity of carbonates on the topsoil changed between 3.8-141.7 g/kg and mean values is 103.3 g/kg. But within the profile the value of carbonates increased (46.7- 215.8 g/kg). A quantity of organic substances changes by 1,5-3%, pH -8-9. On the other hand, salinized–solonetzificated process is characteristic for the soil profile and the dissolved salts are accumulated at 50 cm of depth. These soils correlate to Haplic Calcisols (Gleyic, Salic). These soils spread in an associated form with the saline soils. A main characteristic feature of the Calcisols is accumulation of secondary carbonates in the soil profile. Oxidation-reduction process indications which are created under the groundwater effect are taken into account at a second level. Though this process was clearly given in the national classification, it was distinguished in the gender and sort taxa. The main morphological characteristics of characteristic pedons were given in Table 1. A main characteristic feature in the irrigated meadow–grey soil type is Irragric horizon formation as a result of the long irrigation and cultivation. The irrigated meadow-gray soils of the study area have been formed on the Araks river basin. These soils are irrigated by the channels separated from Araks River. Araks river has high content of mineral and nutritional content. The mineralization of irrigation water is 560-880 mg/l. Morphological and diagnostic indicators of soil have changed considerably as a result of irrigation of cultivated areas using these waters.

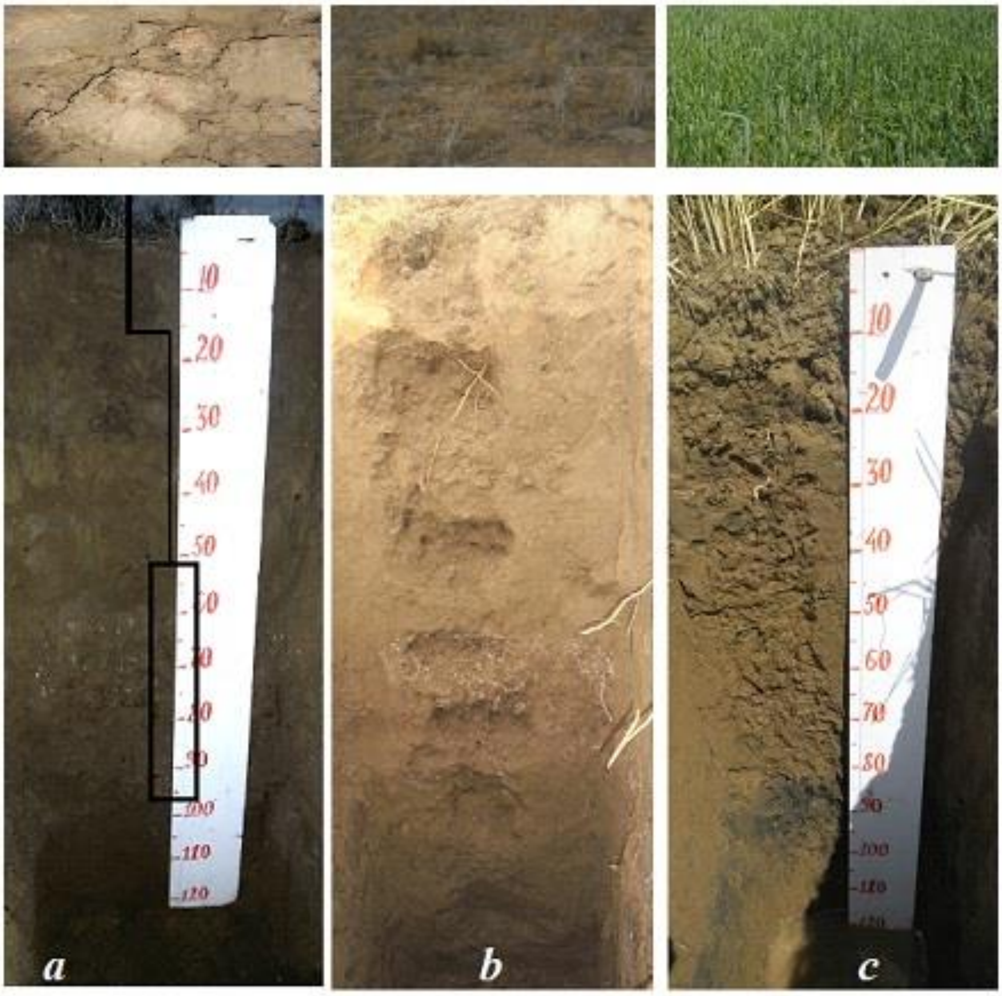

Figure 2. Gleysols (a), Calcisols (c) and Anthrosols (c) of Mugan lowland

The main reason of formation of pedodiversity on the type level is the changing of soil formation processes. In this case, it forms the changes in soil taxa and the current taxon's isn't enough to describe soil classification systems in global way (Bockheim and Gennadiyev, 2000).

In any case, guarding of the natural structure is impossible, because majority of the soil is under anthropogenic impact to some or other degree. Babaev (1984) consolidated such soils in the highest taxa anthropogenic changed soils class paying attention to the long anthropogenic, especially irrigation impact. The agroirrigation horizon was formed in the soil profile by the impact of the long irrigation and cultivation in morphogenetic diagnostics of these soils. Thickness of this layer is more than 50 cm. The soil profile became dark, humus was washed into the deep layers. The burial state exists in these soils (Figure 2). Correlation of this soil type to the WRB confirms with the Anthrosols RSG and the Irragric Anthrosols (Gleyic, Loamic) soils were formed. As is seen the correlation to the WRB occurs in the highest taxon-class level in this soil type.

Table 1. Morphological characteristics of studied pedons

Profile No and cordinates	Horizon	Depth	Boundary	Colour		Consistency (moist)	Structure	Effervescence	Texture
				Moist	Dry				
S13 39º33'02 N 48º36'36 E	Ap	0-16	GS	10YR4/3	10YR7/1	LO	GR	MO	Silty-clay
	A1	16-30	GS	10YR4/2	10YR7/4	FR	AB	ST	Silty-clay
	ABc	30-58	GS	10YR5/4	10YR7/4	VFR	MA	MO	Silty-clay
	Bk	58-92	CS	10YR4/4	10YR7/3	VFR	MA	ST	Clay
	CBz	92-124	CS	10YR5/3	10YR7/2	VFR	BL	ST	Clay
S16 39º26'28 N 48º31'16 E	Ap	0-18	GW	10YR5/3	10YR6/4	FR	SG	ST	Silty-clay
	A1	18-33	GS	10YR5/3	10YR6/2	FR	SA	ST	Silty-clay
	AB	33-57	CS	10YR4/2	10YR7/3	FR	SA	ST	Silty-clay
	Bg	57-94	AS	10YR5/3	10YR6/3	FR	AS	ST	Silty-clay
	Cr	94-132	AS	10YR5/3	10YR7/2	FR	AS	ST	Silty-clay
S19 39º40'30 48º38'41 E	A	0-8	CS	10YR3/2	10YR5/2	VFR	MA	ST	Clay-loam
	A1	8-26	CS	10YR3/2	10YR6/2	VFR	SN	ST	Silty-clay-loam
	Bc	26-49	CS	10YR6/4	10YR7/1	FR	SA	ST	Silty-loam
	BCc	49-78	CS	10YR5/4	10YR8/1	FR	AS	ST	Silty-loam
	Cg	78-109	CS	10YR5/3	10YR8/2	FR	AS	ST	Silty-loam
S20 39º43'35 N 48º33'37 E	A	0-9	CS	10YR3/2	10YR6/3	FR	AB	ST	Silty-loam
	AB	9-30	CS	10YR6/4	10YR8/3	FR	SB	ST	Silty-loam
	Bg	30-58	CS	10YR6/5	10YR7/4	MFR	M	ST	Silty-clay-loam
	BCg	58-89	CS	10YR5/5	10YR7/3	SH	M	ST	Silty-clay
	Cg	89-118	CS	10YR4/3	10YR6/3	SH	M	ST	Silty
S31 39º36'34 N 48º57'11 E	Ap	0-23	GW	10YR6/2	10YR8/2	VFR	SG	ST	Silty-clay-loam
	A1	23-49	GS	10YR5/4	10YR7/2	FR	SB	ST	Silty-clay-loam
	Bk	49-81	CS	10YR5/2	10YR7/3	FR	SB	ST	Silty-clay-loam
	BCk	81-114	CS	10YR7/4	10YR8/2	MFR	SB	ST	Silty-clay-loam
S35 39º56'60 N 48º49'22 E	Ap	0-20	GW	10YR4/3	10YR6/3	FR	GR	ST	Silty-clay-loam
	A1	20-30	GS	10YR4/2	10YR6/2	FR	AS	ST	Loam
	AB	30-74	GS	10YR5/3	10YR7/3	VFR	AS	ST	Silty-clay-loam
	Bg	74-88	CS	10YR4/1	10YR7/1	VFR	SA	ST	Silty-clay-loam
	Cg	88-126	CS	10YR6/3	10YR8/4	VFR	SB	ST	Silty-clay-loam
S41 39º49'20 N 48º25'30 E	Ap	0-26	GS	10YR4/3	10YR8/3	FI	GR	ST	Silty-clay-loam
	A1	26-48	GS	10YR4/4	10YR7/3	FR	AB	ST	Silty-clay-loam
	ABk	48-76	CS	10YR5/4	10YR7/3	FR	SA	ST	Silt-loam
	Bg	76-99	CS	10YR4/3	10YR7/4	FR	MA	ST	Silty-clay-loam
	BCg	99-138	CS	10YR4/4	10YR8/4	FR	MA	ST	Silt-loam

Boundary: distinctness (A-abrupt, C-clear, G-gradual); topography (S - smooth, W - wavy). Consistency: moist (L- loose, VFR - very friable, FR- friable, FI- firm, VFI-very firm). Structure: type (AS-angular and subangular block, AB - angular blocky, SB - subangular blocky, SG-single grain, SA- subangular and angular blocy MA - massive). Effervescence: class (ST - strongly effervescent, MO- moderately).

Conclusion

The Azerbaijan soil classification is called according to morphology, colour, climate, topographic and so on and the chemical parameters are characterized diagnostically. Some of the small taxa aren't used in correlation. For example, the differentiation isn't performed in WRB for the soil profile density and cultured level which include in the small taxon-row and variant. The results showed that the correlation of the low taxa gender, sort and diversity used in the national soil classification to the WRB is possible. The Azerbaijan soil classification correlation based on genetic profile can be occurred at different levels. Sometimes correlation to the WRB does not result positively at a type level and it can conform to the separate Soil Reference Group at the subtypes level that includes in the type. The soil formation direction is assumed a basis while the soil types are divided into subtypes in the Azerbaijan soil classification in any case. Therefore, the correlation is also observed at a subtype level. The smallest taxa conform to the main and additional qualifications. It is possible to correlate the Azerbaijan soil classification based on genetic profile to the WRB at a different taxon level paying attention to an analogy of the soil forming process. But there is a great need for high perceptibility and expert discussion in this problem. While establishing the Azerbaijan soil classification a great scaled map is assumed as a base map and it is mainly local applied characteristic.

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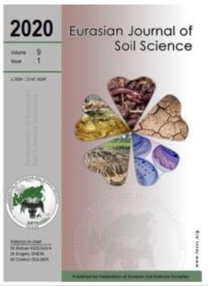

Quantifying some physical properties and organic matter of soils under different management systems in cherry orchard

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Abstract

Effects of different cover crops (CCs), mechanically cultivation and herbicide treatments on soil organic matter (SOM) content and some physical properties of soils were investigated in a cherry orchard with clay soil in 2013 and 2014. The present study was conducted in a cherry orchard located at the Experiment Station of Black Sea Agricultural Research Institute in Samsun province in the Northern region of Turkey. As the CCs, Trifolium repens L. (TR), Festuca rubra subsp. Rubra (FRR), Festuca arundinacea (FA), T. repens (40%)+F. rubra rubra (30%)+F. arundinacea (30%) mixture (TFF), Vicia villosa (VV) and Trifolium meneghinianum (TM) were utilized. Experiment also included plots mechanically cultivation (MC), herbicide treatment (HC) and control (C) plot without CCs. Experiment was conducted in randomized complete blocks design with four replications. The CCs were mowed in the flowering period of the plants. After 90 d following seed harvest, soil samples were collected from two depths (0-20 and 20-40 cm) in each plot. The CCs treatments decreased bulk density, volumetric water content, relative saturation, penetration resistance and increased SOM, field capacity, permanent wilting point, available water capacity, total porosity, gravimetric water content, mean weight diameter, structural stability index. The differences in the SOM contents and physical soil properties of all treatments as compared to the control were not found to be significant for the 20-40 cm soil depth in both years of experiments. The CCs, especially TR and VV treatments as legume plants improved SOM and physical soil properties, but longer term studies are needed to evaluate the long-term effects.

Keywords: Bulk density, penetration resistance, relative saturation, available water capacity, total porosity, cover crops.

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Introduction

Among major countries producing cherry in the world, Turkey ranks the first both in the northern hemisphere and in the world with 599.650 tons of production in 2017, corresponding to 20.3% of world cherry production (FAOSTAT, 2017). Cherry fruit cultivation is carried out in an area of 847.461 da in Turkey (TURKSTAT, 2018). The U.S. with 288.480 tons of production and 13.8% share and Iran with 220.393 tons of production and 11.8% follow it. Turkey is leader country in terms of volume of cherry production in the World (FAOSTAT, 2017). Owing to the temperate zone it is located in and its geographical advantages, Turkey is a country that has appropriate conditions for cherry production. Cherry production in Turkey can be seen that there has been a continuous increase in production since 2004. Proper weather conditions as well as the increase in the interest of cultivators towards cherry were influential on this increase (Anonymous, 2008). It was also understood that Turkish cherry market has been growing due to the demand from the western European countries, especially Germany. While cherry production has been increasing permanently since 1980s, Turkish cherries constitute 19 % of supplies. The most prominent competitors have been the United States, Iran, Poland, Italy, Spain, Romania and Russian Federation, with inclusion of Chile recently (Gul et al., 2016). Many soils in Turkish orchards are low in organic matter and

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nutrients, resulting in poor soil structure and nutrient deficiencies in the fruit trees (Zengin et al., 2016). This situation negatively affects the growth of trees, which is detrimental to the yield and the quality of the fruit. The use of cover crops (CCs) as a source of organic matter may be potential solution to these problems. CCs are described as crops grown primarily for the purpose of improving and protecting soil between periods of regular crop production (Schnepf and Cox, 2006). CCs have been used to improve soil quality and reduce nonpoint sources of nutrient pollution, e.g., NO3. Thus, it is significant from both an environmental and economic standpoint to indicate how cover crop systems effect soil organic matter characteristics and the biogeochemical cycling of carbon (Daliparthy et al., 1994). Spatial variability and the dependence of soil properties are controlled by inherent variations in soil characteristics (e.g., parental material, vegetation, and climate) or are affected by exogenous factors such as crop production practices (tillage, fertilization, and crop rotation) (Gülser et al., 2016). CCs provide canopy cover outside of the normal cropping season and their residues contribute to surface residues and organic matter left by the preceding crop (Reicosky and Kemper, 1995). Cropping treatments are also used to enhance SOM contents and therefore soil quality and fertility (Demir and Gülser, 2015; Demir et al., 2019; Demir and Işık, 2019b). CCs can enhance soil quality through addition of organic matter when incorporated into the soil, helping to reduce compaction and increase infiltration; thus reducing runoff and immobilizing soil nitrogen, and reducing non-point source pollution (Dabney et al., 2001). CCs have been determined to increase soil organic matter and aggregation and generally increasing cropping intensity enhances soil quality (Kabir and Koide, 2000; Fageria et al., 2005; Villamil et al., 2006). CCs also improves soil physical properties which contribute to increase soil water storage. The overall effects of CCs on soil water availability depends largely on the amount of precipitation, water infiltration, evaporation, and transpiration by the CCs (Unger and Vigil, 1998). CCs helps to increase water at field capacity (Bilek, 2007). Intensive rainfall over short periods of time can cause the soil to ‘settle’, thus decreasing the proportion of large pores quickly. Soil physical properties are significant factors that can determine agricultural productivity and environmental sustainability. These properties influence the water and nutrient holding capacity of the soil and they serve as indicators of soil quality. Most CCs are grown in periods when the field is left bare and they can help prime the soil for the corresponding cash crops by influencing soil physical properties (Yunusa and Newton, 2003). The inclusion of CCs can help transpire some water from the soil, and their roots may hold the soils in place. This can help nutrient loss and mitigate soil. Management practices like CCs can improve porosity, increase soil organic matter and aggregation, transpire water and increase infiltration (Joyce et al., 2002). Because of their potential ability to reduce water evaporation (Blanco-Canqui et al., 2011), increase soil organic matter and aggregation (Dabney et al., 2001) and increase porosity (Williams and Weil, 2004). In addition, CCs control soil erosion, improve soil quality and fertility, suppress weeds and provide insect control (Sarrantonio, 2008). At the same time CCs are suitable implements for weed control in orchards (Mennan et al., 2009; Işık et al., 2009). Using cover crops for weed control in cherry orchards is one of the broadly applied alternative methods to the mechanically cultivated and herbicide treatment. Herbicide application and mechanical cultivation treatment are important among the current weed control practices in cherry farming. Herbicide and mechanical cultivation treatment are expected to provide weed-free cherry fields. However, coverless (bare) cherry fields may bring about increased erosion and run-off, reduced organic matter and moisture contents, and damage the soil chemical and physical properties (Keesstra et al., 2016). In addition, a few other problems are also associated with the use of herbicides for weed control in cherry fields. Evolution of herbicide resistance in weeds and environmental pollution are the most important among these (Annett et al., 2014).

Agricultural lands of Turkey are particularly prone to erosion after the crop harvests. Severe rainstorms negatively affect physical properties of the bare soil by deteriorating aggregates and clogging macropores leading to an increased erosion risk. Choosing a crop that increases aggregate stability during the growing season is one strategy to reduce the risk for post-harvest erosion (Yakupoğlu et al., 2011). Previous studies already reported on the positive effects of plant cover and aggregate stability (Gülser, 2006; Gol and Dengiz, 2008). Thus, crop residue management is a key element of sustainable crop production. Crop residues were incorporated into cropping systems in previous studies to improve and maintain soil structure and fertility. Legume and grass species are the most significant annual and perennial forage crops in Turkey and are preferred by farms because of providing sufficient plant cover and improving the percentage of organic matter (Nyakatawa et al., 2001). Soil structure improvements associated with legume based rotations also increase the drought tolerance of soils and moisture holding capacity (Goldstein, 1989).

In this study, influences of the different cover crops, mechanically cultivation and herbicide treatments on some physical properties and organic matter content of soils were investigated in a cherry orchard with clay soil in Samsun province in the Northern region of Turkey in 2013 and 2014.

Material and Methods

The present study was conducted between 2013 and 2014 in a cherry orchard located at the Experiment Station of Black Sea Agricultural Research Institute in Samsun (Latitude, 41° 22′ 93′′ N; Longitude, 36° 50′ 19′′ E) province in the Northern region of Turkey. Annual average precipitation was 685.5 mm and annual average temperature was 14.5 °C as stated by the Turkish Republic General Directorate of Meteorology (Anonymous, 2015). There was 1 m spacing between the plots and 3 m between the blocks. Each plot had a size of 35 m2 (5 × 7 m).

Cover crop treatments (CCs)

The cover crop (CCs) treatments consisted of *Trifolium repens* L. (TR), *Festuca rubra rubra* L. (FRR), *Festuca arundinacea* (FA), *Trifolium repens* (40%) + *Festuca rubra rubra* (30%) + *Festuca arundinacea* (30%) mixture (TFF), *Vicia villosa* (VV) and *Trifolium meneghinianum* (TM). *Vicia villosa* and *Trifolium meneghinianum* are annual legume plants and *Trifolium repens* L. are perennial legume plants. *Festuca rubra rubra* L. and *Festuca arundinacea* are perennial grass cover plants. Experiments also included plots mechanically cultivation (MC), herbicide treatment (HC) and control (C) plot without CCs. *Trifolium meneghinianum* seeds were supplied from Black Sea Agricultural Research Institute and the others were purchased from private seed companies. For the aim of the experiment, the randomized complete block design was used and the experiment was carried out as four repetitions. Throughout the experiment, the CCs were continued to be applied in the same plots. During the vegetation period, no fertilizer was applied. To be able to separate the consecutive plots, buffer zones that did not include CCs were used. Before the plantation of the CCs, existing weeds were manually or mechanically removed. Irrigation was performed twice (one in July and the other in August). CCs were planted through broadcast seeding at 50, 80 and 70 kg ha^{-1} for *T.repens*, *Festuca* spp. and mixture of perennials respectively in April 2012. *V.villosa* (100 kg ha^{-1}) and *T.meneghinianum* (40 kg ha^{-1}) were sown in October 2012 and November 2013. Following the sowing, seeds were incorporated into the soil by shallow cultivation. Primary tillage was performed through chisel plow and disk harrow. The CCs were mowed at the flowering period of the plants. Mowing was carried out with a motorized back-scythe. Following mowing, incorporation of the CCs into the soil was done by disking. While mowing of the CCs was performed on 23 June 2013 during the flowering stage in the first year, it was performed on 26 June 2014 in the second year of the study. Mechanical weed control was practiced with a rotary hoeing machine. In the herbicide control plots, the glyphosate isopropylamine salt (360 g a.i L^{-1}) was implemented at a dose of 2880 ml ha^{-1} (1.39 kg a.i ha^{-1}). Glyphosate was implemented at 3 atm pressure (303.97 kPa) and 250 L ha^{-1} spraying volume with a portable hand sprayer Honda WJR 2225.

Soil sampling

Soil samples were taken from 0–20 and 20-40 cm depths in each plot 90 d after plant harvest using a corkscrew-shaped soil drill and some physical properties of soil were determined. Cover crops mowed when Festuca spp. in the flag leaf periods, others were at the beginning of the flowering periods (Işık et al., 2014; Tursun et al., 2018). Samples were sieved through 2 mm sieve and made ready for soil physical analyses. Soil physico-chemical characteristics at the beginning of the experiments were given in Table 1. Soil analyses revealed that soils in the experimental field site have a fine-textured soil (61.28% Clay, 21.37% Silt, 17.35% Sand), were neutral soil pH (7.1) and low organic matter (1.81%) contents (Soil Survey Staff, 2014).

Table 1. Soil physico-chemical characteristics at the beginning of the experiments

Properties	Soil depth (cm)	
	0-20	20-40
Texture class	C	C
Clay, %	61.28	54.04
Silt, %	21.37	30.86
Sand, %	17.35	15.10
Organic carbon (OC), %	1.05	0.74
pH	7.11	7.28
$EC_{25°C}$, mmhos cm^{-1}	0.81	0.67
Ca, me 100g^{-1}	29.1	25.2
Mg, me 100g^{-1}	10.32	8.41
K, me 100g^{-1}	1.09	0.85
Na, me 100 g^{-1}	0.17	0.15

Soil chemical analysis

Soil reaction (pH) was measured by using a pH meter with glass electrode in a 1:1 (w:v) ratio soil-water suspension (Jackson, 1958). Electrical conductivity ($EC_{25ºC}$) was measured with an EC meter in a 1:1 (w:v) ratio soil-water suspension (Richards, 1954). Exchangeable cations (Ca, Mg, K, Na) were determined with the 1N ammonia acetate (NH_4OAc) extraction (Rowell, 1996). Soil organic matter was determined by the modified Walkley-Black method (Black, 1965).

Soil physical analysis

Soil particle size distribution was determined by using Bouyoucos hydrometer method (Richards, 1954). Bulk density (BD) was carried out with the cylinder method (Black, 1965). After saturating soil samples with tap water for 24 hours, soil water content at the field capacity (FC) was determined through equilibrating soil moisture for 24 hours at 33 kPa on a ceramic plate, and the permanent wilting point (PWP) was measured through equilibrating soil moisture for 96 hours at 1500 kPa on a pressure plate apparatus (Hillel, 1982). Available water content (AWC) was then calculated as the difference between FC and PWP (Hillel, 1982). Soil gravimetric moisture content (W) was determined by drying samples at 105 °C for 24 h. Volumetric water content (θ) was estimated from the following equation (Hillel, 1982);

$$\theta = W \text{ (g } H_2O \text{ g}^{-1} \text{ soil at the sampling time) x BD (g cm}^{-3}\text{)} \qquad (1)$$

Then, Eq. 2 was used to calculate the total porosity (F) (Hillel, 1982):

$$F = 1 - (BD/2.65) \qquad (2)$$

Volumetric water content (θ) was then divided by total porosity (F) to get relative saturation (RS). A standard cone penetrometer was used to determine soil penetration resistance (PR) at 0–20 cm depth (Bradford, 1986).

Hydrometer method and the following equation were used to determine soil structural stability index (SSI):

$$SSI = \sum b - \sum a \qquad (3)$$

Where “b” is clay percantage in suspension and “a” is silt + clay percentage in mechanical analysis (Leo, 1963).

Dry sieving method (with 4.00, 3.35, 2.00, 1.40, 1.20, 1.00, 0.50, 0.425 and 0.25 mm sieves) was used to calculate mean weight diameter (MWD) (Black, 1965):

$$MVD = \sum_{i=1}^{k} W(i)\,\bar{x}i \qquad (4)$$

Statistical analysis

Experimental results were subjected to statistical analyses with SPSS. Data were subjected to ANOVA and treatment means were compared with Duncan’s multiple range test. Correlation analyses were performed to express the relationships between experimental parameters (Yurtsever, 2011).

Results and Discussion

Descriptive statistics for the soil organic matter (SOM) and some soil physical properties in 0-20 cm soil depth of the cherry orchard under cover crops (CCs) and other treatments are given in Table 2. Organic matter (1.84 to 3.95%), FC (38.25 to 45.53%), PWP (21.00 to 24.59%), AWC (17.17 to 20.99%), total F (55.27 to 65.01%), W (31.59 to 36.46%), ϴ (32.86 to 37.55%), RS (53.85 to 63.47%), MWD (0.84 to 1.00 mm), SSI (54.96 to 61.67%) showed variations among the treatments at 0-20 cm soil depth in the cherry orchard. While BD values varied between 0.95 and 1.14 g cm^{-3}, PR values varied between 1.94 and 2.92 MPa in 0-20 cm soil depth (Table 2).

Table 2. Descriptive statistics for the soil properties in the 0-20 cm soil depth at the end of the experiment

Soil properties	Minimum	Maximum	Mean	Std. Deviation	CV, %	Skewness	Kurtosis
SOM (soil organic matter), %	1.84	3.95	2.97	0.76	25.7	-0.411	-1.441
BD (bulk density), g cm^{-3}	0.95	1.14	1.02	0.07	6.5	0.716	-1.405
FC (field capacity), %	38.25	45.53	42.75	2.05	4.8	-0.794	-0.646
PWP (permanent wilting point), %	21.00	24.59	23.23	0.97	4.2	-0.826	-0.239
AWC (available water capacity), %	17.17	20.99	19.56	1.14	5.8	-0.765	-0.892
Total F (total porosity), %	55.27	65.01	61.34	2.76	4.5	-0.787	-0.533
W (gravimetric water content), %	31.59	36.46	34.38	1.67	4.9	-0.478	-1.378
ϴ (volumetric water content), %	32.86	37.55	34.58	1.30	3.7	0.751	-0.281
RS (relative saturation), %	53.85	63.47	57.55	3.45	6.0	0.684	-1.430
PR (penetration resistance), Mpa	1.94	2.92	2.35	0.34	14.5	0.515	-1.458
MWD (mean weight diameter), mm	0.84	1.00	0.92	0.06	6.4	-0.310	-1.541
SSI (structural stability index), %	54.96	61.67	58.73	2.26	3.9	-0.489	-1.424

Soil organic matter (SOM)

Cover crop (CCs) treatments increased soil organic matter (SOM) contents at 0-20 cm soil depth as compared to the soil of an untreated control plot (Figure 1). CCs increased SOM content from 1.95% in the herbicide treatment (HC) to 3.47% in Vicia villosa (VV) treatment in the first year of the experiments (2013). SOM contents at 0-20 cm soil depth in the second year of the experiment (2014) were ordered as; HC (1.91%) < MC (1.98%) < C (2.00%) < FA (3.18%) < TM (3.24%) < TFF (3.27%) < FRR (3.40%) < TR (3.84%) < VV (3.89%) (Figure 1). Because CCs are normally grown during fallow periods of cropping systems, the addition of CCs to a cropping system may increase total residue C inputs to soil and has the potential to increase soil organic carbon (Jarecki and Lal, 2003). Bertin et al. (2003) reported that SOM content was increased in the cover crops through root activity, i.e. exudation of low-molecular weight organic compounds. In addition, the root systems of perennials are more randomly branched and have larger diameter roots than annual. This may explain the nutrient conservative strategy of perennials and the high nutrient uptake capacities of annuals (Roumet et al., 2006). On the other hand, salt tolerance in Trifolium repens appears to be correlated with (i) a capacity to restrict and regulate the transport of these ions from the roots to the shoots, leading to lower concentrations of Na^+ and Cl^- in the shoot, and (ii) lower uptake rates of Na^+ and Cl^- per unit of root tissue (Rogers et al., 1997). Sainju et al. (2002) and Villamil et al. (2006) have determined higher organic matter content when cover crop roots decompose and when their above ground biomass is incorporated into the soil. Most studies determined that the amelioration of soil physical properties is largely based on increases of organic carbon in the soils (Gülser and Candemir, 2015). Previous studies conducted on same experimental plots with the similar treatments showed that soil organic matter increased by CCs (Wegner et al., 2015; Demir and Işık, 2020). Blanco-Canqui and Lal (2007) reported that CCs treatments also found an increase in soil organic carbon with higher residue retention rate in silt loam soil. The increase in soil organic carbon was due to the input of additional carbon in the soil surface (Stetson et al., 2012). These other studies support the present results of increasing SOM with cover crop treatments. Sainju et al. (2006) reported that soil organic carbon at 0-10 cm fluctuated with plant carbon input and was greater from the CCs (hairy vetch, rye, mixture of hairy vetch and rye) as compared to the control. Gülser (2004) reported that cropping treatments increased the SOM content from 2.28% for bare soil to 3.18% for bromegrass treatment. In this study, the greatest increases in SOM contents at 0-20 cm soil depth of cherry orchard as compared to the control were observed in VV treatment in 2014. CCs have also been reported to increase SOM compared to the bare control (Sainju et al., 2002; Villamil et al., 2006) and this can lead to improved soil aggregate formation and increased water infiltration (Joyce et al., 2002). This better soil cover and aggregation can be the difference in agricultural sustainability after a few decades.

The differences in SOM contents were not found to be significant for the 20-40 cm soil depth in both years of experiments (Figure 1). SOM contents ranged from 1.19% in HC treatment to 1.36% in VV treatment for the 20-40 cm soil depth in 2013. SOM contents ranged from 1.21% in MC treatment to 1.40% in VV treatment for the 20-40 cm soil depth in 2014.

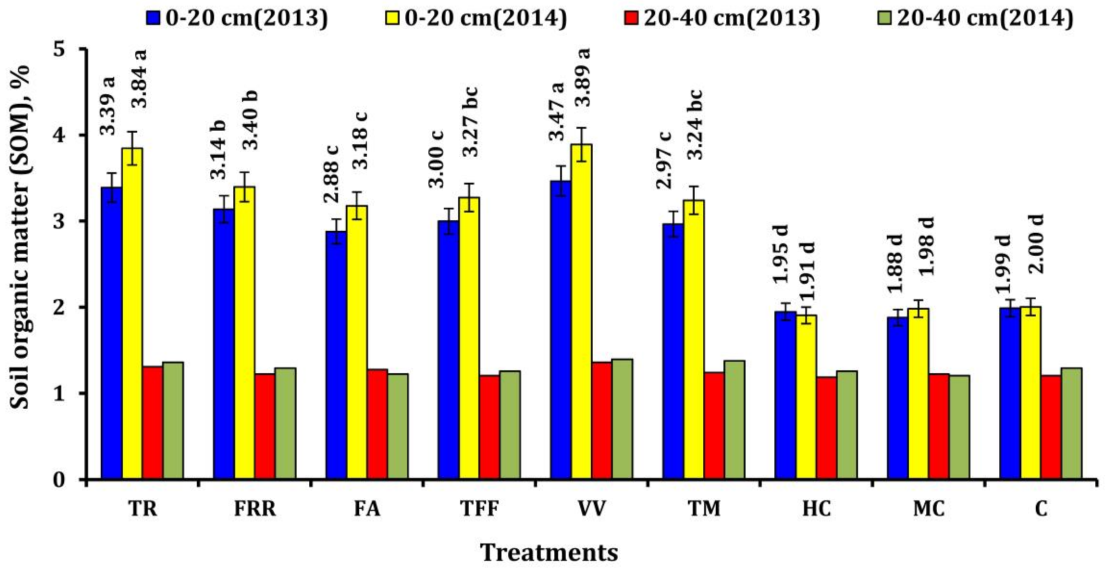

Figure 1. Effects of cover crops (CCs) and other treatments on soil organic matter (SOM) at 0-20 cm and 20-40 cm soil depths in a cherry orchard with clay soil (*Trifolium repens* L. (TR), *Festuca rubra rubra* L. (FRR), *Festuca arundinacea* (FA), *T. repens* (40%)+*F. rubra rubra* (30%)+*F. Arundinacea* (30%) mixture (TFF), *Vicia villosa* (VV), *Trifolium meneghinianum* (TM), a plot mechanically cultivated (MC), herbicide treatment (HC) and control plot (C)).

Bulk density (BD) and total porosity (F)

There were significant decreases in bulk density (BD) values (P< 0.01) and significant increases in total porosity (F) (P< 0.01) with CCs treatments at 0-20 cm soil depth when compared to values of the control (Table 3). As compared to control, the percent changes in BD and total F are provided in Table 4.

Table 3. Effects of the treatments on some soil physical properties at 0-20 cm soil depth in a cherry orchard

Treatments	BD, $g\ cm^{-3}$	FC, %**	PWP, %**	AWC, %**	Total F, %**	W, %**	Θ, %**	RS, %**	PR, Mpa**	MWD, mm**	SSI, %**
2013											
TR	0.987 b	44.2 a	23.5 a	20.6 a	62.8 a	35.2 a	34.7 ab	55.4 bc	2.09 c	0.984 a	60.23 a
FRR	1.015 b	43.3 a	23.0 ab	20.3 a	61.7 a	33.5 b	34.0 b	55.1 c	2.35 b	0.942 b	59.20 ab
FA	1.017 b	43.6 a	23.2 a	20.3 a	61.6 a	34.3 ab	34.9 ab	56.6 b	2.33 b	0.926 b	59.03 ab
TFF	1.001 b	43.9 a	23.4 a	20.5 a	62.2 a	34.1 ab	34.1 b	54.9 c	2.29 b	0.976 a	58.57 b
VV	0.988 b	44.1 a	23.5 a	20.6 a	62.7 a	35.3 a	34.9 ab	55.6 bc	2.07 c	0.979 a	60.20 a
TM	1.011 b	43.5 a	23.2 a	20.4 a	61.8 a	33.6 ab	34.0 b	54.9 c	2.28 b	0.933 b	58.67 ab
HC	1.150 a	40.0 b	21.5 c	18.5 b	56.6 b	31.0 c	35.7 a	63.0 a	2.89 a	0.835 c	55.86 c
MC	1.130 a	40.1 b	21.9 bc	18.2 b	57.4 b	31.7 c	35.8 a	62.5 a	2.80 a	0.848 c	55.89 c
C	1.124 a	40.1 b	21.8 bc	18.3 b	57.6 b	31.7 c	35.6 a	61.9 a	2.85 a	0.849 c	55.68 c
2014											
TR	0.965 b	44.5 a	24.0 a	20.5 a	63.6 a	36.1 a	34.84 b	54.8 b	2.00 d	0.988 a	60.76 a
FRR	0.982 b	43.7 a	23.6 a	20.1 a	62.9 a	35.1 bc	34.47 b	54.8 b	2.28 b	0.940 b	59.64 a
FA	0.992 b	43.9 a	23.6 a	20.3 a	62.6 a	34.6 c	34.33 b	54.9 b	2.24 b	0.933 b	60.25 a
TFF	0.982 b	44.0 a	23.8 a	20.2 a	62.9 a	35.3 bc	34.67 b	55.1 b	2.10 c	0.981 a	59.85 a
VV	0.975 b	44.3 a	23.9 a	20.4 a	63.2 a	36.2 a	35.30 b	55.8 b	1.98 d	0.986 a	60.73 a
TM	0.986 b	44.1 a	23.9 a	20.2 a	62.8 a	35.5 ab	35.02 b	55.8 b	2.14 c	0.944 b	60.11 a
HC	1.130 a	40.1 b	21.8 b	18.3 b	57.4 b	31.8 d	35.93 a	62.6 a	2.83 a	0.849 c	55.83 b
MC	1.110 a	40.5 b	22.4 b	18.0 b	58.1 b	32.5 d	36.08 a	62.1 a	2.77 a	0.847 c	55.51 b
C	1.114 a	40.1 b	22.1 b	18.0 b	58.0 b	32.3 d	35.98 a	62.1 a	2.80 a	0.844 c	55.88 b

Numbers followed by different letters, within columns, are considered to be significantly different according to Duncan's new multiple range test (**P < 0.01, *P < 0.05). (BD: bulk density, FC: field capacity, PWP: permanent wilting point, AWC: available water capacity, F: total porosity, W: gravimetric water content, Θ: volumetric water content, RS: relative saturation, PR: penetration resistance, MWD: mean weight diameter, SSI: structural stability index).

BD values at 0-20 cm soil depth in 2013 were ordered as; HC > MC > C > FA > FRR > TM > TFF > VV > TR treatments. While the highest BD was found in HC treatment (1.130 g cm^{-3}), the lowest bulk density was obtained in *Trifolium repens* (TR) treatment (0.965 g cm^{-3}) at 0-20 cm soil depth in 2014. The greatest increase in total F (9.70%) and the greatest decrease in BD (13.38%) were observed in *Trifolium repens* (TR) treatment in 2014. Troeh et al. (2003) reported that the influence of CCs on changes in BD and total F usually results from the roots of the crops. CCs have been found to decrease soil BD and increase soil macroporosity (Villamil et al., 2006; Candemir and Gülser, 2010; Blanco-Canqui et al., 2011; Demir and Işık, 2019a). These researchers explained that CCs protect the soil from compaction, as well as it increases soil organic carbon concentrations which lowered the soil BD in the subsurface depth. The hairy vetch treatment also had a lower BD, greater porosity, and greater water holding capacity than the control in the surface soil (Patrick et al., 1957). Subedi-Chalise (2017) found that those cover crop treatments reduced the BD for the 0-5 cm soil depth. Similar results were determined by the study of Moebius-Clune et al. (2008) where stover returned had 5% lower BD compared with stover harvested. Gülser (2004) reported that cropping treatments significantly reduced BD from 1.45 g cm^{-3} for control plot to 1.27 g cm^{-3} for bromegrass treatment. In addition, due to cropping effects, total F significantly increased from 45% for control plot to 52% for bromegrass treatment. The increases in total porosity were determined in the following order; control < ryegrass < alfalfa < crownvetch < small burnet < subterranean clover < bromegrass treatments. A similar finding was determined in a research conducted on silt loam with cover crop treatments which showed reduced soil compatibility by 5% (Blanco-Canqui et al., 2011). The study of Lorenz and Lal (2005) showed that if organic matter is higher in the upper surface, it can be transported to the deeper soil to promote soil micro and macro organisms. Therefore, crop residues provide food and habitat for micro and macro organisms those preserve and increase porosity in the soil. In this study, CCs treatments significantly increased total F for surface depth in both years of the experiment. While the highest total F was found in TR treatment (63.6%), the lowest total F was obtained in HC treatment (57.4%) at 0-20 cm soil depth in 2014. In the study conducted by Villamil et al. (2006), they found that the introduction of winter CCs decreased BD and therefore significantly increased total F at the soil surface. Auler et al. (2014) found that annual ryegrass used as a cover crop decreased soil BD and microporosity, but increased macroporosity and total F, which can lead to better water flow in the soil. This is similar to the findings of Lal et al. (1991), Villamil et al.

(2006), and Haruna and Nkongolo (2015). Bodner et al. (2013), in a study conducted on an arable field in Austria found that was importantly effected by the soil cover treatment was the pore radius, with cereal rye (*Secale cereal* L.) having a significantly higher average pore radius compared to no cover crop. CCs as an effective way to alleviate soil compaction due to root-induced biopores being used by the following crop to penetrate the soil (Williams and Weil, 2004). The differences in mean BD and total F values of CCs treatments were not found to be significant for the 20-40 cm soil depth in both years of the experiment. The mean BD values varied between 1.17-1.21 g cm^{-3}, the mean total F values between 54.3-55.8% in the 20-40 cm soil depth (Table 5).

Table 4. Percent (%) changes in physical soil quality variables as compared to control at the 0-20 cm soil dept

2013											
Treatments	BD	FC	PWP	AWC	Total F	W	ϴ	RS	PR	MWD	SSI
TR	-12.19	10.24	7.94	12.99	8.98	11.04	-2.49	-10.53	-26.73	15.90	8.18
FRR	-9.70	8.07	5.51	11.13	7.14	5.68	-4.57	-10.93	-17.68	10.95	6.32
FA	-9.52	8.74	6.65	11.24	7.01	8.20	-2.10	-8.51	-18.32	9.07	6.02
TFF	-10.94	9.57	7.39	12.17	8.06	7.57	-4.20	-11.35	-19.69	14.96	5.19
VV	-12.10	10.05	7.80	12.74	8.91	11.36	-2.12	-10.13	-27.41	15.31	8.13
TM	-10.05	8.59	6.24	11.40	7.40	5.99	-4.66	-11.24	-20.18	9.89	5.37
HC	2.31	-0.09	-1.33	1.40	-1.70	-2.21	0.05	1.79	1.38	-1.65	0.33
MC	0.53	0.14	0.41	-0.19	-0.39	0.00	0.53	0.93	-1.72	-0.12	0.37
2014											
TR	-13.38	11.04	8.64	13.99	9.70	11.76	-3.18	-11.75	-28.56	17.06	8.74
FRR	-11.84	8.90	6.70	11.60	8.58	8.67	-4.19	-11.77	-18.61	11.37	6.72
FA	-10.94	9.37	6.83	12.49	7.93	7.12	-4.60	-11.61	-20.01	10.55	7.83
TFF	-11.84	9.77	7.83	12.16	8.58	9.29	-3.65	-11.27	-24.96	16.23	7.10
VV	-12.48	10.35	8.05	13.16	9.05	12.07	-1.91	-10.05	-29.25	16.82	8.68
TM	-11.45	9.92	7.92	12.38	8.31	9.91	-2.68	-10.14	-23.71	11.85	7.57
HC	1.44	-0.10	-1.49	1.61	-1.04	-1.55	-0.13	0.92	1.12	0.59	-0.09
MC	-0.36	0.90	1.54	0.13	0.26	0.62	0.26	0.00	-0.93	0.36	-0.67

TR: *Trifolium repens* L., FRR: *Festuca rubra* subsp. *rubra*, FA: *Festuca arundinacea*, TFF: *T. repens* (40%) + *F. rubra rubra* (30%) + *F. arundinacea* (30%) mixture, VV: *Vicia villosa*, TM: *Trifolium meneghinianum*, MC: Mechanically cultivated, HC: Herbicide treatment, C: Control. (BD: bulk density, FC: field capacity, PWP: permanent wilting point, AWC: available water capacity, F: total porosity, W: gravimetric water content, ϴ: volumetric water content, RS: relative saturation, PR: penetration resistance, MWD: mean weight diameter, SSI: structural stability index).

Table 5. Effects of the treatments on some soil physical properties at 20-40 cm soil depth in a cherry orchard

Treatments	BD, g cm^{-3}	FC, %	PWP, %	AWC, %	Total F, %	W, %	ϴ, %	RS, %	MWD, mm	SSI,%
TR	1.20	41.0	22.1	18.9	54.7	25.5	30.6	55.9	0.812	53.4
FRR	1.17	40.6	22.1	18.4	55.8	26.3	30.8	55.1	0.786	52.8
FA	1.19	40.2	22.2	18.0	55.1	26.0	30.9	56.2	0.795	53.3
TFF	1.19	40.2	22.3	17.9	55.1	26.1	31.1	56.4	0.835	52.9
VV	1.17	41.1	22.3	18.8	55.8	26.4	30.9	55.3	0.823	53.5
TM	1.18	40.5	22.3	18.2	55.5	25.6	30.2	54.5	0.796	53.7
HC	1.21	40.1	21.1	19.0	54.3	25.0	30.3	55.7	0.798	52.7
MC	1.20	40.0	21.1	18.9	54.7	25.5	30.6	55.9	0.789	52.1
C	1.19	40.1	21.6	18.5	55.1	25.3	30.1	54.6	0.801	52.3

TR: *Trifolium repens* L., FRR: *Festuca rubra* subsp. *rubra*, FA: *Festuca arundinacea*, TFF: *T. repens* (40%) + *F. rubra rubra* (30%) + *F. arundinacea* (30%) mixture, VV: *Vicia villosa*, TM: *Trifolium meneghinianum*, MC: Mechanically cultivated, HC: Herbicide treatment, C: Control. (BD: bulk density, FC: field capacity, PWP: permanent wilting point, AWC: available water capacity, F: total porosity, W: gravimetric water content, ϴ: volumetric water content, RS: relative saturation, MWD: mean weight diameter, SSI: structural stability index).

Field capacity (FC), permanent wilting point (PWP) and available water content (AWC)

There were significant increases in field capacity (FC), permanent wilting point (PWP), available water capacity (AWC) ($P<0.01$) with cover crop treatments at 0-20 cm soil depth in the second year of the experiment (2014) as compared to control (Table 3). As compared to control, the percent changes in FC, PWP and AWC are provided in Table 4. Percent increases in AWC values as compared to the control treatment at 0-20 cm soil depth in 2013 varied between 11.13% in the FRR treatment and 12.99% in the TR treatment (Table 4). As compared to control, the greatest increase in FC, PWP and AWC values was respectively observed as 11.04%, 8.64% and 13.99% in TR treatment in 2014. Soil organic matter supplementation to soils improves water holding capacity (Candemir and Gülser, 2010; Demir and Gülser, 2015; Demir, 2019). Daigh et al. (2014) reported that CCs increased soil water storage by 1.9 cm compared with no cover crop plots. A similar result was found in a research conducted in Boone County, IA where long-term incorporation of CCs increased the plant available water by 21–22% and field capacity water content by 10–11% (Basche et al., 2016). Many studies showed that CCs increase soil organic matter concentration on soil and have a positive relationship with soil water storage (Rawls et al., 2003; Olson et al., 2010). A review showed that addition of CCs may improve soil water condition by improving the soil infiltration rate, reducing evaporation, and increasing soil water storage (Unger and Vigil, 1998). Similar results were observed in a study, where cover crops improved soil moisture (Obalum et al., 2011). Demir et al. (2019) found that highest rises were in the Vicia villosa Roth treatment, diminishing the BD by 12.7% while rising the AWC by 19.4%, SOM by 63.5%, and SSI by 9.4% in the 0-20 cm soil depth in the apricot orchard with clay soil. Steele et al. (2012) reported that CCs improved physical properties of soils. The differences in FC, PWP and AWC values of cover crop treatments were not found to be significant for the 20-40 cm soil depth in both years of the experiment. The mean FC values varied between 40.0-41.1%, the mean PWP values between 21.1-22.3%, the mean AWC values between 17.9-19.0% (Table 5).

Gravimetric water content (W)

There were important increases ($P< 0.01$) in gravimetric water content (W) with CCs treatments when compared to values of the control in both years of the experiment (Table 3). As compared to control, the percent changes in W are provided in Table 4. The greatest increases in W values at 0-20 cm depth in the cherry orchard in 2013 were observed in VV treatment (11.36%). The greatest increase in W was observed in VV treatment (12.07%) and the smallest increase in W was observed in FA treatment (7.12%) at the 0-20 cm soil depth in 2014. Data from the present study observed that in general CCs treatments have higher W compared with no cover crop treatments. Similar results were determined in another study conducted in Iowa uses of winter rye CCs increased soil water storage (Basche et al., 2016). It was observed that use of cereal rye as cover crop helped to enhance soil water in maize-soybean cropping system (Qi and Helmers, 2010). Similarly, another study conducted for 7 years by successive use of winter rye as cover crop in maize-soybean cropping system determined to be effective in enhancing soil water table and conserving soil moisture. Addition of organic matter to soils increases water holding capacity (Candemir and Gülser, 2010). CCs help to decrease evaporation from the soil surface, conserves moisture from the rainfall and irrigation, and help in soil moisture availability to the subsequent crops. CCs increased water retention in soil at water potentials related to plant available water and field capacity by 21-22% and 10-11%, respectively (Basche et al., 2016). Blanco-Canqui et al., (2011) showed that a cover crop conserved more soil water compared with a no cover crop treatment. These researchers found that CCs improved the field gravimetric water content and buffered soil temperature by acting as a cover, reducing sunlight penetration and water evaporation. Soil water content was greater under CCs compared to no CCs by an average of 35% at the 0-20 cm depth (Blanco-Canqui et al., 2011). The previous study found that preserving high moisture on surface depth was due to less evaporation and high soil organic matter on surface depth (VanLoocke et al., 2012). This is consistent with several researcher findings on the effect CCs has on soil physical properties (Jokela et al., 2011; Ward et al., 2012; Haruna and Nkongolo, 2015). The reason for higher W with CCs treatments may be due to that adopting cover crop protects the aggregates from breakdown and enhances soil physical properties (Stetson et al., 2012; Osborne et al., 2014; Johnson et al., 2016). The differences in W values of CCs treatments were not found to be significant for the 20-40 cm soil depth in both years of the experiment. Mean W values ranged from 25.0% in HC treatment to 26.4% in VV treatment for the 20-40 cm soil depth (Table 5).

Soil penetration resistance (PR)

The results of this study showed that there was an important effect of CCs treatments compared with no CCs treatments on soil penetration resistance (PR) (Table 3). In both years of the experiment, the PR significantly decreased with CCs treatments. Thus, CCs treatment reduced compaction. The reason behind low soil penetration resistance under CCs treatment may be due to cover crop and crop residue protect soil aggregates from the break down, protects soil from direct compaction due to raindrops (Blanco-Canqui et al., 2014). In addition, CCs reduce compaction by the accumulation of soil organic matter. Soil penetration resistance determines the soil compaction (Abdollahi et al., 2014). In this study, the greatest decrease in PR value was observed in VV treatment (-29.25Mpa) and the least decrease was observed in FRR treatment (-18.61Mpa) (Table 4). In 2013, PR value was the highest (2.89Mpa) in the HC treatment followed by HC > C > MC > FRR > FA > TFF > TM > TR > VV treatments. The highest PR value (2.83Mpa) was seen at 0-20 cm in 2014 in the HC treatment while the lowest PR value (1.98Mpa) was seen in VV treatment. The pervious study showed that soil penetration resistance is affected by soil moisture (Whalley et al., 2007). Cover crops play an important role to preserve the soil moisture. Gupta et al. (1987) explained that silt loam and clay loam soils have greater surface area, so the addition of residues will not allow physically separate mineral particles which result in friction force reduction and less compaction. Acuña and Villamil (2014) reported that similar results were obtained in a study conducted in Illinois on silty clay loam. Subedi-Chalise (2017) found that CCs reduced soil penetration resistance value by 23% compared with that under no cover crop treatment. In this study showed that CCs treatment reduced soil PR value by mean 24.2% compared with that under no cover crop treatment.

Mean weight diameter (MWD)

As compared to control, CCs treatments significantly increased the mean weight diameter (MWD) values at 0-20 cm soil depth in both years of the experiments (Table 3). CCs treatments increased MWD value from 0.835 mm in the HC treatment to 0.984 mm in TR treatment in 2013. The greatest MWD value (0.988 mm) was observed in TR treatment and least (0.844 mm) in control treatment in 2014. Changes in the MWD showed that aggregates were more resistant to physical abrasion under CCs. This study clearly shows that the legumes are the most effective plants in increasing MWD in 0-20 cm soil depth. *Trifolium repens* and *Vicia villosa* treatments as legume plant seems to perform best in increasing MWD of the 0-20 cm soil depth. This can be attributed to differences in efficiency of different species to different root densities in the upper soils. A closely related species, *Trifolium repens* has a robust root system that could provide drought resistance as compared to grass cover crops (Roumet et al., 2006). This study clearly shows that aggregate stability improvement by legume forage plants strongly depends on the plant species. Soil management with the use of CCs favored an increase in the number of macroaggregates, which can be due to the growth of these plants as they release their root exudates in the soil medium, favoring the formation and stabilization of aggregates in the A horizon, developing links among soil mineral particles (Bronick and Lal, 2005; Ferreira et al., 2007). In this study, the greatest increases in MWD values at 0-20 cm depth in the cherry orchard in both years were observed in TR treatment (15.90% in 2013 and 17.06% in 2014) (Table 4). Increasing SOM content in a clay soil by the different forage treatments increased MWD values according to the fallow control treatment (Gülser, 2006). Improved soil organic matter in a kiwifruit orchard with loamy textured soil increased MWD values (Demir and Işık, 2019a). Yakupoğlu et al (2011) reported that annual *V. lutea* L. and *Sphaericus* L. under Mediterranean climate significantly increased aggregate stability by 73% and 63%, respectively, in surface soil when compared to values of the control plot. The building of macroaggregates from microaggregates is a function of time and availability of SOM as the major binding agent (Hammerbeck et al., 2012). CCs improve soil aggregate stability (Mbah et al. 2007, Bhattacharyya et al. 2012) by contributing organic matter, which in turn contain active soil-binding agents (Liu et al., 2005; Mbah et al., 2007; So et al., 2009). The higher rates of soil aggregation may be due to the diversity of plant species with different sizes and shapes of the root system, which provided greater stability to the aggregates (Nascente et al., 2004). Patrick et al. (1957) found that after 25 yr of continuous cotton with tillage on a loam soil, 21.3% of the aggregates had diameters >0.21 mm when a hairy vetch cover crop was included in the cropping system compared with the 11.8% for a common vetch (*Vicia sativa* L.) cover crop treatment and 9.5% for a control without a cover crop. The differences in MWD values of CCs treatments were not found to be significant for the 20-40 cm soil depth in both years of the experiment. MWD values at 20-40 cm soil depth varied between 0.789-0.835 mm with a mean value of 0.804 mm (Table 5).

Structural stability index (SSI)

The highest SSI value was obtained from in TR treatment in 2013 (60.23%) and in 2014 (60.76%) (Table 3). Percent increase in the SSI values with CCs treatments as compared to control varied between 8.18 % in 2013 and 8.74% in 2014 in the TR treatment (Table 4). High root density produced by CCs provided exudates to the soil and importantly effected soil structure and enhanced microbial activity, and this also plays a fundamental role in soil aggregation (Bayer et al., 2000; Lovato et al., 2004). Souza et al. (2009) determined that the vigorous root system of forages contributed to the formation of aggregates and to enhancing soil physical properties, which could be obtained in the aggregates larger than 8 mm under the fallow, B. brizantha, B. ruziziensis and P. maximum treatments, especially at the soil depth of 0-5 cm. Gülser (2004) found that due to cropping effects, SSI values increased from 57.4% for control soil to 63.0% for the bromegrass treatment. Many studies indicated that CCs sustained a better soil structure, increased soil total F, aeration and water holding capacity and thus decreased BD (Steele et al., 2012). Similarly, Demir et al. (2019) determined that different CCs (Vicia pannonica Crantz, Vicia pannonica Crantz (70%) + Triticale (30%) mixture, *Phacelia tanacetifolia Benth*., *Vicia villosa*, and *Fagopyrum esculentum* (Moench.) increased available water capacity, total F, MWD, SSI values and significant decreases in BD values in an apricot orchard with clay soil.

The differences in SSI values of cover crop treatments were not found to be significant for the 20-40 cm soil depth in both years of the experiment. SSI values at 20-40 cm soil depth varied between 52.1-53.7% with a mean value of 53.0% (Table 5).

Relationships among the selected soil properties

The SOM had important positive correlations with AWC (0.924**), total F (0.896**), W (0.868**), MWD (0.862**), SSI (0.750**) and important negative correlations with BD (-0.954**), RS (-0.821**) and PR (-0.869**) at the 0-20 cm soil depth in a cherry orchard (Table 6). This could be because SOM directly effects on soil macroporosity and aggregation by the density of organic matter itself or through the affect of increasing the biological activity of the soil (Franzluebbers, 2002). Similar results were found by Stone and Silveira (2001) and Silveira Neto et al. (2006), who obtained that soil BD reduced through an increase in SOM in the surface layer. Significant negative correlations were observed between BD and total F (-0.873**), between BD and AWC (-0.907**), between the BD and MWD (-0.816**), total F and PR (-0.861**), W and PR (-0.950**). Gülser and Candemir (2012) reported that PR values gave significant negative correlations with F (-0.551**), W (-0.439**) and MWD (-0.509**), and significant positive correlations with BD (0.550**) and RS (0.374*). Veronese-Júnior et al. (2006) observed that reduces in soil moisture content increased PR in soil. Similarly, another study conducted by Gülser et al. (2011) spatial variability of PR values in a cultivated soil and determined that PR values had negative correlations with W. Gülser (2006) reported reduced the BD and increased total F with increasing the soil organic carbon contents. Gülser (2006) reported that MWD increased by the forage cropping treatments over the control had important negative correlations with PR and BD values. In this study, significant positive correlations were observed between BD and PR (0.948**), AWC and total F (0.963**), BD and θ (0.814**), between AWC and W (0.897**), between the AWC and MWD (0.793**). Although the W usually increased according to the control, the θ reduced due to decreasing BD by the CCs treatments. While the highest θ content (36.08%) was observed in the HC treatment, the lowest θ content (34.33%) was obtained in the FA treatment in the 0-20 cm soil depth at the end of the experiment.

Table 6. Correlation matrix among the soil properties in the 0-20 cm soil depth at the end of the experiment

	BD	FC	PWP	AWC	Total F	W	θ	RS	PR	MWD	SSI
SOM	-0.954**	0.916**	0.874**	0.924**	0.896**	0.868**	-0.702**	-0.821**	-0.869**	0.862**	0.7 50**
BD		-0.893**	-0.848**	-0.907**	-0.873**	-0.920**	0.814**	0.784**	0.948**	-0.816**	-0.833**
FC			0.782**	0.891**	0.677**	0.691**	-0.549**	-0.685**	-0.873**	0.879**	0.853**
PWP				0.963**	0.772**	0.872**	-0.455**	-0.632**	-0.844**	0.846**	0.719**
AWC					0.963**	0.897**	-0.592**	-0.897**	-0.895**	0.793**	0.765**
Total F						0.883**	-0.485**	-0.866**	-0.861**	0.866**	0.629**
W							-0.684**	-0.872**	-0.950**	0.666**	0.817**
θ								0.832**	0.747**	-0.693**	-0.666**
RS									0.925**	-0.682**	-0.718**
PR										-0.771**	-0.711**
MWD											0.810**

**correlation is significant at 0.01 level, *correlation is significant at 0.05 level. (SOM: soil organic matter, BD: bulk density, FC: field capacity, PWP: permanent wilting point, AWC: available water capacity, F: total porosity, W: gravimetric water content, θ: volumetric water content RS: relative saturation, PR: penetration resistance, MWD: mean weight diameter, SSI: structural stability index).

Conclusion

This study was conducted to investigate the effect of cover crops (CCs) soil management practices, mechanically cultivation and herbicide treatments on the organic matter and some physical properties of soil in a cherry orchard with clay soil in 2013 and 2014. SOM and soil physical properties were compared for two years at two soil depths (0-20 cm and 20-40 cm). In the present study, CCs treatments generally improved soil physical properties at 0-20 cm soil depth compared to the control. Mean SOM contents at 0-20 cm soil depth in a cherry orchard was ordered as; HC < MC < C < FA < TM < TFF < FRR < TR < VV treatments. CCs treatments in the cherry orchard with clay soil reduced PR and increased total F by decreasing bulk density and increasing SOM and MWD. Total F was one of the most significant soil properties that effected BD and PR in the clay textured soil. Soil organic matter supplementation to soils improved water holding capacity. The different CCs treatments had different effects on PR of clay soil due to changing soil structure with increasing total F and MWD. A combination of CCs also improved macroporosity. The differences in the SOM contents and physical soil properties of all treatments as compared to the control were not found to be significant for the 20-40 cm soil depth in both years of experiments. The results of this study showed that CCs treatments, especially Trifolium repens (TR) and Vicia villosa (VV) treatments as legume plants improved organic matter content and the selected physical properties of soils in the short term, but longer term studies are needed to evaluate the long-term effects.

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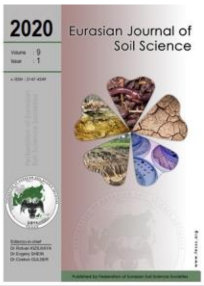

Quality of fresh plant residue affects sequestration of residue derived organic material by humic acid

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Abstract

Increasing the retention and sequestration of plant residue carbon in agricultural soils by incorporating humic acid is the main focus of this study. This study aims to examine the effect of humic acid addition on the decomposition of plant residues of varying degrees of lability. Respiration experiments were conducted to estimate the ability of humic acid to protect plant derived organic compounds from decomposition. Humic acid reduced mineralization from all added residues and this protection effect followed the lability of the residues: vetch> wheat> oak. This could be attributed to the chemical interaction between different plant-derived organic compounds and the humic acid. Lysine was strongly adsorbed to humic acid and mineralization was reduced by 23% as a result of the strong electrostatic interaction. Applying humic acid with vetch reduced all microbial indices as a result of less substrate availability for miroorganisms. On the other hand, applying humic acid with wheat might have simulated the synthesis of extracellular enzymes and the co-metabolism of humic acid (brimming effect), resulting in an enhanced microbial structure toward a higher fungal population. This study suggests: (1) Applying humic acid to ecosystems that receive labile residues (such as vetch) to reduce mineralization and enhance carbon sequestration (2) Applying less labile residues (such as wheat) in combination with humic acid to recover degraded soils and enhance carbon sequestration.

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Introduction

Sequestration of carbon in soils depends on the amount of carbon that is conserved during the decomposition process as plant residues are added to soil. Residue carbon inputs are the major source of Soil Organic Matter (SOM), thus higher residue carbon retention influence both soil structure (Pituello et al., 2016) and fertility. In addition to its importance in agronomy, higher residue carbon retention in soil is beneficial from an environmental point of view, mainly for the mitigation of greenhouse gases (ESA, 2000). Wang et al. (2017) pointed out that about 55% to 70% of residue C can be mineralized and released as CO2 within one year after addition. Therefore, residue management practices should aim to enhance long-term SOC sequestration, thus minimizing the adverse impact on global climate change (Mahmoodabadi and Heydarpour, 2014).

Physical and chemical protection of organic materials against mineralization is a crucial way to enhance the potential of soil as a sink of carbon. Organic materials can be physically protected in the interior of soil aggregates, where microorganisms and their enzymes have limited access and where oxygen concentration may also be low (Rovira and Vallejo, 2003). In addition, organic material can be chemically protected by adsorption to mineral surfaces (Rasmussen et al., 2006). Recently, some studies showed the ability of stable organic materials to sequester carbon of easily degradable materials. Biochar is the by-product of the pyrolysis of organic wastes and is regarded as a stable carbon pool. Jien et al. (2015) emphasized on the role

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of biochars in carbon sequestration. Melas et al. (2017) also showed that labile organic materials (such as glucose) can be protected against mineralization by sorption on biochar amended soils. Tian et al. (2015) showed that greater crop residue carbon was retained in biosolids-amended soil (32.5 % of total crop residue carbon) compared to unamended soil (11.8 %). Spaccini et al., (2002) showed that applying humic acids to soil could exert a protection effect of organic carbon by reducing mineralization of the relatively labile aliphatic alcohol such as 13C-2-decanol.

Testing the potential of humic acid to retain plant residue carbon in agricultural soils is the main focus of this study. Previous studies that tested the sequestration ability of humic acid are limited (Piccolo et al., 1999; Spaccini et al., 2002; Piccolo et al., 2004). The effect of plant residue quality and different plant derived organic molecules on the interaction with humic acid needs further investigation. Therefore, this study aims to examine the effect of humic acid addition on the decomposition and stability of plant residues with varying degrees of lability. Soil with a relatively low soil organic carbon content was used. Humic acid was applied with three different plant residues having varying degrees of lability (vetch, wheat and oak). Soil respiration experiments as well as different microbial quality indicators were performed to assess the effect of humic acid addition on carbon mineralization and sequestration. We hypothesized that plant residue quality and composition, mainly the soluble fraction and the proportion of different low molecular weight organic substances (LMWOS) might affect the interaction between plant residue and humic acid. Understanding the interaction between different plant residues and humic acid may give us some insight for the efficient application of humic acid to promote residue carbon sequestration in agricultural soils.

Material and Methods

Soil

Surface (0- 20 cm) representative composite soil sample was collected from Ajloun area in Jordan. The sample was taken from a land that was forest in the past, but the forest was cleared and the land was converted to cultivation. The sample was brought to the laboratory and air dried. For physical and chemical analysis, portion of the soil was sieved through 2 mm mesh size sieve. The remaining was passed through a 6.3 mm sieve in order to be used for biological analysis as well as for respiration and incubation experiments. The soil has a relatively low carbon content of 1.32%. A summary of the soil characteristics is shown in Table 1.

Table 1. Physical, chemical and biological characteristics of the soil used in the study

Soil property	Value
Moisture (%)	15.8
Total soil organic carbon %	1.32 ± 0.05
Total nitrogen %	0.13 ± 0.01
Soil pH	7.93 ± 0.20
Electrical conductivity	192.50 ± 0.05
Soil Texture	Clay
Microbial biomass (μg C g^{-1} soil)	621.00 ± 8.65
Basal respiration (μg CO_2 g^{-1} soil hr^{-1})	0.57 ± 0.13
β- glucosidase activity (μg pNP g^{-1} soil hr^{-1})	238.17 ± 0.01
Dehydrogenase activity (μg TPF g^{-1} soil hr^{-1})	5.24 ± 0.30
β- glucosaminidase activity (μg pNP g^{-1} soil hr^{-1})	15.50 ± 0.89

* Values of standard deviations are a mean of triplicates

Soil additives

Three plant residues were selected for this study area; stem and leaf portions of vetch (*Vicia sativa)* and wheat ***(****Triticum aestivum*), in addition to oak (*Quercus Calliprinos*) leaves. Collected plant materials were dried at 60 Cº and ground to pass through 1 mm sieve. These plant residues were selected according to their lability and affinity to degradation. This study requires the use of plant residues with varying degrees of lability. Oak is a tree litter with higher degree of recalcitrance compared to vetch and wheat. Vetch has lower lignin content and C/N ratio thus is the most labile plant residue in this study (Baumann et al., 2009). In addition to the plant residues, the following materials were used to treat soil samples: Glutamine, Lysine, glucose and octanol.

K-humate, Humic acid material isolated from leonardite, was brought from Rehana Agricultural Middle East Company, Jordan. This material was 90% pure; humic acid consists 90% of it. Throughout this study we will refer to this material as humic acid (HA). The characteristics of the humic acid used in this study are illustrated in Table 2.

Table 2. Characteristics of the humic acid used in this study

Property	Value
Purity (percentage of humic acid in the organic material) (%)	90
C, %	71.05
K, % as K_2O	12.00
N, %	0.72
Si, %	0.89
Ca, %	0.78
Mg, %	0.45
Na, %	1.45
Fe, %	3.00
Boron (ppm)	29.00
Specific gravity	0.82
pH (ambient temperature)	8-9
CEC, mmole/g	3.22
Acidic functional groups, mmole/ g/ ml	5.03

Respiration experiments

A preliminary experiment was done to determine the appropriate amount of humic acid to be used. Air dried soil (equivalent to 50 g oven dry weight) was incubated, in duplicate, with 2% of vetch residue and different rates of humic acid; 5 g, 10 g and 15 g of humic acid per 50 g of (oven dried) soil equivalent to 10%, 20% and 30% respectively. Microcosms were incubated for 13 days at 25 ˚C. Soil moisture was adjusted and maintained at 60% of water holding capacity (WHC).

To quantify the protection provided by humic acid for different plant residues, a respiration experiment was performed by amending 50 g soil with 2% of different plant residues, namely; vetch, wheat or oak in addition to 20% of humic acid. Treatments were: i. Control: soil alone ii. HA: humic acid+soil iii. Vetch: soil+vetch iv. Vetch+HA: soil+ vetch+ humic acid v. Wheat: soil+ wheat vi. Wheat+HA: soil+ wheat+ humic acid vii. Oak: soil+ oak viii. Oak+HA: soil+ oak+ humic acid. Treatments were conducted in triplicates.

Another respiration experiment was performed to study the protection of humic acid to individual plant-derived low molecular weight organic substances (LMWOS); lysine, glutamine, glucose and octanol. In triplicate, 50 g oven dry weight of soil was treated with 0.2 g (0.4%) substrate with or without 20 % of humic acid.

In all respiration experiments evolved CO_2 was trapped by a 1 N NaOH solution and measured periodically by back titrating the residual NaOH with 0.1 N HCl.

Incubation Experiment

Wheat and vetch residues were used in this study. 2% of each residue was applied to 300 g of soil, either alone or in combination with humic acid. Incubations were performed in jars (1.5 L). The jars were opened daily to facilitate air circulation. The jars were weighted periodically; water was added to maintain soil moisture at 60% of WHC to keep moisture constant. Treatments in this study were: i. Control: soil ii. Wheat: soil + wheat iii. Vetch: soil + vetch iv. Vetch+ HA: soil + vetch+ HA v. Wheat+ HA: soil + wheat+ HA.

About 30 g moist soil sample was taken from each jar at the intervals 0, 3, 8, 16, 23 and 30 days of incubation for enzymatic activity assays. At the end of the incubation period, fungal respiration, bacterial respiration and microbial biomass were evaluated by conducting substrate induced respiration (SIR) as described below.

Analytical methods

Chemical and physical analysis

Water content was measured according to Walter (1986). Soil pH was determined in 1:1 soil: water extract (Thomas, 1996). Organic matter was estimated by measuring organic carbon, using Walkley Black method (Nelson and Sommers, 1996). The hydrometer method was used for determining soil texture (Gee and Bauder, 1986). Kjeldal method (Bremner, 1996) was used to measure total nitrogen.

Biological analysis

Microbial biomass carbon (MBC): MBC was determined by fumigation extraction method (Brookes and Joergenseu, 2005). Briefly, 10 g soil was extracted with 50 ml 0.5 M K_2SO_4 after being fumigated for 5 days with ethanol free chloroform. MBC was calculated as follows:

$$\text{MBC (mg C g}^{-1}\text{ soil)} = \text{EC}/\text{K}_{\text{EC}}$$

Where EC is the difference in organic carbon extracted from fumigated soil and the non- fumigated soil and K_{EC}= 0.38 (Moscatelli et al., 2007).

Soil basal respiration: A respiration experiment was conducted and the value of carbon dioxide production rate was recorded after 8 days when the respiration activity reached a steady state.

Enzymatic activities: β-glucosidase activity was evaluated by the method described by Tabatabai (1994) using 0.05 M pNP β-D- glucopyranoside as a substrate. NAGase activity was assayed using 0.065 M pNP-N acetyl- β-D- glucosaminidine (Parham and Deng, 2000). The intensity of the yellow color was measured calorimetrically with a spectrophotometer at 405 nm. Dehydrogenase was assayed by reducing 2.3.5-Triphenyltetrazollium chloride (TTC) to Triphenyl formazan (TPF). The absorbance of TPF was measured calorimetrically at 485 nm (Tabatabai, 1994).

Substrate induced respiration (SIR): SIR was done according to Lin and Brookes (1999). Preliminary experiments were done to determine the optimal concentration of glucose and the optimal incubation period. 15 g soil (oven dry weight bases) was amended with 4 mg g^{-1} glucose and the rate of respiration was measured after 18 hours.

Fungal and bacterial respiration: Fungal and bacterial respiration were done following the procedure described by Bailey et al. (2002). Briefly, preliminary Tests were done to determine the amounts of antibiotics required to maximally suppress respiration. Afterwards, SIR with selective inhibition was conducted with the following three treatments: (A) No addition of antibiotics (B) Soil treated with 8 mg g^{-1} cycloheximide and (C) Soil treated with 8 mg g^{-1} streptomycine. This experiment was done in duplicate.

Statistical analysis

A one-way ANOVA was carried out to look at the effect of HA addition on different treatments. Significant (P=0.01) differences between treatments were Identified.

Results

The effect of humic acid addition on respiration and carbon mineralization

Less carbon was mineralized as humic acid rate increased. For example, the treatment of 30% of humic acid released 1452.80 mg C kg^{-1} soil, while 10% resulted in 2544.80 mg C kg^{-1} soil (Figure 1). The relationship between humic acid amount and total mineralized carbon within 13 days was linear (r^2=0.99) and quantitative within the tested range of humic acid. Accordingly, 20% was chosen to conduct the other experiments in order to facilitate the recognition of the effect of humic acid application in the experimental level that we performed. In real situation, this high concentration will not be applied at once; however, continuous additions of humic acids might result in the accumulation of high concentration of humic acid.

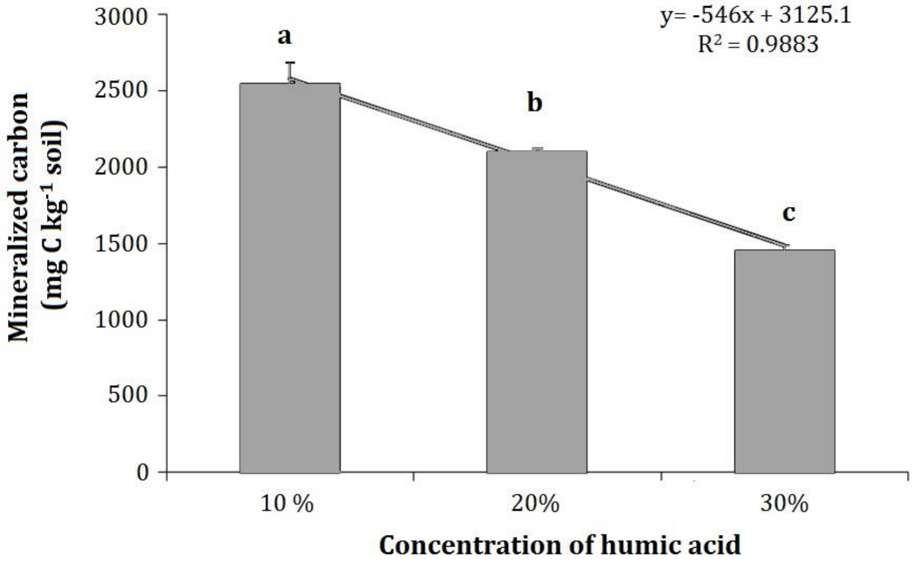

Figure 1. Total carbon mineralization over 13 days of incubation of 2% vetch residue with different concentrations of humic acid. Error bars are standard deviations from means of duplicate. No significant difference between treatments with same letter (P=0.01)

Figure 2a shows the pattern of carbon mineralization from vetch, wheat and oak. Initially humic acid significantly (P=0.01, day 3) reduced the amount of carbon mineralized from all the plant residues. The effect of humic acid in reducing mineralization then decreased with time. Later on, humic acid caused higher mineralization from the wheat and oak treatments. Humic acid seemed to be more effective in reducing decomposition of vetch litters.

Cumulative carbon mineralization from soil amended with either 0.4% of lysine, glutamine or glucose is shown in Figure 3. Applying humic acid significantly reduced carbon mineralization from lysine throughout the incubation period; it was significantly (P= 0.01, day 13) reduced from 1210.09 mg C kg^{-1} to 930.26 mg C kg^{-1}. For both glutamine and glucose, humic acid significantly (P= 0.01) reduced mineralization for the first three days, later on this effect diminished. Among the LMWOS in our study, the effect of humic acid protection was the least for octanol, which is the most hydrophobic (least soluble) compound in this study (data not shown).

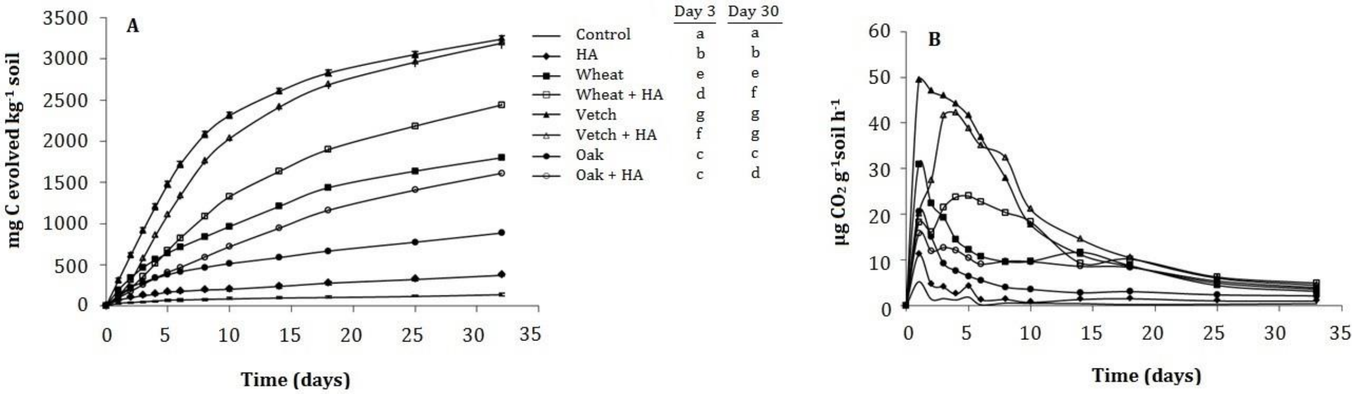

Figure 2. Respiration experiment of 2% of different plant residues with or without 20% humic acid a) Cumulative carbon C-CO$_2$ evolved (mg C kg^{-1} soil) and b) Rates of CO$_2$- C evolution (µg CO$_2$ g^{-1}soil h^{-1}). Error bars are standard deviations from means of duplicate. No significant difference between treatments with same letter within the same day (P=0.01)

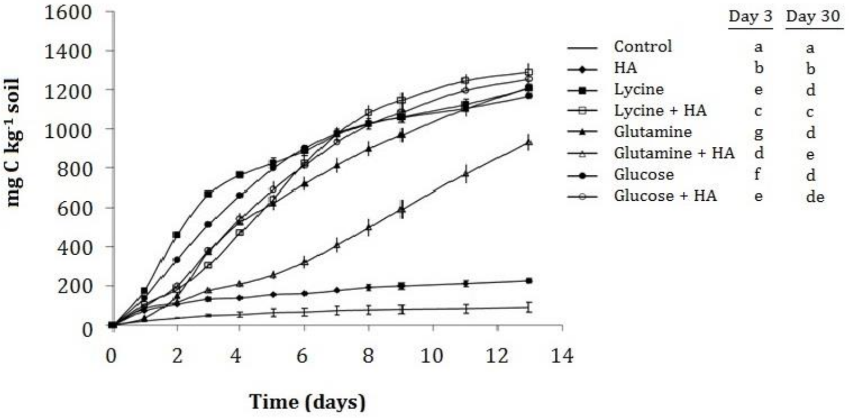

Figure 3. Cumulative carbon C-CO$_2$ (mg C kg^{-1} soil) mineralization over 13 days of incubation of 0.4% glutamine, lysine, and glucose with and without 20% humic acid. Error bars are standard deviations from means of triplicate. No significant difference between treatments with same letter within the same day (P=0.01)

Enzymatic activities

As shown in Figure 4a when vetch and wheat residue were applied alone the peak of β-glucosidase was achieved at day three; 378.17 and 377.7 µg pNP g^{-1} h^{-1} for vetch and wheat treatments respectively. Throughout the incubation period the activity of β-glucosidase was higher for vetch than for wheat. When the soil was treated with humic acid, the initial activity of β-glucosidase was higher for vetch but at late stages of decomposition its activity was higher for wheat. Differences between treatments were not significant (P=0.01) at the end of the incubation.

As illustrated in Figure 4b, initially, vetch treatments (Vetch and Vetch+HA) showed higher levels of NAGase activity than wheat treatments (Wheat and Wheat+HA). The activity of NAGase for wheat+HA treatment increased to reach its peak (286.12 µg pNP g^{-1} h^{-1}) at day 16 and its activity remained significantly (P= 0.01) the greatest until the end of the incubation. On the other hand, when soil was amended with wheat alone, activity of NAGase didn't increase and stayed at low level throughout the period of incubation.

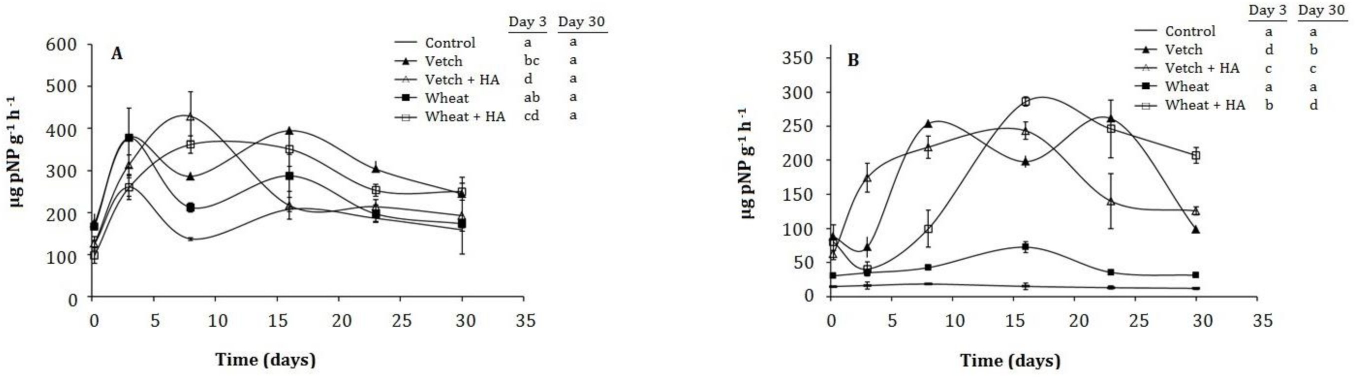

Figure 4. Time course changes of (a) β-glucosidase activity and (b) NAGase activity after incubating wheat and vetch residues with or without humic acid. Error bars are standard deviation from the means of duplicates. No significant difference between treatments with same letter within the same day (P=0.01)

Fungal and bacterial activities

Applying plant residues either alone or in combination with humic acid resulted in an increase of SIR (Table 3 (A)), which can be used as an indicator of soil microbial biomass. Vetch residues caused the highest fungal respiration (19.6 µg CO_2 g^{-1} soil h^{-1}), but it was reduced to 7 µg CO_2 g^{-1} soil h^{-1} when humic acid was added. While bacterial respiration for vetch was nearly the same in the presence or absence of humic acid. Adding humic acid to wheat caused and increase in both fungal and bacterial respiration.

Table 3. Substrate induced respiration with selective inhibition. No significant difference between treatments with same letter.

Treatment	Respiration (µg CO_2 g^{-1} soil h^{-1})					MBC* (µg g^{-1})
	A	B	C	Fungal Respiration	Bacterial Respiration	
Control	13.96 ± 0.64(a)	10.80 ± 0.00(a)	12.61 ± 0.00(a)	1.35	3.16	284.56
Vetch	52.45 ± 0.95(d)	32.86 ± 1.91(d)	47.72 ± 1.27(d)	4.73	19.6	1069.44
Vetch+ HA	33.54 ± 2.23(c)	26.56 ± 0.64(c)	29.26 ± 1.95(c)	4.28	7.00	683.85
Wheat	16.43 ± 0.32(a)	13.06 ± 0.64(a)	15.31 ± 1.27(a)	1.12	3.37	335.10
Wheat+ HA	24.76 ± 0.64(b)	19.80 ± 0.00(b)	22.07 ± 0.00(b)	2.69	4.95	504.87

*MBC (µg g-1)= (40.04×µl CO2 g-1 Soil h-1) + 0.37

A:Total substrate induced respiration (respiration with no application of inhibitors)

B: Respiration after treating with cycloheximide

C: Respiration after treating with Sterptomycin sulfate

Fungal respiration: A-B

Bacterial respiration: A-C

Discussion

Residue quality determines humic acid protection

Different factions of plant derived organic molecules such as LMWOS, such as sugars and amino acids, can be released from plant residues into the soil. We hypothesize that interaction of these plant derived organic molecules with humic acid might have regulated the interaction of different residue with the applied humic acid.

The reduction in carbon mineralization from all LMWOS (Figure 3) can be explained by them being sorped to humic acid. Vieublé Gonod et al. (2006) showed that sorption of amino acids to soil solid phase retards their biodegradation. Several studies have shown that the sorption affinity of amino acids to soil minerals or organic material is controlled by properties of amino acids: basicity, hydrophobicity and molecular weight (Rothstein, 2010). In our study, among the LMWOS, the highest protection exerted by humic acid was for lysine and this effect lasted for the 13 days experimental period. This strong sorption can be explained by the strong electrostatic interaction through cation exchange of this positively charged hydrophilic compound (Liu and Lee, 2007). The humic acid used in this study has high CEC with 5.03 mmole/g of acidic functional groups (Table 2), which facilitate cation exchange. Sorption of glutamine and glucose (neutral hydrophilic soluble molecules) does not involve ion exchange and probably occurred through hydrogen bonding, which might explain their weaker interaction. Witthuhn et al. (2005) reported that covalently bound compounds are non- biodegradable, while weakly bound compounds may desorb and become available again for degradation. Piccolo et al. (1999) emphasized in the role of humic acid in protecting 2-decanol, a highly hydrophobic aliphatic alcohol, through hydrophobic- hydrophobic interactions. In our study the protection effect of humic acid to octanol (insoluble in water) was the least important due to the weaker hydrophobic interaction by the alkyl group compared to other forces. In this study, the adsorption affinity of the LMWOS to humic acid was in the order: soluble charged Lysine> soluble uncharged glutamine and glucose> insoluble octanol.

Vetch is the most labile plant residue in this study. It contains higher amount of protein and soluble compounds and it has low lignin content (Baumann et al., 2009). Therefore, compared with wheat and oak, decomposition of vetch residue might have produced more amino acids and soluble compounds that might be preferentially sorped to the humic acid. Therefore, this protection effect lasted longer throughout the experimental period.

There was a recognizable higher mineralization for wheat+HA, oak+HA, glutamine+HA and glucose+HA compared with wheat, oak, glutamine and glucose alone at later stages of decomposition, this can be explained as 1. Our humic acid is 90% pure and the remaining 10% might represent a source of bioavailable carbon, which can stimulate microbial growth and increases carbon mineralization in soils. This is also confirmed as applying humic acid alone increased carbon mineralization compared to the control 2. Priming effect, this will be explained in the next section.

Humic acid- plant residue interaction and microbial community characteristics: Fungi the main actor

The limited substrate availability when humic acid was applied with vetch caused a reduction in all microbial indices: β-glucosidase (Figure 4a), fungal respiration, bacterial respiration and MBC (Table 3). The reduced activity of *β*-glucosidase can be explained as:

i) humic acid sequestered the cellobiose, therefore, limiting the activity of β-glucosidase,
ii) humic acid might adsorb β-glucosidase enzyme itself causing its limited activity. An experimental work by Lammirato et al. (2010) showed that adsorption of β-glucosidase on mineral surface retarded the hydrolysis of cellobiose.

In contrast to vetch, applying humic acid with wheat resulted in higher microbial indices Figure 4 and Table 3. This can be attributed to brimming effect. Wang et al. (2015) identified two theories of priming effect:

i) co-metabolism theory: applying high quality material (optimum C:N and low lignin) will serve as an energy source to microorganisms to synthesize extracellular enzymes capable of degrading recalcitrant SOM
ii) N-limiting theory: adding fresh organic material with high C:N and high lignin simulates the degradation of SOM as a source of nutrients.

Our results agree with the first theory since the higher carbon mineralization for Wheat+ HA (Figure 2) was coupled with higher enzymatic activities (Figure 4). Fungi are the main actor of priming effect (Fontaine, 2011); therefore, we postulate that in the Wheat+HA treatment, humic acid was preferentially mineralized by fungi. This hypothesis is further confirmed as our results showed a higher fungal biomass, as indicated by a higher NAGase activity (Figure 4b) and a higher fungal respiration (Table 3) for the Wheat+HA treatment compared with the Wheat treatment. Six et al. (2006) reported that fungi have higher microbial growth efficiency (MGE) than bacteria. Ecosystems that promote fungal growth and activity are more efficient in sequestering carbon (Bailey et al., 2002).

Priming effect was not observed when humic acid was applied with vetch. This can be explained by the high soluble fraction and the high nutrient availability provided by vetch. Fontaine et al. (2011) reported that priming effect is low in high nutrient treatments. Fresh carbon source with high lability (high fraction of easily degradable compounds) and high nutrient availability will encourage fast-growing r-strategist microbes, which are not able to degrade recalcitrant material such as SOM. On the other hand, more polymerized carbon source will stimulate slow-growing K-strategist microbes, which are capable of co-mineralizing recalcitrant SOM with the fresh C source.

Insights for carbon sequestration

Our finding regarding the effect of applying humic acid with different plant residues on carbon sequestration can be explained as:

i) Applying plant residue with very low nutrient availability, such as oak, might result in the mineralization of recalcitrant organic materials such as humic acid as a source of nutrient (2nd theory of brimming effect (Wang et al., 2015)). This will adversely affect carbon sequestration.
ii) Applying plant residue with medium level of nutrient availability, such as wheat, will enhance the synthesis of extracellular enzymes and the co-metabolism of humic acid (1st theory of brimming effect

(Wang et al., 2015)). This will cause a shift in the microbial community structure toward higher fungal population and positively affecting carbon sequestration.

iii) Applying plant residue with high nutrient availability, such as vetch, that has high mineralization rate. In this case humic acid will serve as a protecting agent of the plant residue against mineralization and hence enhancing carbon sequestration.

For enhancing carbon sequestration in agricultural soils, this study proposes two main protocols:

i) Applying humic acid to ecosystems that receive high amounts of easily degradable organic material as a mean of enhancing carbon sequestration through reducing carbon mineralization from these materials.

ii) Applying organic material with less degree of lability (such as wheat) in combination with recalcitrant organic material (such as humic acid) might be an efficient way to recover degraded soils and enhance carbon sequestration as a result of a better carbon retention, fungal activity and MGE.

Conclusion

This study proposes the application of humic acid as a way to reduce mineralization and enhance carbon sequestration. According to this study, the following conclusions could be withdrawn:

i) The protection exerted by humic acid against mineralization followed the lability of plant residue, where the degree of protection by humic acid was in the order vetch>wheat>oak.

ii) The protection excreted by humic acid is probably related to the mechanism of interaction between different organic compounds and the humic acid. For example, the positively charged lysine form a strong electrostatic interaction with humic acid, while neutral compounds (such as glutamine) are weakly bounded to the humic acid.

iii) Compared to vetch, wheat residue had low carbon mineralization and a very low fungal activity throughout the incubation period. However, applying humic with this medium nutrient residue resulted in a priming effect and enhanced microbial structure toward a higher fungal population.

iv) Applying humic acid to areas that receive labile (high nutrient) residue is recommended as an innovative practice to reduce the emissions of CO_2 to the atmosphere.

v) Applying humic acid in combination with less labile (medium nutrient) residue, such as wheat residue, is recommended as a way to recovering degraded soils.

vi) The results of this laboratory scale study must be further confirmed with long term field studies.

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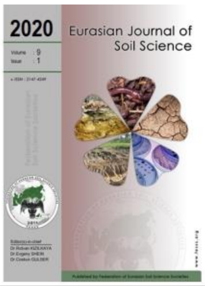

Assessment of extraction methods for studying the fractional composition of Cu and Zn in uncontaminated and contaminated soils

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Abstract

This study is aimed at elucidating the fractionation of Cu and Zn in Haplic Chernozem and its alteration under the contamination to evaluate the extraction selectivity of different extractants and thus the efficiency of three sequential extraction schemes (Tessier and McLaren five-step and modified BCR three-step methods). General trends in the distribution of Cu and Zn speciations revealed with these three fractionation schemes suggest that they can be applied for the soils and the obtained results can be compared. Low mobility of potentially toxic metals (PTM) in the studied soil is suggested not only by their high content in residual fraction, but also by low contents of exchangeable and carbonate bound fractions (not more than 4-5 % Cu and 5-7 % Zn in the first two fractions). The highest contribution to the absorption and retention of Cu delivered from anthropogenic sources is made by organic matter and sesquioxides (up to 29 %); for Zn, by the nonsilicate Fe and Mn compounds (up to 25 %). However, the pattern of PTM extraction from soils varies during the application of different fractionation methods. The Tessier method is distinguished by a higher extractability relative to organic matter and sesquioxides. Therefore, this method is more informative for the contaminated soils. The McLaren method makes it possible to track the weakly bound species of compounds without the risk of involving other soil components. The BCR method is marked by simplicity of application and, therefore, recommended only for the noncalcic or low-calcic soils.

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Introduction

In recent decades, contamination of soils with the potentially toxic metals (PTM) has become a global ecological problem. In contrast to the majority of organic pollutants, which lose toxicity due to biodegradation, PTMs can provoke prolonged toxic effects serving as a source of contamination (Tashakor et al., 2014; Acosta et al., 2015). Urgency of this problem lies in the following fact: when PTMs fall into soil, they do not break down and can migrate to other compartments of ecosystem, creating severe problems for the human life and environment (Yaylali-Abanuz, 2011; Hu et al., 2013). Their contamination effect in soil

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depends on chemical associations. Therefore, determination of the chemical form of metal in soil has an important significance for evaluating its mobility and bioaccessibility (Naji et al., 2010; Gabarrón et al., 2017). Soil is a complex interface containing organic and inorganic compounds, as well as solution and gases, reacting under various physicochemical conditions. For example, it is known that ions of metals (Cu and Zn included) become more soluble, mobile and toxic for plants under conditions of high acidity (Anju and Banerjee, 2010). Metal speciation in the soil, i.e. determination of the phase (or phases) of chemical elements, is a scientific challenge (Doelsch et al., 2008).

The sequential extraction schemes (SES) methods are most widely used for determining the PTM speciation in soils. They yield additional information about the associations of metals with the geochemical phases of soil. Thus, they provide insight into processes, which regulate the mobilization of metals, and possible ecological risks (Silveira et al., 2006; Kennou et al., 2015). Although these methods do not give direct characteristics of metal speciation, they can imitate the chemical reactivity of metals during natural and anthropogenic alterations in the environment. Moreover, these methods comply with all requirements for the realization over large areas: simplicity, applicability to all soil and rock species, low cost, and understandable results (Rosado et al., 2016).

Metals can occur in soils as various physicochemical phases that govern different modes of their occurrence. The most commonly isolated phases in different SES methods are: exchangeable, carbonate bound, Fe–Mn oxide bound (reducible), organic (oxidizable) and residual (Rao et al., 2007;He et al., 2013).

Numerous different procedures of the sequential extraction have been proposed for the TM extraction from soils (Filgueiras et al., 2002; Hlavay et al., 2004). The most widely used SES methods are those proposed by McLaren and Crawford (1973) and Tessier et al. (1979). The McLaren method was initially designed for fractionating copper compounds. In practice, however, it is applied long ago for fractionating compounds of various PTMs. The classic sequential extraction method reported in Tessier method (Tessier et al., 1979), initially designed for bottom sediments, is widely known and applied often in Russia and Europe for evaluating the fractional PTM composition in soils (Rao et al., 2007; Orecchio, Polizzotto, 2013; Minkina et al., 2015; Rosado et al., 2016; Evans et al., 2019).

Diversity of the sequential extraction procedures makes it possible to use various reagents under different experimental conditions of the storage and preparation of samples, temperature, fraction size, reagent concentration, extragent application sequence, solid/liquid phase ratio, pH value control, and duration of stirring and filtration relative to centrifugation (Sutherland et al., 2000). Because of a lack of conformity in the applied procedures, however, it is rather difficult to compare the results obtained in different laboratories. The Community Bureau of Reference (BCR) of the European Commission attempted to standardize the three-step extraction (acid-soluble/exchangeable, reducible, and oxidizable fractions) scheme that was supplemented later with a new step providing determination of the residual fraction (Rauret et al., 1999; Ure et al., 1993; Pueyo et al., 2008). This method is used widely because of the presence of a certified reference material (BCR 701) as a control sample (Leermakers et al., 2019).

Although the sequential fractionation schemes are very popular, impeccability of these methods and univocacy (accuracy) of results are now being challenged with increasing frequency. The main problem in the sequential extraction is related to non-selectivity of extragents and redistribution of elements between phases in the course of extraction (Rapin et al., 1986; Raksasataya et al., 1996; Sulkowski and Hirner, 2006). In addition, the preliminary sample treatment (freeze drying, air drying, oven drying, and others) also play a crucial role often leading to determination errors (van Hullebusch et al., 2005). Despite all these shortcomings described well in literature, the sequential extraction methods are widely used so far for evaluating the accessibility and mobility of metals in solid matrices (soils included) both in separate methods (Wisawapipat et al., 2017;Hasan et al., 2018; Minkina et al., 2018a; Al-Mur, 2020) and in combination with other instrumental identification methods (Scheinost et al., 2002; Hsu et al., 2015; Arenas-Lago et al., 2016; Mekapogu et al., 2018; Minkina et al., 2018b, 2019).

The present paper is aimed at a comparative evaluation of the results of Cu and Zn fractionation in Haplic Chernozem samples obtained by different sequential chemical extraction schemes to determine the most suitable version for specific features of soil without and with anthropogenic contamination.

Material and Methods

Soils

For the purpose of this study, two important locations were selected: (I) soil located away from any source of contamination (pure uncontaminated soil); (II) soil subjected to aerosol contamination by products of the fuel combustion. In both cases, soil is represented by Haplic Chernozem.

The pure (uncontaminated) soil was sampled from the upper layer of virgin Haplic Chernozem sector, where the thick medium-humic, calcaric heavy loam overlies the loess-type sandy clay in the specially protected Persianovo steppe area (Rostov region).

Soil in this area, marked by a constant anthropogenic load related to aerosol emissions from the Novocherkassk power station (NPS), was sampled at 1 km northwest (direction of the prevailing winds) from the emission source. It is believed that areas located at a distance of up to 4 km from power stations are subjected to the highest ecological disturbances (Minkina et al., 2013a). The power station, a year-round functioning, high electric and thermal output station mainly based on coal, is one of the largest producers of electricity in southern Russia and at the same time the major source of environment pollution (Linnik et al., 2019). Moreover, it is the sole hydroelectric station functioning on the local coal mining wastes and beneficiation products (anthracite fines).

All soils were sampled in the 0–20 cm layer. Only steel or plastic tools were used for sampling in order to avoid the PTM contamination of soils. After air-drying and declumping the aggregates, the soil was sieved through a 2-mm mesh sieve. In the collected soil samples, we analyzed the following main physicochemical parameters: pH value by the potentiometric method (soil:water ratio 1:2.5); C_{org} content by the titrimetric method using the biochromatic oxidation procedure according to Tyurin (Tyurin, 1965; Vorob'eva, 2006); cation exchange capacity (CEC) and exchangeable cations Ca^{2+} and Mg^{2+} according to the method proposed by Shaimukhametov (1993); carbonate content by the complexometric method according to S.A. Kudrin; Fe-Mn (hydr)oxides using the Meer–Jackson's reductive complex (Vorob'eva, 2006); and grain size composition of soil by the pipette method with the pyrophosphatic sample preparation (Vadyunina and Korchagina, 1986). Properties of the studied soil are presented in Table 1.

Table 1. Physical and chemical properties of Haplic Chernozem

Soil	Silt and clay (soil particle <0.01 mm), %	Silt (soil particle <0.001), %	C_{org}, %	pH_{H2O}	$CaCO_3$, %	Fe-Mn (hydro) oxides	Exchangeable cations, cmol kg^{-1}		CEC, cmol kg^{-1}
							Ca^{2+}	Mg^{2+}	
Uncontaminated soil	47.1 ± 3.8*	26.8 ± 1.8	3.7 ± 0.3	7.5 ± 0.1	0.03	3.8 ± 0.3	3.0 ± 0.1	0.6 ± 0.1	3.71 ± 0.3
Contaminated soil	55.3 ± 4.2	30.9 ± 2.0	2.6 ± 0.2	7.4 ± 0.1	0.07	3.9 ± 0.4	2.6 ± 0.1	0.5 ± 0.1	3.22 ± 0.3

* ±Standard deviation

Sequential extraction procedures

Sequential extraction was performed using two five-step (Tessier and McLaren) methods and the modified three-step BCR sequential extraction procedure (Table 2). After each extraction, the liquid and solid phases were separated by centrifuging. The TM content in extracts was determined by the atomic absorption spectrometry (AAS). The above-mentioned methods make it possible to extract the fractions of metals differing from each other in the strength of bond with soil components (Table 2).

Results and Discussion

Overview

The bulk content of Zn and Cu in soil of the virgin sector matching the background metal content in Haplic Chernozem (Mandzhieva et al., 2017) was almost two times lower than in the soil located under the influence of aerosol emissions (Tables 3, 4). The sum of fractions obtained by all sequential fractionation methods under consideration was very close and similar to the bulk content of Cu and Zn in soil, suggesting credibility of the data obtained by different methods (Tables 3, 4).

Exchangeable fraction

The exchangeable fraction includes the weakly adsorbed metals retained on the surface of soil particles by a relatively weak electrostatic interaction: (i) metals that can be released due to ion exchange processes; (ii) metals that can co-precipitate with carbonates occurring in many types of sediment. To determine the exchangeable forms of metals, $CaCl_2$ and $MgCl_2$ are used in the McLaren and Tessier methods, respectively. These extragents represent neutral salts that are used most commonly for extracting the above compounds (Rauret, 1998; Hall and Pelchat, 1999). In the BCR method, the exchangeable fraction is extracted not separately but together with the acid-soluble fraction.

Metals corresponding to the exchangeable fraction represent a minor portion of the total metal content in soil (Emmerson et al., 2000). In the uncontaminated Chernozem, the share of exchangeable Cu and Zn is low and makes up 1 % of the sum of fractions obtained by the McLaren and Tessier methods (Figure 1, 2).

Table 2. Different sequential extraction schemes of potential toxic metal extraction from soils

Authors of the method	Toxic metal fractions	Reagent and operating conditions
McLaren and Crawford (1973)	Exchangeable	0.05 M $CaCl_2$ was shaken for 16 h. (the ratio of soil:solution 1:10)
	Acid soluble (bound to carbonates)	2.5% CH_3COOH was shaken for 1 h. (the ratio of soil:solution 1:10)
	Bound to organic matter	1 M $K_4P_2O_7$, pH 11 was shaken for 1 h. (the ratio of soil:solution 1:10)
	Bound to Fe-Mn oxides	0.14 M $H_2C_2O_4$ + 0.2 M $(NH_4)_2C_2O_4$ (oxalate reagent) was shaken under ultraviolet irradiation for 1 h (the ratio of soil:solution 1:10)
	Residual	$HF+HClO_4$, then concentrated HNO_3 after evaporation (the ratio of soil:solution 1:10)
Tessier et al (1979)	Exchangeable	1M $MgCl_2$ (pH 7.0) with continuous agitation (1 h) (the ratio of soil:solution 1:8)
	Bound to Carbonates	1M $NaCH_3COO$, pH 5.0 (CH_3COOH) was leached at room temperature for 5 h. (the ratio of soil:solution 1:8)
	Bound to Fe-Mn oxides	0.04M $NH_2OH{\cdot}HCl$ in 25% CH_3COOH was heated at 96±3ºC for 8 h. with continuous agitation (the ratio of soil:solution 1:20)
	Bound to Organic Matter	0.02M HNO_3 and 5 ml of 30 % H_2O_2 adjusted to pH 2 with HNO_3, and the mixture was heated to 85 ± 2 ºC for 3 h with intermittent agitation. After cooling, 3.2 M NH_4CH_3COO in 20 % (v/v) HNO_3 was added and agitated continuously for 30 min (the ratio of soil:solution 1:20)
	Residual	$HF+HClO_4$, then concentrated HNO_3 after evaporation (the ratio of soil:solution 1:25)
Modified BCR three-step sequential extraction procedure (Pueyo et al., 2008)	Acid soluble (Exchangeable and weak acid soluble fraction, first step)	0.11 M CH_3COOH solution by shaking in a mechanical, end-over-end shaker for 16 h (the ratio of soil:solution 1:40)
	Reducible (bound to hydroxides and oxides of Fe and Mn, «ferrous», second step)	0.5 M $NH_2OH{\cdot}HCl$ was shaken for 16 h, pH 2, The acidification of this reagent is by the addition of a 2.5 % (v/v) HNO_3 solution (the ratio of soil:solution 1:40)
	Oxidisable (bound to organic matter, «organic», third step)	8.8 M H_2O_2 when heated (for 1 h at 85 ± 2 ˚C) to evaporate almost to dryness, then 50 ml 1 M NH_4CH_3COO, pH 2, The acidification of this reagent is by the addition of a 2.5 % (v/v) HNO_3 solution (the ratio of soil:solution 1:70)
	Residual (Residue from the third step)	aqua regia (HCl (37 %) and HNO_3 (70 %)) were added, after evaporation (the ratio of soil:solution 1:9)

Contents of Zn and Cu are increased appreciably (up to 4-6 %) in the exchangeable fraction due to pollution, resulting in their accumulation in plants (Figure 1, 2). The concentration of metals in the exchangeable fraction shows a high correlation with their content in plants (Gupta, 1993; Novozamsky, 1993; Aten, 1996).

Table 3. Fractional composition of Cu in Haplic Chernozem, mg kg^{-1}

Soil	Fractions						Total content
	Exchangeable	Bound to carbonates	Bound to Fe-Mn oxides	Bound to organic matter	Residual	Sum	
The McLaren method							
Uncontaminated	0.3	1.5	3.9	6.5	26.7	38.9	44
Contaminated	5.7	11.5	21.1	38.5	85.6	162	180
The Tessier method							
Uncontaminated	0.5	1.9	5.1	8	27.2	42.7	44
Contaminated	7.4	10.6	30.6	49.1	73.8	172	180
Modified BCR method							
Uncontaminated	1.2		4.5	7.7	28.5	41.9	44
Contaminated	14.5		25.5	43.2	80.6	164	180

Table 4. Fractional composition of Zn in Haplic Chernozem, mg kg^{-1}

Soil	Fractions						Total content
	Exchangeable	Bound to carbonates	Bound to Fe-Mn oxides	Bound to organic matter	Residual	Sum	
The McLaren method							
Uncontaminated	0.9	5	9	3.5	62.4	80.8	90
Contaminated	12.6	28.5	54.2	25.0	194.0	314.3	338
The Tessier method							
Uncontaminated	1	4.5	13.2	9.5	57.5	85.7	90
Contaminated	19.5	28.1	82.8	32.5	173.0	335.9	338
Modified BCR method							
Uncontaminated	6.0		11.3	6	60.7	84	90
Contaminated	38.6		67.6	23.3	192.8	322.3	338

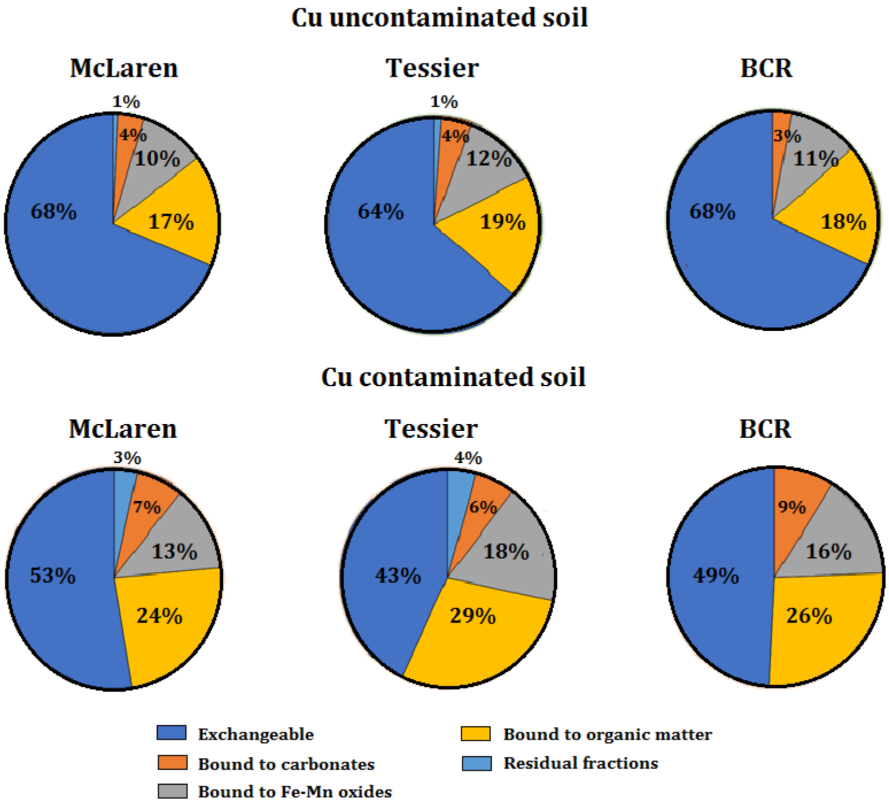

Figure 1. Cu distribution in uncontaminated and contaminated soils, % of the sum of fractions

Acid-soluble fraction

The acid-soluble fraction is represented by metals precipitating on carbonates (Clevenger, 1990). These compounds are sensitive to changes of pH values (Zorpas et al., 2000) and are commonly extracted with weak acids (Coetzee, 1993; Gauthreaux et al., 1998). To extract this fraction, researchers use the acetic acid (BCR and McLaren methods) or sodium acetate (Tessier method). In addition to the acetate buffer solution, these acids are the most commonly used extragents for the above-mentioned compounds (Diks and Allen, 1983; Lee and Kittrick, 1984; Gibson and Farmer, 1986; Ure et al., 1993).

The acid-soluble fraction is next to the exchangeable fraction in terms of the metal content. Similar results of metal content in this fraction are related to identical extraction conditions. The content of Cu and Zn in this fraction in the uncontaminated soil is 3-4 and 5-6 %, respectively.

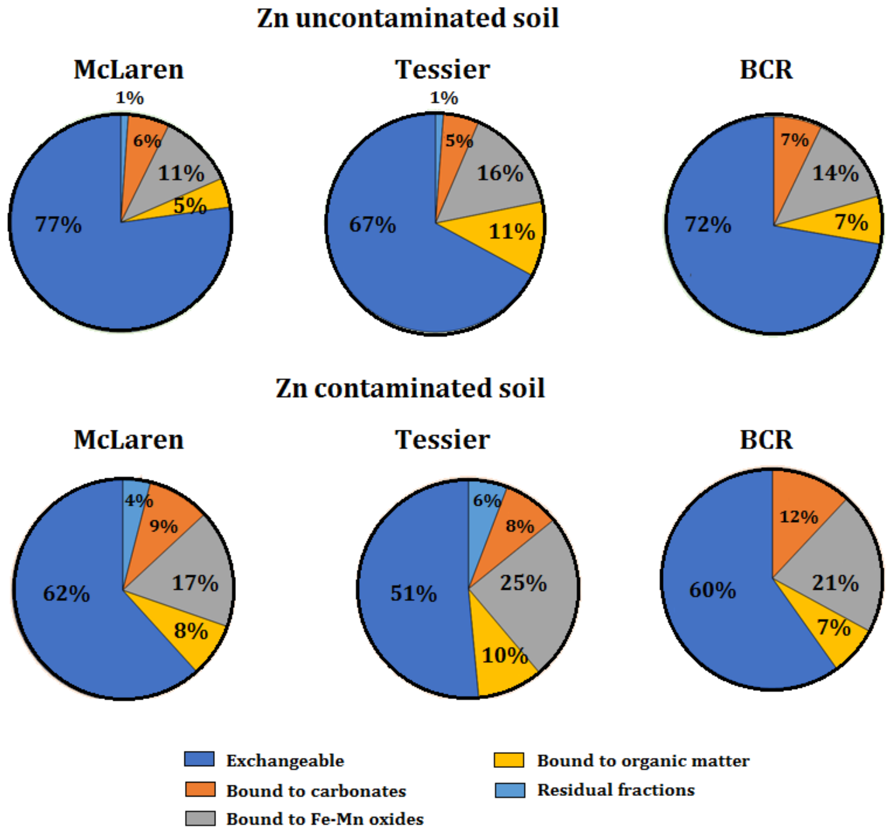

Figure 2. Zn distribution in uncontaminated and contaminated soils, % of the sum of fractions

Zn shows a higher affinity to carbonates than Cu (Stone and Marsalek, 1996; Li et al., 2001). Under conditions of anthropogenic load, this fraction accumulates up to 6-9 % Cu and 9-12 % Zn. Although the carbonate content in the studied soil samples is <1 % (Table 1), they demonstrate high activity in the retention of metals because of a highly dispersed mycelial form of carbonate occurrence in Haplic Chernozem, which is a specific feature of these soils (Minkina et al., 2017). The dispersed carbonates have a high specific surface area, which increases their interaction with PTM. Analysis of the K-edge XANES spectra of Zn adsorbed by the carbonate showed that Zn ions replace Ca ions in the octahedral positions and display 1s→4p electronic transition. Moreover, they are coordinated with carbonate ions as ligands making up adsorbed complexes on the surface of mineral calcite (Minkina et al., 2013a).

Usually, PTMs in the exchangeable and acid-soluble fractions are considered bioaccessible, whereas the fractions of metals bound to organic matter and Fe-Mn oxides are considered more stable under natural soil conditions (Wong et al., 2002).

Reducible fraction

In order to simplify the extraction of metals, the McLaren, Tessier and BCR methods, as well as the majority of recently proposed SES methods, take into consideration the reducible fraction that includes not only Mn oxides, but also the amorphous and crystalline Fe oxides (Lee and Kittrick, 1984; Gibson and Farmer, 1986; Ure et al., 1993).

During the analysis of this metal fraction, Fe and Mn (hydr)oxides are extracted jointly. They are good PTM absorbers (Baruah et al., 1996; Stone and Droppo, 1996). The absorption of metals by sesquioxides occurring in soil as a coating on the mineral surface or as separate fine particles can take place by one or combination of the following mechanisms: co-precipitation, adsorption, surface complexation, ion exchange and penetration into the mineral lattice (Hall and Pelchat, 1999).

In the case of low Cu ion concentration in the uncontaminated soil, a significant portion of this metal is associated with Fe-Mn oxides (10-12 %). Zn content in this fraction amount to 11-16 %. Under conditions of contamination, the content of Cu and Zn in the reducible fraction increases to 13-20 % and 17-25 %, respectively (Figure 2), because of a big role of hydroxides in the fixation of metals (Mellis et al., 2003). Metals are likely to fixed in the nonexchangeable form by chemosorption due to the replacement of two H^+ ions incorporated in OH or OH_2 groups on the oxide surface by the bivalent metal cation (Pinskii et al., 2014).

The highest increase of metals in this fraction was observed during the application of Tessier method. This method is marked by a higher extractability of PTMs bound to the ferruginous minerals because of the application of hydroxylamine hydrochloride in acid–a strong reducer with high processing temperature (96^{o}C) and high CH_3COOH concentration (25 %). The hydroxylamine hydrochloride can affect other soil components to a greater extent than Tamm's reagent used in the McLaren method. One of the problems arising in this case is as follows: the hydroxylamine hydrochloride releases a significant amount of metals bound to organic matter and, hence, reduction of metals in the Fe-Mn oxide fraction can be enhanced at the expense of the oxidizable fraction (Ahnstrom and Parker, 1999).

The lowest contents of Cu and Zn in soils are observed in the McLaren method. Moreover, discrepancies increase in the contaminated soil. The McLaren method uses ammonium oxalate, oxalic acid or oxalate buffer solution with pH=3. When evaluating the fraction of metals tightly bound to ferruginous minerals in the contaminated soils, this method, probably, yields not quite adequate estimate. Processing of soil samples is accompanied by the formation of low-soluble sediments of PTM oxalates, as suggested by low solubility products of salts. Influence of oxalate as PTM extragent decreases with the degree of soil contamination. In addition, the oxalate has a low selectivity (Vodyanitsky, 2010). Thus, the Tamm's reagent used in this method, probably, cannot decompose completely all ferruginous minerals even during the ultraviolet radiation (Ladonin and Karpukhin, 2003), which is particularly important for studying metals in the uncontaminated soils. It should also be noted that the extraction of organic fraction by the McLaren method precedes the extraction of Fe-Mn oxide (Shuman, 1983). At the same time, the ferruginous coating can partially be dissolved, resulting in a lower content of the fraction associated with ferruginous minerals.

Oxidizable fraction

Trace elements can be associated with the process of complexation or bioaccumulation with different forms of organic matter, such as living organisms, dead organic matter (detritus), or coating on mineral particles (Tokalioglu et al., 2000). It is presumed that metals bound to organic matter remain in soil in the tightly bound state for a long time (Kennedy et al., 1997). However, the decomposition of organic matter can foster the release of soluble metals associated with this component (Clevenger, 1990; Gauthreaux et al., 1998; Davidson et al., 1999). Since the metals bound to organic matter can easily be released in an oxidizing environment, the technique of oxidation is usually applied for their extraction. One of the most widespread oxidizers is represented by hydrogen peroxide in acid medium used in the BCR and Tessier methods due to requirement of more stringent extraction conditions. In the uncontaminated soil, the share of Cu and Zn bound to organic matter is 17-19 % and 5-11 %, respectively (Figure 1, 2). The highest metal content in this fraction was yielded by the Tessier method.

In the contaminated soil, the share of Cu bound to organic matter increases to 24-29 % (Figure 1). Physical analytical methods confirmed the organophilic nature of Cu substantiated by high values of the stability constant of this metal with organic ligands (McBride, 1989; Manceau, 2010; Nielsen, 2015). Analysis of the fine XANES spectra revealed that the interaction of Cu^{2+} ions with humic acids of soils can lead to the octahedral (intraspshere) coordination of chelate complexes (Xia, 1997; Strawn, 2008;Minkina et al., 2016).

The Zn content in the organic matter fraction does not practically change with the increase of contamination when the Tessier and BCR methods are applied (Figure 2). An appreciable increase of the metal (from 5 % to 8 %) in this fraction is only observed in the McLaren method, possibly, due to specific features of the latter method, which is based on the dissolution of humic acids in an alkaline medium and the retention of PTM ions in the solution owing to a strong complex-forming capacity of the pyrophosphate ion. However, though this reagent is likely selective for organic matter, efficiency of extraction remains low (Papp et al., 1991).

In the case of Tessier and BCR methods, the extraction takes place under rather aggressive conditions that affect the amorphous Fe-Mn oxides and needs a preliminary dissolution of the organic fraction (Elliot et al., 1990).

Residual fraction

Since PTM ions fixed in the crystal lattices of minerals are most firmly associated with the soil, they are difficultly extractable. Therefore, they are least accessible for plants and involvement in the biological cycle. The results obtained by the three fractionation methods revealed: in uncontaminated and contaminated soils the highest Cu content (45-68 % of the sum of Cu fractions) is recorded in the residual fraction associated with silicates (Figure 1). The distribution of Zn is similar to that of Cu: the Zn content in Haplic Chernozem is highest in the residual fraction (51-77 %). Moreover, the maximum Zn content is recorded in the uncontaminated soil (Figure 2), and Zn is more prone to interaction with soil mineral components (Mellis et al., 2003; Manceau, 2004; Ladonin and Karpukhin, 2011).

However, the share of this fraction of metals in the contaminated soil is lower than in the uncontaminated soil, probably, because of a very slow uptake of alien metal compounds into the crystal lattices of clay minerals. Moreover, metals can penetrate mineral lattices and replace Al or make up intrasphere $MeOH^+$-type complexes (Manceau, 2002; Minkina et al., 2013b, 2015; Bauer et al., 2018).

Fractional composition of PTM

Thus, the results of fractionation made it possible to unravel regularities in the fractional distribution of metals in the uncontaminated and contaminated soils and to define the role of soil components in the fixation of metals. Both uncontaminated and contaminated varieties of Haplic Chernozem are marked by the following Cu distribution pattern in fractions: residual > bound to organic matter > bound to Fe-Mn oxides > acid-soluble/exchangeable. However, when the BCR method is used, the Fe-Mn fraction prevails over the organic fraction, possibly, because of the sequential application of extragents.

The Zn distribution pattern in fractions of the uncontaminated and contaminated soils is similar: residual > bound to Fe-Mn oxides > bound to organic matter > acid-soluble/exchangeable.

Hence, irrespective of the level of Zn concentration, its distribution between different soils components is mainly governed by the interaction with soil mineral components (Fe-Mn silicates and oxides), as well as by the interaction with organic matter in the case of Cu contamination.

Conclusion

In general, the application of different sequential extraction methods revealed similar trends in the distribution of Cu and Zn in fractions. The obtained values are consistent and showed similar pattern for mobility of toxic metals. The results of Cu and Zn fractionation revealed that the Tessier method is more suitable for determining the total anthropogenic component in contaminated soils, but low-informative for the fractions nontightly bound to the soil. This is a significant shortcoming of this method, because precisely the most mobile PTM compounds are important for soil processes. The BCR method, which is broadly similar to and based on the Tessier method, has the following advantages: simplicity and accessibility. Besides, precisely this method is considered as standardized procedure in the global practice. This method can successfully be applied for the noncalcic or low-calcic soils. The McLaren method provides a better insight into metals in the nontightly soil-bound fractions owing to the application of “weaker” extragents, which is important in the study of their accessibility for plants. When evaluating the content of metal compounds tightly bound to the organic matter and ferruginous fraction, the McLaren method is likely less reliable.

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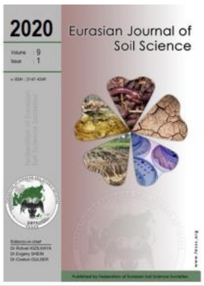

PAHs accumulation in soil-plant system of *Phragmites australis* Cav. in soil under long-term chemical contamination

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Abstract

Distribution and level of 16 individual and total polycyclic aromatic hydrocarbons (∑PAHs) were assessed in soils, roots and above-ground tissues of reed (*Phragmites australis*) on monitoring plots in the city of Kamensk-Shakhtinskyi (Southern Russia, Rostov Region). The total concentration of the 16 PAHs in soil samples ranged between 499.0 to 7177.9 μg kg^{-1}. Samples from the plot no. 4 had the highest PAHs concentrations of 7177.9 μg kg^{-1}. The mean concentration of ∑16PAHs in plot no. 3 was noticeably higher than those in other monitoring sites for both root (363.0 μg kg^{-1}) and above-ground tissues (239.8 μg kg^{-1}). The distribution of PAHs ring size was in the order of 5-6>4>3>2 in soil samples and HMW PAHs fractions represent 57.3% of the total PAHs. The concentrations of 3-4 ring PAHs were higher than HMW PAHs with 5–6 aromatic rings in all *P. australis* tissues. Diagnostic ratios of PAHs indicated that anthropogenic activities were probably major sources of PAHs. Accordingly, the maximum accumulation was found for phenanthrene among the 16 priority PAHs in the most of the soil and plants samples. More PAHs were accumulated in roots, as reflected by its higher mean concentration of PAHs in each plot. In addition, the BCF and TF values of LMW PAHs with 2- and 3-rings were higher than those of HMW PAHs. Taken together, our results indicated that there were an intensive accumulation of PAHs in the zone of industrial sewage tanks and sludge reservoirs as well as an obvious translocations of PAHs from the polluted soils to plant tissues, therefore, more attention is required to be paid to the PAH contamination in this area.

Keywords: Plant uptake, polycyclic aromatic hydrocarbons, phragmites australis, soil contamination, translocation.

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Introduction

Polycyclic aromatic hydrocarbons (PAHs) are considered as one of the most significant organic pollutants existing in environment as complex mixtures of many components. These compounds are ubiquitous and persistent in the nature and consist in two or more condensed benzene rings which arrange in linear,

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angular, or cluster structures (Haritash and Kaushik, 2009; Li et al., 2014). PAHs are capable of accumulation in plants (as a main food source for human and animals) and subsequently they can easily enter into food chain, therefore, affect living organisms owing to their toxic, mutagenic, teratogenic, and carcinogenic properties (Keith and Telliard, 1979; Čvančarová et al., 2013). In general, PAHs are originated from a broad range of natural and especially anthropogenic sources, such as traffic and industrial activity, through the combustion of oil, wood, and other organic materials (Gworek et al., 2014; Serafimovski et al., 2014; Krzebietke et al., 2018).

The contamination of soil, sediment, and groundwater can led to subsequent plant contamination (Chang et al., 2013). Plants have also the capacity of uptake and accumulation of PAHs from polluted areas playing a vital role in their remediation. The accumulation of PAHs in plants as a primary component of terrestrial ecosystems will cause the subsequent transfer of these pollutants into the higher trophic levels (Aina et al., 2006; Zuo et al., 2006). Considering this regard, it is important to distinguish the ways of these pollutants' absorption by plants. PAHs, similar with other organic pollutants, are taken up by plants through two major routes (either from soil or air) (Chang et al., 2013; Gworek et al., 2016). However, there are other pathways for PAHs entering into plant tissues including shoot uptake via root (Burken and Schnoor, 1997), shoot uptake via soil or air (Li and Chen, 2014). There is increasing evidence showing that roots are one of the main routs responsible for the absorption and accumulation of most of the detected PAHs in the soil–plant system as being first tissues that comes in contact with pollutants (Zhang et al., 2017a,b). Subsequently, they translocate from root to shoot through transpiration stream flux (Dettenmaier et al., 2009; Chen et al., 2015). Some studies carried out by fluorescence microscopy have illustrated the uptake and translocation mechanism in roots of maize *Zea mays* (Dupuy et al., 2016) and in the roots and shoots of *Medicago sativa* (Alves et al., 2017). In general, the absorption of organic contaminants by roots can occur via passive diffusive partitioning (i.e. apoplastic) and/or active (i.e. symplastic) transport which the latter is mediated by H+-coupled symporters (Zhan et al., 2018). However, to date, little information is available about the mechanisms involved in these movements (Dupuy et al., 2016; Zhan et al., 2018). PAHs uptake, distribution as well as their toxic impacts on plants depend on initial concentrations and chemical properties of PAHs, physico-chemical properties of soil, and plant species (Zhang et al., 2017a,b) and the movements of PAHs through soil-plant system mostly occur due to their low solubility and high octanol-water partition coefficient. It appears that most of the PAHs with log Kow < 4 are more likely accumulate via roots, then translocate to other plant tissues, whilst high hydrophobic PAHs with log Kow > 4 may strongly adsorb on the root epidermis and are unlikely to be penetrate into the inner roots and translocated within the plant (Zhang et al., 2017a,b).

Previous studies suggested that some plants have the capacity to efficaciously uptake and accumulate PAHs (Gałuszka, 2007; De Nicola et al., 2008; Klánová et al., 2009). It seems that the impact of PAHs on wetland plants is species-specific regardless of PAH types and media (Zhang et al., 2010). However, *Phragmites australis* Cav. can serve effectively as PAHs bioaccumulator for the removal of different organic and inorganic contaminants (Davies et al., 2005; Bonanno, 2011, 2013). The accelerated removal of pyrene and benzo(a)pyrene by the root exudates of *P. australis* was previously reported by Toyama et al. (2011). They also suggested that phenolic compounds in root exudate of *P. australis* can support the *Mycobacterium spp.* growth resulting in stimulation of biodegradation of pyrene and benzo(a)pyrene in the *P. australis* rhizosphere sediment. It seems that *P. australis* accelerated the oxidation of the PAHs by rhizodegradation. Also, in some other studies this species was introduced as an efficient aquatic plants for the depletion of an aged PAHs contamination as well as tolerating high levels of organic contaminants (Di Gregorio et al., 2014; Alwan, 2016). Furthermore, the capability of *P. australis* in accumulation of PAHs were examined by Cui et al. which found that *P. Australis* accumulated PAHs effectively compared to native plants with higher accumulation of MMW and HMW PAHs in leaves than in roots and stems (Cui et al., 2015).

The objective of current study was the assessment of plant efficiency in PAHs accumulation and to determine biological concentration factor (BCF) and translocation factor (TR) of PAHs in *Phragmites australis* plants grown in soils located in the Seversky Donets River floodplain, southern Russia, Rostov Region. Furthermore, a compositional profile of LMW and HMW PAHs compounds in soil, root and above-ground parts of this species were investigated, as well.

Material and Methods

Object of study

The research soils were located in the Seversky Donets River floodplain, southern Russia, Rostov Region. The studied soils situated on the territory of the Lake Atamanskoe former used as a wastewater disposal basin for the chemical factory after 1950s and was a dried up oxbow in 1990s (Figure 1.). The plants under the study were represented by mesophilic succession of the Lake Atamanskoe by common reed (Phragmites australis Cav.), which adapts due to its remarkable ecological resilience (Jiang and Wang, 2008). Other dominant plants of floodplain lakes were reed mace (*Typha* spp.), bulrush (*Scirpus lacustris*), water plantain (*Alisma plantago-aquatica*), rush flower (*Butomus umbellatus*), sedges (*Carex* spp.), etc. These plants are not tolerate to high water mineralization, high level toxic metal salts, or organic pollutants (Privalenko et al., 2000).

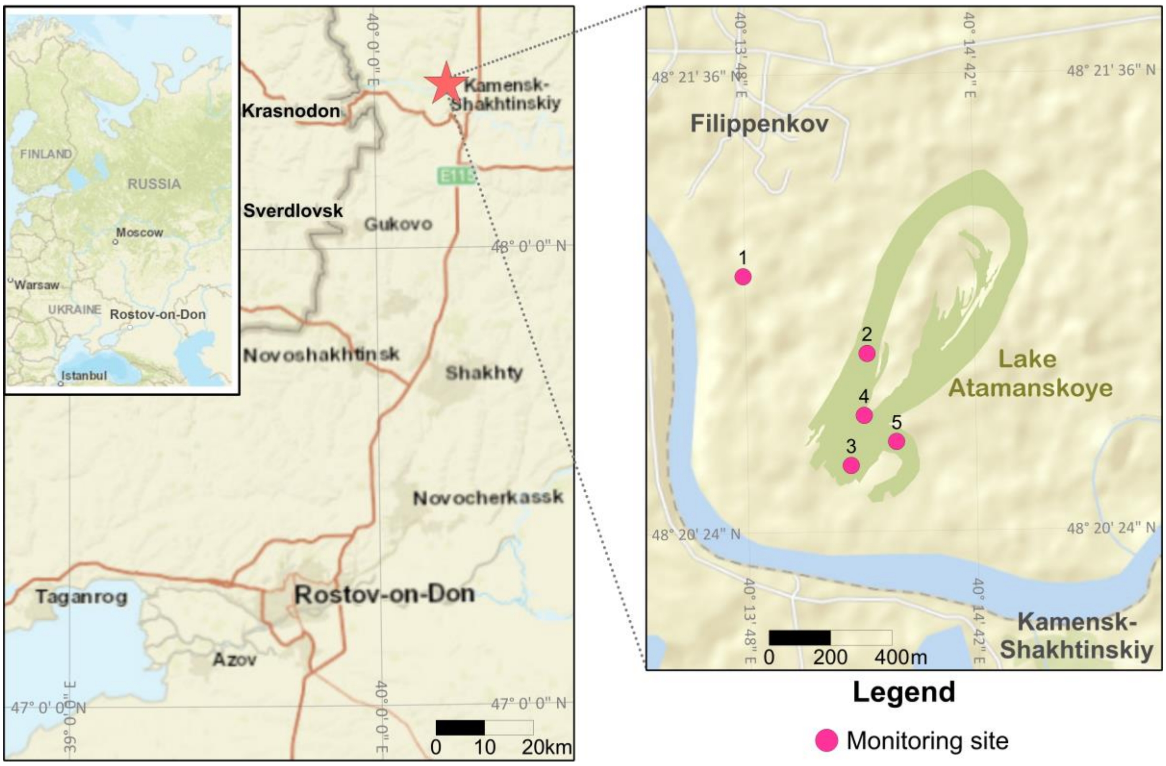

Figure 1. General schematic of the region under study. Monitoring plots are denoted with circles

Monitoring plots

The 100 soil monitoring plots grid was established in 2015–2017 for the object study. The sampling depth was 0 to 20 cm and soils were selected according ISO 10381-1 (2002). Four monitoring plots with high levels of anthropogenic pollution adjacent to the lake were selected. The soil on monitoring plot no. 1 was taken as a background, because it was located at 2 km from the pollution source and represents an analogue of the initial soil (Figure 1), which was classified as a Fluvisol (according to IUSS, 2015). The anthropogenically transformed soils on plots no. 2-5 are classified as Spolic Technosols.

Chemical analysis

Soil

In each area, four soil samples were collected from the surface (0- to 20-cm) horizons. They were stored in polyethylene bags and taken to the laboratory. The soil samples from the same area were mixed, air-dried, sieved through a 2 mm-sieve, and homogenized. The main physical and chemical characteristics of the soil were determined at the certified analytical laboratory (Certificate No. POCC RU 0001.511127) in following ISO Guide 34 (2009) methods. The particle size distribution was determined by the pipette method (with pyrophosphate procedure preparation) (Shein, 2009). In this method carbonates, organic matter, Fe, and Al oxides were not removed before the pipette procedure. The pH in H_2O suspension was determined using a glass electrode in a 1:5 (volume fraction) suspension of sample in water according to ISO 10390 (2005), organic carbon content by sulfochromic oxidation, ISO 14235 (1998), carbonates content by using a Scheibler apparatus, ISO 10693 (1995), and exchangeable cations using a hexamminecobalt trichloride solution, ISO 23470 (2011).

Plants

Common reed (*Phragmites australis* Cav.) is a cenosis-forming species on the established monitoring plots. Standard cuts for the morphobiometric measurements of reed plants on the monitoring plots were performed in the second half of August, when the vegetative and generative organs of reed plants reach maximum. On each monitoring plot, an average sample was formed from 8–10 individual samples containing plants of the same species. Soil was removed from roots and rhizomes.

PAHs extraction and determination

PAHs were extracted from the soils and plants of the objects under study by the standard method using for the removal of the interfering soil components by saponification (Directive document 52.10.556-95, 2002; Sushkova et al., 2015; 2016).

A 1-g portion of the prepared soil was put in a pear-shaped flask for rotary evaporator; 20 mL of 2% KOH solution in ethanol was added, and the mixture was refluxed on a water bath for 3 h. The saponification of lipids and gummy soil components occurred during the refluxing, which increased the recovery of PAHs and reduced the amount of coextracted substances in the extract. The supernatant was decanted into an Erlenmeyer flask, and 15 mL of n-hexane and 5 mL of distilled water were added for the better separation of the layers. The mixture was shaken on a rotary shaker for 10 min and transferred into a dividing funnel. The hexane layer was poured into a separate vessel. The residue in the flask was extracted twice more in a similar way. The combined hexane extract was washed with distilled water to neutral pH (using litmus as an indicator), transferred into a dark vessel with a close lid, and desiccated by adding 5 g of anhydrous Na_2SO_4. After exposure at +5^{o}C for 8 h, the desiccated extract was decanted into a dry round-bottomed flask and evaporated to dry on a rotary evaporator at a bath temperature of 40^{o}C. The dry residue was dissolved in 1 mL of acetonitrile.

The content of PAH in the test samples was determined by the external standard method (Procedure of measurements…, 2008). The content of PAH in the soil was calculated from the equation

$$C_s = k\, S_i \times C_{st} \times V/(S_{st} \times m) \qquad (1)$$

where C_s is the content of PAH in the soil sample (µg/kg); S_{st} and S_i are the BaP peak areas for the standard solution and the sample, respectively; Cst is the concentration of the standard PAH solution (µg kg^{-1}); k is the recovery factor of PAH from the sample; V is the volume of the acetonitrile extract (mL); and m is the mass of the sample (g). The efficiency of target PAHs extraction from soils was calculated using a spike matrix (Yaroshchuk et al., 2003; Sushkova et al., 2017).

Samples were analyzed for PAHs with an Agilent 1260 Infinity high performance liquid chromatograph (HPLC) equipped with a fluorescence detector following the ISO 13859:2014 requirements. The HPLC system was fitted with reversed phase column Hypersil BDS C18 (125×4.6 mm, 5 µm) and a mixture of acetonitrile and ultrapure water as the mobile phase. Sixteen priority PAHs were measured in the present study: naphthalene (NAP), biphenyl (BIP), anthracene (ANT), acenaphthene (ACE), acenaphthylene(ACY), fluorene (FLU), phenanthrene (PHE), benzo[a]anthracene (BaA), pyrene (PYR), fluoranthene (FLT), chrysene (CHR), benzo[a]pyrene (BaP), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[ghi]perylene (BghiP), dibenzo[a,h]anthracene (DBA). Compounds were identified by comparing the retention times to that of the analytical standard samples.

Quality control of every HPLC detection was performed according Agilent Application Solution (ISO 13877-2005). Individual standard solutions were purchased from the Sigma-Aldrich (Merch). A calibration standard of PAH mixture was injected after every six samples to correct for drift in retention time within a run. The certified reference materials and calibration curves were used for calculation of the limits of detection (LODs) and limits of quantification (LOQs) have been presented previously (Minkina et al., 2019). For the developed methods of extracting the target PAH in the soil, a random component of the measurement error was estimated, which for the concentration range of 2-200 µg kg^{-1} was 3.5-14%.

Data are expressed as a root concentration factor (RCF) where RCF) root concentration (mg g^{-1})/solution concentration (mg mL^{-1}), shoot concentration factor (SCF) where SCF) shoot concentration (mg g^{-1})/solution concentration (mg mL^{-1}) and as a transfer factor (TF) where TF) shoot concentration (mg g^{-1})/root concentration (mg g^{-1}).

Calculation of BCF and TF

PAHs uptake from soil to roots and ground-parts was calculated using following equations according to Bech et al. (2014):

$$BCF = C_{\text{root}}/C_{\text{soil}}$$

$$TF = C_{\text{above-ground parts}}/C_{\text{root}}$$

Where TF is BCF is the Biological Concentration Factor and Translocation Factor, respectively.

Statistical analysis

All data were processed using Microsoft Excel 2018 software and statistically analyzed using SPSS-22 software. The data presented as mean ± standard division (S.D.) of three independent replications using one-way ANOVA. Statistically comparison among the means was carried out by Tukey's range tests and differences were considered significant at $p \le 0.05$.

Results

PAHs content and distribution

The determination of PAHs content in the 20 cm soil layer of monitoring plots showed an intensive accumulation of PAHs. The total concentration and composition of 16 priority PAHs according to their ring numbers in the 20 cm soil layer of monitoring sites and, also in plants grown in contaminated sites were shown in Figure 2. The data on the content of $\sum_{16}$PAHs in 20 cm layer in soil of monitoring site no. 1 was 499.0 μg kg^{-1}. The total concentration of $\sum_{16}$PAH in soil of site no. 2, 3, 4, and 5 were 5073.2, 1941.1, 7177.9, and 1465.8 μg kg^{-1}, respectively (Figure 2A). The most affected monitoring site was no. 4 with maximum of 16 priority PAHs total concentration (7177.9 μg kg^{-1}) in soil (Figure 2A). As it is shown in figure 2A, the comparison data analysis for the dominant PAHs type in 20 cm soil layer of plot no. 1 (located far from the dry lake) and all soil samples from the monitoring plots situated within the former lake area showed significant differences. The mean concentration of two- and three-ring PAHs in soil samples were in the range of 14.6 to 511.4 μg kg^{-1} and 120.7 to 1423.1 μg kg^{-1}, respectively. Two rings PAHs was in their lowest amounts followed by three rings in all plots. By contrast, the concentration of five-six ring PAHs was significantly high in soil samples except for plots no. 1 and 5 which replaced by four rings (Figure 2A).

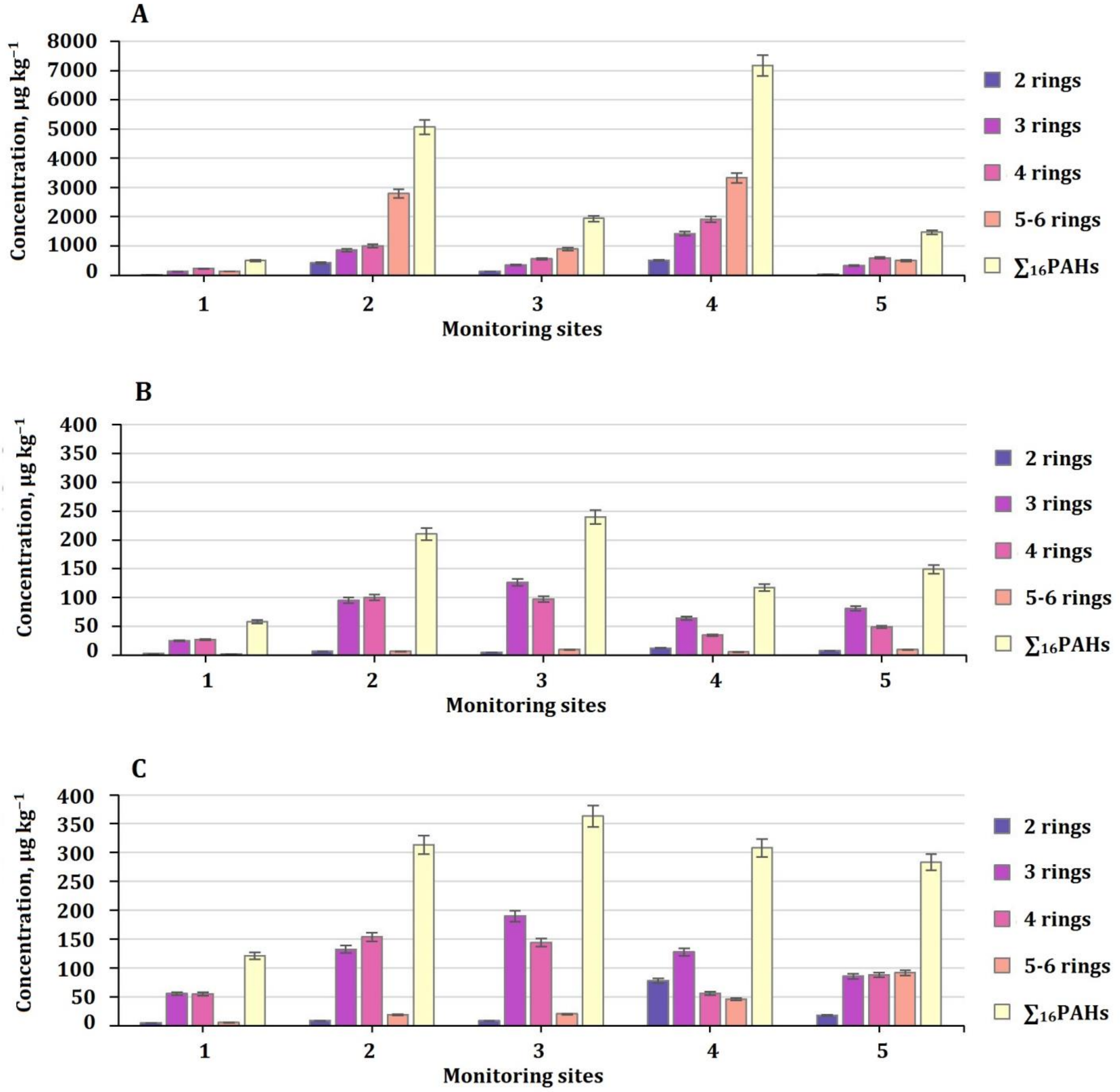

Figure 2. The concentration of ∑16PAHs in the 20 cm soil layer of monitoring sites and in P. australis grown in contaminated sites. The hanging bars is the average means standard division between three replications.

As it is shown in Figures 3A, the comparison data analysis for the dominant PAHs type in 20 cm soil layer of plot no. 1 (located far from the dry lake) and all soil samples from the monitoring plots located within the former lake area showed significant differences. It was found that the content of high molecular weight (HMW) PAHs such as DBA (no. 2), BbF (no. 4) and BghiP (no. 2, 3 and 5) prevailed to low molecular weight (LMW) PAHs. However, in all area, PHE, CHR=BbF, BghiP, and DBA were the major PAH compounds, accounting for 13.4%, 12.2%, 11.5%, and 9.2% of the total PAHs, respectively, followed by FLT=PYR (8.4%). The contribution of ACY, ACE, FLU, and BIP was low, and they accounted for <2% of the total PAHs. LMW (2 and 3 rings) and MMW-HMW (4 and 5-6 rings) PAHs accounted for 25.9% and 74.1% (57.3% for HMWs only) of the total PAHs, respectively (Figure 3A).

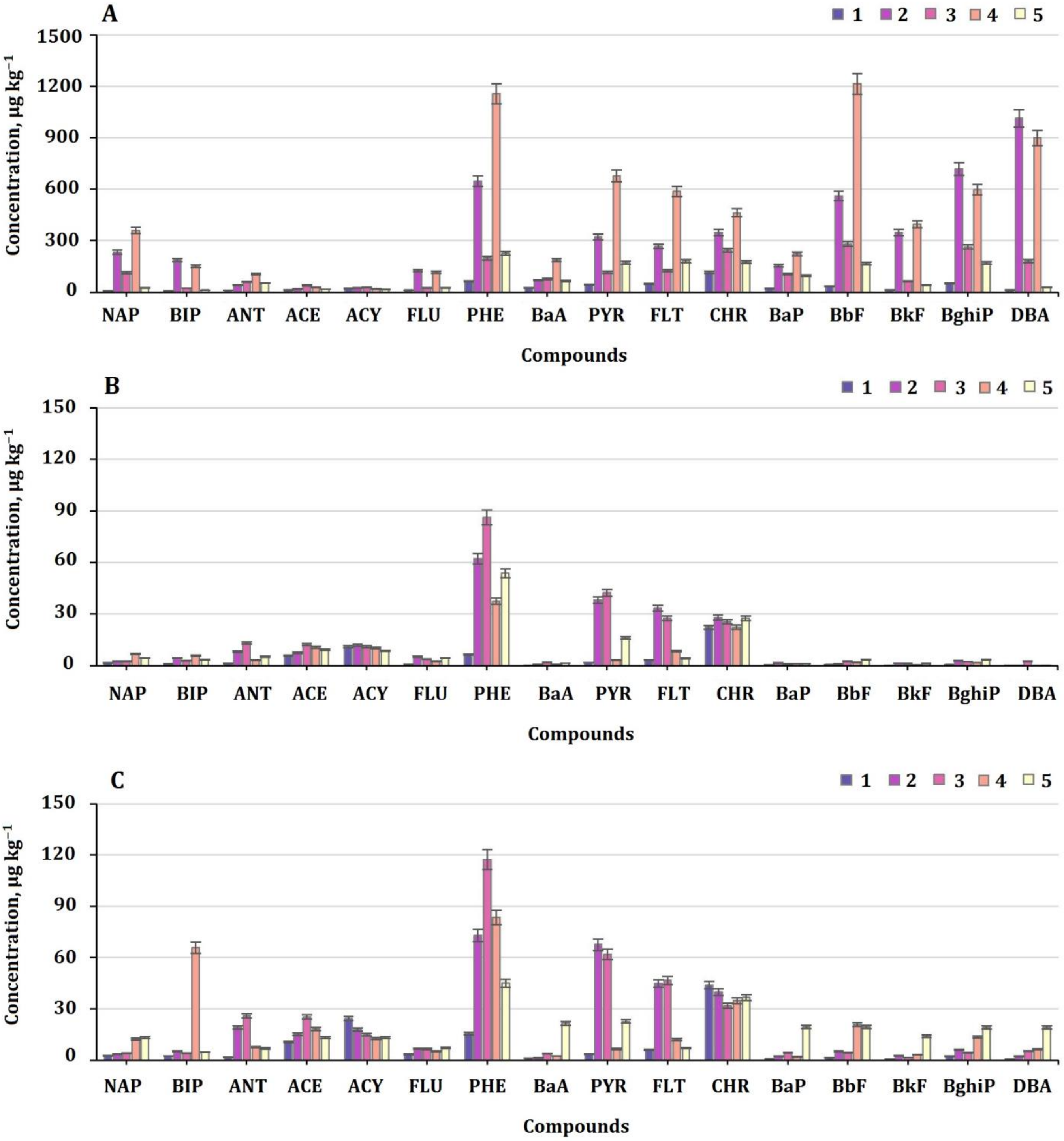

Figure 3. Individual PAHs concentrations in 20 cm soil layer of monitoring plots (A), Phragmites australis Cav. roots (B) and above-ground parts (C), µg kg^{-1}. The hanging bars is the average means standard division between three replications.

After determining the content of PAHs in soils, the content of PAHs in the *P. australis* plants growing in them was also analyzed. The total concentration of 16 priority PAHs in plants grown in contaminated sites is shown in Figures 2B and C. The total concentration of 16 priority PAHs in the above-ground parts of *P. australis* plants for site no. 2, 3, 4, and 5 were 210.2, 239.8, 117.6, and 149.0 µg kg^{-1}, respectively (Figure 2B). The content of 16 PAHs in roots of *P. australis* also varied among contaminated plots and were 313.5, 363.0, 308.0, and 283.1 µg kg^{-1} for plots no. 2, 3, 4, and 5, respectively (Figure 2C). In both root and above-ground parts, plot no. 1 was the least polluted and samples taken from this plot had the lowest content of total PAHs in both roots (120.9 µg kg^{-1}) and above-ground parts (58.1 µg kg^{-1}) of *P. australis* (Fig. 2B and C). Plants growing in site no. 3 prevailed with total PAHs concentration of 363.0 and 239.8 µg kg^{-1} for roots and above-ground parts, respectively (Figure 2B and C).

The dominant PAHs content was found to be mostly LMW and MMW PAHs in *P. australis* plants, including PHE (no. 2-5) and CHR (no. 1) (Figure 3B and C). The composition and dominant PAHs in *P. australis* plants were similar and the mean concentrations of PHE and CHR in different sample materials on the control monitoring plot (no. 1) and other plots were 22.3% and 16.4% in roots, and 28.9% and 20% in above-ground parts, respectively. PYR was the third most abundant PAH with respective percentage concentration of 10.3% and 10.5% in roots and above-ground parts, followed by FLU and ACY. The least amount of PAHs was for 5-6 rings and 2 ring PAHs. On the other words, BkF, BbF, DBA, BaP, NAP, and BIP occurred in lowest amounts in the plants collected from monitoring sites (Figure 3B and C).

Bioconcentration Factor (BCF) and Translocation Factor (TF)

The BCF of $\sum_{16}$PAHs in monitoring plots were presented in Figure 4. The mean BCF of LMW PAHs with 2- and 3-rings (0.4) was 3.6 times higher than that of HMW PAHs (0.11) (except for DBA as a HMW PAHs which showed higher BCF only in plot no. 5), as LMW PAHs were easier to be absorbed by *P. australis* plants than the HMW ones. The greatest BCF value was for ACY (21%) followed by ACE (20%) and BIP (8%) (Figure 4). Root to above-ground part transfer factors (TFs), defined as the ratio of the contents of PAHs in the above-ground part to the roots, were also calculated (Figure 4). Although there was no big difference between TF values for LMW and HMW PAHs (all values were less than 1), TF recorded the highest values for the 2-4 ring PAHs in monitoring sites (Figure 4) and generally decreased by increment in KOW of PAHs. TF values from the highest to the lowest ones were as: PHE > FLT=ACY=CHR followed by mostly LMW and MMW PAHs including ACE, ANT, BIP, NAP, FLU, and PYR with almost the same TF values ($p < 0.05$) (Figure 4).

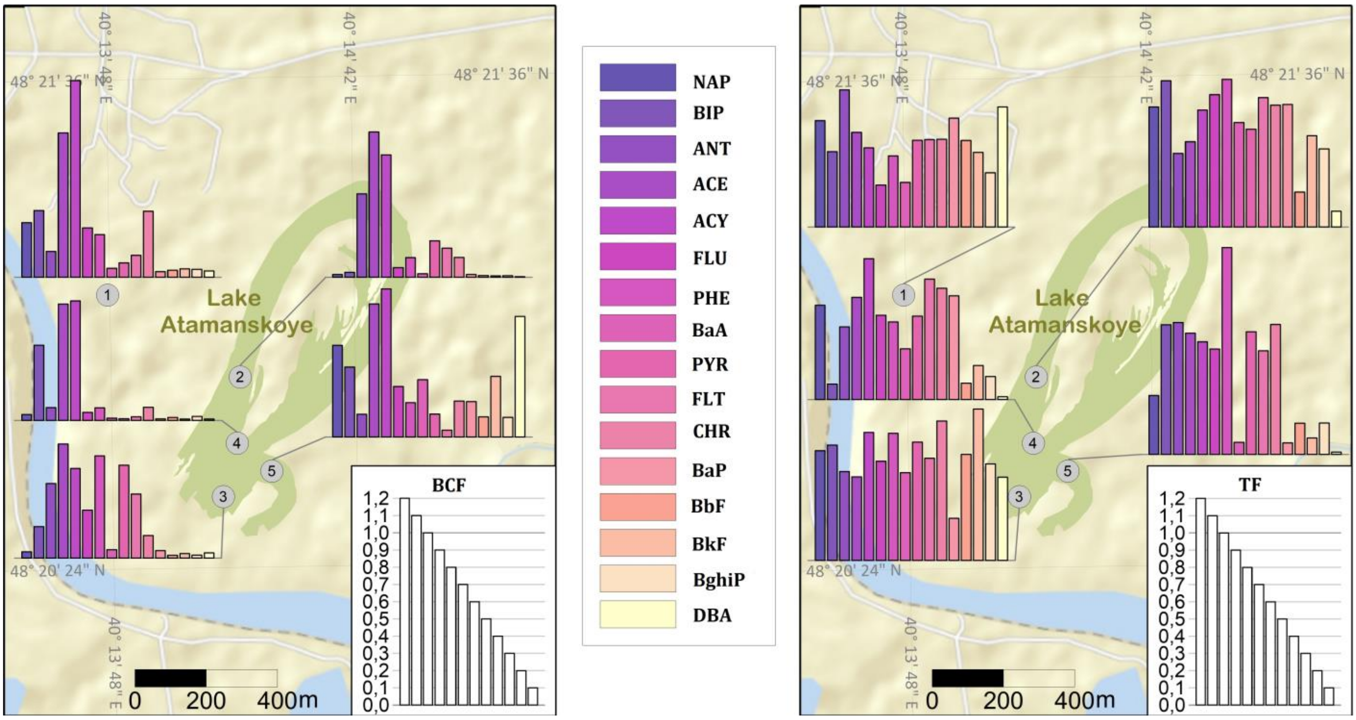

Figure 4. The Bioconcentration factor (BCF) and translocation factor (TF) of 16 priority PAHs compounds in P. australis.

Discussion

PAHs do not present as a separate entity in the environmental media and mostly their mixture concentration is considered for explaining their distribution. Here, the distribution pattern of the 16 priority PAHs recommended by the United State Environmental Protection Agencies (US EPA) were investigated. According to the number of aromatic rings, the 16 PAHs were divided into four groups: 2-ring, 3-ring, 4-ring, and 5-6ring PAHs and the sequence of proportion in all surface soils were 5.6%, 20.4%, 32.4%, and 41.6%, respectively. The obtained results illustrated that the soil samples in each plot had different content and compositions of PAHs. As mentioned above, the most affected monitoring site was no. 4 with maximum of $\sum_{16}$PAHs concentration in soil, however, plants growing in site no. 3 prevailed with total PAHs concentration in both root and above-ground parts (Figure 2). It seems that plants grown in highly contaminated plot (no. 4) probably prevent the uptake of PAHs in order to manage the contamination. In the reports by Gworek et al., it has been suggested that the difference in the PAH content in different plots is likely associated with the significant difference in organic matter content of soils (Gworek et al., 2016). Based on the data, 5-6 ring PAHs were registered in soils of studied territory as the most abundant (Fig. 2A). Therein, BbF, BghiP, and DBA were found to be the most prominent compounds in all soil samples except for CHR and PHE in soils no. 1 and 4, respectively. The predominance of HMW PAHs in monitoring plots (57.3%) suggests that the sources of PAHs might be caused by high level of anthropogenically contributed PAHs such as emission from industrial activity and heavy traffic in these areas (Jiao et al., 2017). In accordance with this regard, another report also demonstrated that high-temperature combustion process including incomplete fuel combustion and vehicle engine emissions are the main source for HMW PAHs, while LMW PAHs were mainly generated by low-or moderate-temperature combustion processes (such as biomass combustion and domestic coal burning) (Sun et al., 2010).

PAHs uptake and distribution can differ remarkably depending on many factors including plant species, initial soil concentrations of PAHs and microbial population (Ryan et al., 1988). Due to direct connection, roots are usually the major reservoir for pollutants and despite strong adsorption to soil particles, high hydrophobicity, and low bioavailability of PAHs, some of them are taken up by plants (Wang et al., 2015). Plants can accumulate PAHs from a polluted area through two main pathways: (1) root uptake from contaminated soils: (2) deposition on the waxy leaf cuticle or through stomata. PAHs can subsequently transfer to above-ground parts of plants (Li et al., 2016; Zhang et al., 2017a,b). The amount of total PAHs in soils is generally higher than in plants (Wang et al., 2012) which is in accordance with present study.

All the 16 PAHs were detected in *P. australis* tissues, however, the dominant PAHs in roots and above-ground parts of *P. australis* possessed mostly three rings (PHE) and four rings (CHR). This may be attributed to their higher solubility and volatility due to their molecular size and lower persistence in the environment which in turn result in their easy biodegradation or translocation when compared to HMWs (Zohair et al., 2006; Gworek et al., 2016). Similarly, higher LMW PAHs accumulation in vegetable with the contribution of 60% and 45.8% of Σ_{16}PAH in Pakistan and China, respectively (Waqas et al., 2014). In addition, there are other reports showing that a LMW compounds such as PHE were the most abundant compounds in tissues of the some mangroves, ryegrass (*Lolium multiflorum* Lam.) and *Arabidopsis thaliana* with high uptake and accumulation (Gao and Zhu, 2004; Lu et al., 2005; Alkio et al., 2005; Kang et al., 2010). The high concentration of PHE is presumably because of its mobility through the transpiration stream from roots to shoots. In another report, the soil-to-plant transfer was considered as one of the major pathways of PAHs transport into vegetable, and LMW PAHs are more susceptible to uptake and translocate than the HMW PAHs (Khan et al., 2008; Waqas et al., 2014). Furthermore, it was shown a three-ring PAH e.g. anthracene can bind to several cell-wall components such as cellulose, hemicellulose, pectin, and lignin (Wild et al., 2005), which may the reason for absorption and transduction of PAHs such as PHE in roots and shoots of *P. australis* in our study, as well. Rice also uptake PAHs with LMW from soil (Wang et al., 2015). Also, the identities of PAHs with LMW in shoots of oil-treated *R. mucronata* were similar to our study. It has been reported that shoots mostly possess two to three rings PAHs with the maximum amount (50%) for PHE (Naidoo and Naidoo, 2018).

It seems that the detected PAHs in roots mostly uptake from soil particles adjoined to the root surfaces, then translocate from the root to the shoot either by the transpiration stream flux or by a carrier system which is reported for PHE absorption by wheat (*Triticum aestivum* L.) (Zhan et al., 2010). In present work, the highest accumulation rate for PAHs was found in the case of ACY in *P. australis* tissues collected from the monitoring sites (Figure 4). The calculated BCF for ACY reached the highest value of 1.1 for plot no.1. However, it is the fact that the values of BCFs for this compound in the samples from other locations were lower than 1. Also, in other cases, the calculated BCFs for $\sum_{16}$PAH compounds were lower than 1, as well. It can also be said that in general, the calculated BCFs for LMW and MMW PAHs were higher than in the case of HMW PAHs. There are some outcomes in accordance with the present work showing the higher values of BCF for LMW PAHs when it compares with HMWs (Gworek et al., 2014; Li et al., 2014; Li and Ma, 2016). Gworek et al. (2014) has illustrated that three-ring PAHs have several times higher BCF than four-ring ones; moreover, the BCF values of four-ring were several times higher than five-ring PAHs; finally, the BCF values of six-ring PAHs were the lowest one. Based on the data shown in Figure 4, only ACY showed BCF higher than 1 in plot no. 1. Also, the mean BCF of this compound was the highest among other PAHs and it was 0.9. The BCF values lower than 1 may support the presence of mechanisms for PAH accumulation mostly from soil/root pathway. DBA was the only HMW PAHs which recorded higher BCF in plot no. 5. It appears that PAHs behavior is unpredictable in chemically contaminated area, however, high BCF value of DBA can be explained by its less content in soil where means high values of BCF. The high PAH contents probably reduced their sorption and thereby improved their availability. However, it worth mentioning that the BCF values of DBA was still less than 1 in plot no. 5.

It should be noted that the LMW and MMW PAHs containing 2–4 rings tend to take up more than the HMW PAHs which mostly are retained in soils or roots. LMW and volatile PAHs are potentially uptake from the atmosphere through the leaves, as well as by roots (Fismes et al., 2002; Li and Ma, 2016). It appears that the level and origin of contamination and type of plant species are main factors in comparison of BCF values in plants grown in different soils (Li and Ma, 2016), however, in the case of HMWs root uptake occurs regardless of these factors. For three-ring PAHs, the uptake pathway can be differed based on plant type, namely for monocotyledones through root but for dicotyledones, through either roots or leaves (Gworek et al., 2014). Li and Ma (2016) suggested that differences in BCFs are probably due in part to the difference in soil total organic carbon, resulting in different bioavailability of PAHs. In addition, a positive linear relationship between log RCF and log Kow was reported for the uptake of PAHs by tea plants cultivated in a hydroponic solution (Lin et al., 2006). The TF values were in PHE > FLT=ACY=CHR order. In general, transfer of PAHs from root to shoot is driven by the transpiration stream flux which is associated with the solubility of the individual PAH rather than the KOW. It seems that better water solubility of PAHs with 2 and 3 rings in the molecular structure enhances their bioavailability to roots. HMW PAHs, characterized by weak water solubility, high values of Henry's Law constants and Kow, are strongly adsorbed onto the roots preventing them from being drawn into the xylem. They stay trapped at the epidermis surface of root tissues (Kang et al. 2010). We should also add that the territory of the Atamanskoye Lake has no atmospheric emissions with PAHs and mostly characterized by chemical contamination, hence, there is no big difference between TF values for HMW and LMW PAHs.

Conclusion

The results of the present study carried out on monitoring soils and plants in the city of Kamensk-Shakhtinskyi (Southern Russia, Rostov Region) demonstrated that the total PAHs level in the area of Atamanskoye Lake was very high up to 7177.9 μg kg−1 in soil of monitoring plot no. 4 and increased the control soil level up to 14.4 times. The $\sum_{16}$PAHs concentrations for *P. australis* plants reached the maximum for plot no. 3 and mounted to about 3.0 and 4.1 times the PAHs level in control plant roots and above-ground parts, respectively. The most dominant PAH in the monitoring soils were PHE, BbF and DBA. In roots and above-ground parts of *P. australis*, the mean percentages of PHE reached to the highest amount followed by CHR and PYR. The BCF values of LMW PAHs with 2- and 3-rings were about 3 times higher than those of 4-6 ring PAHs. In general, the BCF and TF of total PAHs in plants were lower than 1. Altogether, the results indicated that the PAHs in contaminated soils were taken up and accumulated in plant tissues and the distribution of these pollutants in the samples indicates a possible health risk to humans and other organisms, therefore, this problem needs further attention.

Acknowledgments

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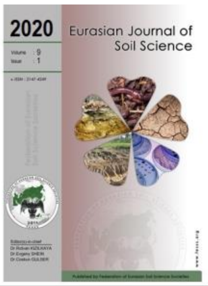

Effect of heavy metals on soil microbial quality of an abandoned mining area Sidi Kamber, North-East of Algeria

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Abstract

The ecological importance of soil bacteria is not limited to their number or biomass, although these parameters contribute greatly. Indeed, their main asset lies in their great genetic and functional diversity.This study aims to determine heavy metal contamination levels of the soils of an abandoned mining area of Sidi Kamber (Skikda), impact of heavy metals on bacterial communities and the possible risks that can affect the ecological balance of this area. Soil samples from three zones (Zone A, B and C) were collected from the top layer (0–20 cm) of mining area. Chemical analysis (pH, organic matter, total organic C, total N, available P, and cation exchange capacity, metal content of (Pb, Cu, Cd, Zn and Ni) and bacterial analysis were carried in center for biotechnology research CRBt. Our results show that the mining area is characterized by an acid pH. Significant variations were observed for edaphic parameters (organic matter, total organic C, total N, available P and cation exchange capacity) between three sampling zones. The overall area was severely polluted with Cu, Cd, Pb, Ni and Zn with a total concentration far exceeding international standards. The bacterial load and diversity were relatively high with a significant variation between the three zones. The PCA analysis of the soil's characteristics indicates that the organic matter and the cation exchange capacity affect the distribution of the metallic trace elements in the soil and allowed us thus to a clear separation of the studied zones.

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Introduction

Mine tailings or mill tailings, which can account for more than 80-99% of the raw ore by weight (Edraki et al., 2014) are the remained materials after the economically extraction of the minerals from the ore (Diaby et al., 2007). The mine spoils and tailings generated by this industry generally present a hostile environment to the organisms' growth. Due to low nutriment availability, low organic matter content, high acidity and a very often elevated trace of metal content (Batty, 2005; Barrutia et al., 2011). Since these wastes aren't treated or even disposed of at the end of the activity, the former mine sites remain a local source of metal contamination (Navarro et al., 2008). Heavy metals are stable and environmentally persistent contaminants since they can neither be degraded nor destroyed (Sevgi et al., 2009). They produce an opposite effect on the human being's health as well as other living beings on both terrestrial and aquatic scale, as it can affect the food chain (Mishra and Nayak, 2009). Thus, it is absolutely necessary to evaluate the quality of the soil resources. Conceptually, the quality of the soil is defined as the capacity of the soil to function within ecosystem boundaries to sustain the biological productivity, to maintain the environmental quality and to

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promote both fauna and flora health (Alexander, 1977). The soil biology is a significant component of soil quality. Microorganisms play a vital role in the soil's fertility and the primary production through the organic matter decomposition and nutriment cycling (Doran and Parkin, 1994). Despite these unfavorable conditions, bacteria have evolved biological mechanisms to resist metal toxic contaminations and colonize this type of substrate (Whiting et al., 2004; Valverde et al., 2011). Bacteria are considered as efficient bio-indicators of the soil's quality because they respond faster and they are more sensitive to environmental changes than higher organisms. Either functional or structural bacterial diversity can be used as bio-indicators of the soil pollution (Nielsen et al., 2002). The ecological importance of the soils bacteria is not limited to their number of biomass even though these parameters contribute greatly. Indeed, their main asset lies in their great genetic and functional diversity (Torsvik and Øvreås, 2002). Our selected area was an abandoned mining area of Sidi Kamber in the North east of Algeria. Unexploited since 1984, with major mining wastes left. It represents a serious source of heavy metal contamination for this area and for the surrounding region.

The main purposes of this study were; a) To assess the soil heavy metals (Pb, Cu, Cd, Zn and Ni) contamination in the mining area called Sidi Kamber; b) To assess edaphic parameters such as pH, organic matter (OM), cation exchange capacity (CEC), total organic C, total N, available P; c) To spot the microbiological characteristics of the soil such as microbial communities and diversity; d) To determine the relation between total heavy metal content and bacteriological characteristics of the soils.

Material and Methods

The study area

This study was accomplished in the abandoned mine of Pb and Zn, precisely located in Sidi Kamber in the region of Oum Toub, Skikda,North eastern Algeria. (Boukhalfa, 2007) (Figure 1). The exploitation of this mine began in 1890 with a very low production and frequent stops and it's only in 1913 that a regular production began (Beddai, 1976). All the metallurgical activities on the site have been definitively ceased in 1984 (Oumdjbeur, 1986) (Figure 2). The studied area was characterized by rugged mountains which the altitudes are ranging between 200 to 500 m. The slope varies between 2 and 25%. It is very strong in the North Eastern land and low in the Southeastern valleys. According to the data collected from Skikda's weather station (SWS, 2014) for a period of 16 years (from 1997 to 2013). The studied area is characterized by a Mediterranean sub humid climate with a hot dry summer and mild winter. The annual temperature average is 19°C; the cumulative annual rainfall is estimated to 742.35mm. Monthly average value of relative humidity is 68.8 % (SWS, 2014). The vegetation inventory of the studied area allowed us to spot 104 plant species most of them are annual or perennial herbaceous plants that belong to the families of *Asteracea*e and *Poaceae* .The studied vegetation was characterized by therophytes to ruderals affinities. They are considered as the ultimate stage of ecosystem degradation. Spoil was colonized mainly by a short lawn composed of herbaceous such as *Rumex bucephalophorus*, *Lamarckia aurea*, *Virbascum sinuatum*, *Trifolium compestre* and *Medicago minima*. The ligneous stratum is small, it is mainly represented by *Cistus monspeliensis*, *Calicotome spinosa*, *Pistacia lentiscus* and some feet of *Erica arborea* (Lehout et al., 2017).

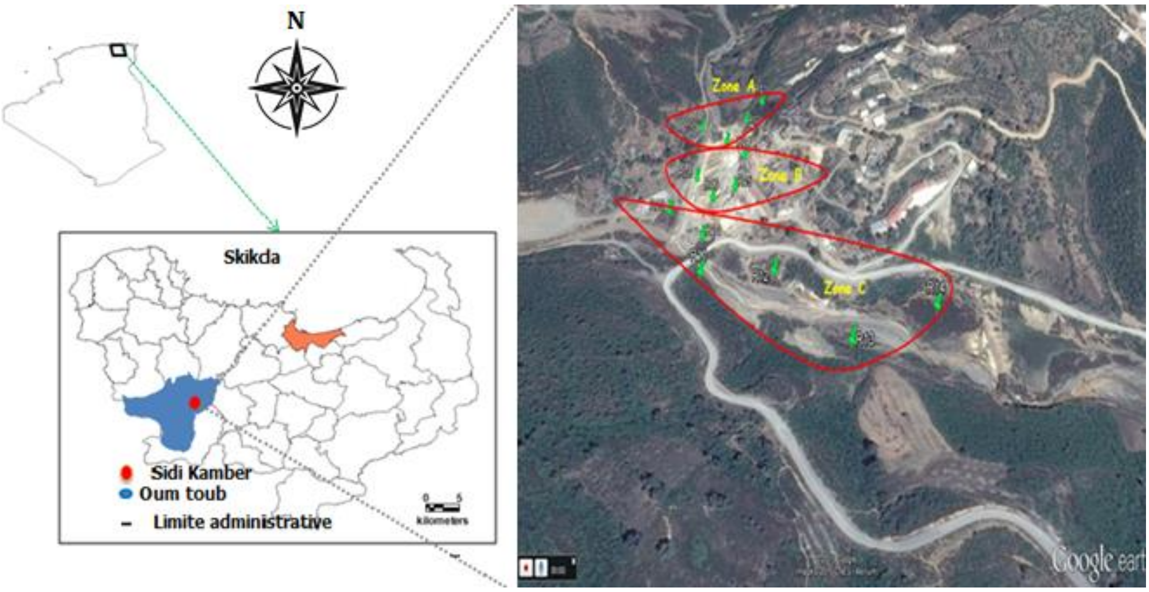

Figure 1. Geographic location of study area and sampling points.

Sampling points

Due to the big surface of the mining area (Sidi Kamber 700 h), the multiplicity and diversity of the metal extraction and the waste discharge sites. We have selected 14 sampling points distributed on three zones (Figure 2).

- ✓ Zone A: upstream of the mining zone with 4 sampling points.
- ✓ Zone B: in the center of the mine, close to both the waste discharges and the metal extraction sites with 4 sampling points.
- ✓ Zone C: the exit of the mining zone downstream, with 6 sampling points.

Figure 2. General view of the studied area

Some chemical analysis of soil samples

Soil samples (non-vegetated soil) were collected from the top layer (0-20cm) of the mining area. These samples were air-dried in a room temperature and sieved through 2mm mesh (to remove as far as possible plants materials and stones) before doing any further analysis of the physico-chemical characterization according to the standards methods. pH of soil (soil:distilled water; 1:2.5, w/v) was measured using the glass-electrode method, organic matter (OM%) and carbon content (C%) were determined using wet oxidation method of Walkey and Black (Walkley and Black, 1934), total nitrogen (TN) was measured using the Kjeldahl method described by Pansu and Gautheyrou (2006), available phosphorus (P) was determined by the method of Olsen (Olsen et al., 1954). Finally, cation exchange capacity (CEC) was measured by titration method with H_2SO_4 using ammonium oxalate in the presence calcium carbonate according to the method adopted by Pansu and Gautheyrou (2006).

Heavy metals analysis in soils

For heavy metal contents (Pb, Cu, Zn, Cd and Ni) analysis, 1g of soil was digested with 15 ml of Aqua-regia method (HNO_3: HCl in 3:1 ratio) in Teflon PFA vessels using speed wave accelerated reaction system (MWS-Berghof speed wave) at 80°C until obtaining a transparent solution according to the method of Allen (Allen et al , 1986). The digested samples were filtered and diluted with de-ionized water up to 50 ml. Heavy metals contents (Pb, Cu, Cd, Zn and Ni) have been carried out by ICP-MS Agilent 7700X at the Environmental Analysis Laboratory of CRBt.

Bacterial analysis

Soil samples were collected using sterile techniques, preserved at 4°C and analyzed within 48h. The total bacteria in the soil were determined by the plate count method. For each soil sample 10g of fresh soil shaken in 90ml of distilled water for 30 minutes, serially diluted in sterile saline buffer (0.85% NaCl) and 100 µl of each soil sample was placed on nutrient agar plates. The plates were incubated at 30°C for 48h and the formed bacterial colonies were counted according to the method described by Grunda (1985). These bacteria were streaked on fresh nutrient agar plates to procure for identification. The bacterial isolates were characterized based on cultural, morphological and biochemical characteristics as described in the Cowan and Steel's Manual for the identification of Medical Bacteria. Oxidase, catalase, methyl red, indole production, citrate and carbohydrate utilization (glucose, sucrose, maltose, xylose and lactose) were biochemically analyzed according to the method followed by Barrow and Feltham (1993).

Statistical analysis

Statistical analyses are performed using the XLSTAT (XlStat-Pro. V.7.5.2) software, including: ANOVA one-way, followed by a multiple comparison analysis test (Fischer LSD test and Duncan test) to compare differences between zones. Principal Component Analysis (PCA) and Pearson matrix correlation test to check the dependencies of studied parameters.

Results

Soil physico-chemical properties

The results of the edaphic parameters of soils samples are given in Table 1. The overall area was characterized by an acid pH (5.58-6.05), organic carbon (C%) was relatively high (2.31 to 4.75%), total nitrogen (TN) was also uniformly high (0.35-0.63) resulting a low C/N ratios (5.41-9.44). The CEC ranged from (7.29-13.44 cmol.kg^{1}). The available phosphorus (P) content varies between (3.26-7.11 mg kg^{1}) compared to soil fertility standards, the soil of the studied area is moderately poor in phosphorus. Organic matter (OM%) is the store house of plant and bacteria nutrients and mineral recycling (Rattan et al., 2005) ranged between (3.97-8.18%). The comparison of these edaphic parameters of the three sampling areas shows that, for the pH values there were not a significant variation only the zone B which presented a lower pH 5.58. However, for the other parameters we have recorded that zone A is characterized by the lowest values of C%, TN, OM%, CEC, C/N and a high concentration of available phosphorus 7.11 mg.kg^{-1} comparing to zone B and C.

Heavy metal content in the soil

The soil samples heavy metal contents from all the sites are shown in Table 1. Generally, the concentrations of metallic trace elements are higher compared to the international soil standards. Total Cd, Pb, Zn, Cu and Ni ranged from 5.54 to 200.01 mg Cd kg^{-1}, 1844.08 to 18048.83 mg Pb kg^{-1}, 987.78 to 42928.99 mg Zn kg^{-1} 259.56 to 995.10 mg Cu kg $^{-1}$ and 56.02 to 220.32 mg Ni kg^{-1}. Heavy metal concentrations show a significant variation between the zones; mainly, the lowest concentrations were observed in zone A compared to the other zones, whereas, the highest concentrations of heavy metals are recorded in zone B*.*

Bacteriological analysis

Plate counting analysis revealed that, bacterial load (bacterial biomass) of zone A is thirty times higher than the one from zone B (Table 1). According, to the bacteria load, we can classify the sites into two groups; Group I includes the zones B and C with the highest concentration of heavy metals and a less number of bacteria, Group II includes zone A, with a low concentration of heavy metals and high amount of bacteria. The differences in bacteria quantity of each zone are shown in Table 1. From the prospected mining area, we have isolated a sum of 31 strains that belong to 31 genus. *Pseudomonas* genus was the most represented with 5 species, followed by *Aeromonas, Enterobacter* and *Seratia* with 3 species, for *Pasteurella* and *Chrysebacterium* we have isolated 2 species for each genus and one species for the rest of the genus Table 3. Zone A shows the highest value of bacterial diversity with an index of Shannon Weaver calculated H=1.81 and 19 species, followed by zone B with H=1.56 and 15 species and finally comes zone C with H=0.95 and only 11 species (Figure 3).

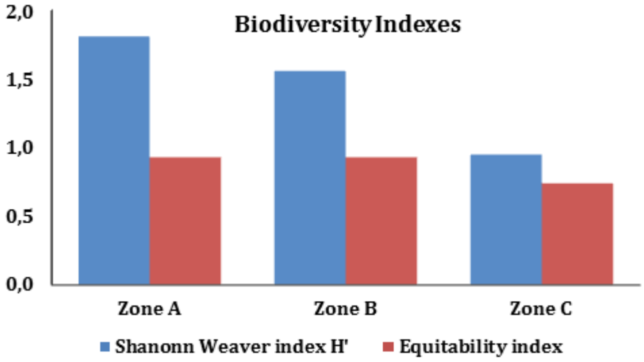

Figure 3. Biodiversity indexes of bacterial communities

Statistical analyses

Processing data by the LSD test (least significant difference) using one–way Anova analysis revealed a significant difference ($p<0.05$) of Pb, Cd, Cu, Zn and Ni content between zone B and the zones A and C. No-significant differences were recorded between zone B and C with respect to pH and TN. A significant difference was also observed between zone B and the zones A and C for OM%, C%, P, CEC and bacterial load (bacterial biomass). The correlation coefficients computed between the some chemical and bacterial parameters are presented in the Table 2. A strong correlation was observed between CEC and Cd, Zn and Ni (r=0.59*, r=0.58 * and r=0.36 * respectively). A low correlation between pH and organic matter (r=-0.44*). A correlation between bacterial biomass and organic matter, Ni and available phosphorus (r=-0.34*, r=-0.44* and r=0.60* respectively).

Table 1. Physico-chemical and bacteriological characteristics of soil samples

	Zone A (n=4)			Zone B (n=4)			Zone C (n=6)		
	Max	**Min**	**Mean± SD**	**Max**	**Min**	**Mean± SD**	**Max**	**Min**	**Mean± SD**
pH	7.18	4.64	6.05 ± 0.99	7.05	3.81	5.58 ± 1.02	6.58	3.74	5,66 ± 1.00
C, %	5.48	0.87	2.31 ± 1.76	9.17	3.17	4.75 ± 1.81	4.52	0.51	2,89 ± 1.08
P, mg kg^{-1}	8.18	4.68	7.11 ± 1.19	7.84	1.83	4.19 ± 2.3.	7.30	1.87	3,26 ± 1.26
TN, %	0.59	0.22	0.35 ± 0.18	1.45	0.22	0.61 ± 0.35	1.46	0.27	0,63 ± 0.28
OM, %	9.43	1.50	4.97 ± 3.04	15.77	5.45	8.18 ± 3.11	8.82	3.23	5,48 ± 1.55
CEC, cmol kg^{-1}	9.75	2.75	7.29 ± 1.90	24.00	3.00	13.44 ± 6.89	12.75	3.00	7,55 ± 2.49
C/N	18.20	2.00	6.80 ± 4.88	17.55	4.52	9.44 ± 3.76	13.08	1.00	5,41 ± 3.00
Pb, mg kg^{-1}	7066.99	270.53	1844.08 ± 2697.60	61004.44	1800.12	18048.83 ± 23209.94	8468.52	1269.49	3615,60 ± 1967.16
Cd, mg kg^{-1}	16.87	1.86	5.54 ± 6.21	742.27	13.89	200.01 ± 293.22	46.30	14.18	23,60 ± 7.66
Zn, mg kg^{-1}	4178.03	16.87	987.78 ± 12841.14	156140.42	3457.27	42928.99 ± 32165.50	11376.63	3369.72	6811,11 ± 2504.62
Cu, mg kg^{-1}	3787.12	39.06	543.48 ± 706.52	2459.22	285.64	995.10 ± 834.92	608.48	100.75	259,56 ± 149.97
Ni, mg kg^{-1}	59.12	46.45	56.02 ± 4.81	357.33	73.00	169.89 ± 106.93	301.73	45.78	220,32 ± 81.56
Bacterial number in soils, CFU g^{-1}	9.2x10^{7}	1.2x10^{6}	4.2x10^{6} ± 1.8x10^{6}	3.9x10^{4}	2.02x10^{3}	1.3x10^{4} ± 1.05x10^{4}	4.6x10^{6}	3.05x10^{4}	4.3x10^{5} ±, 2.4x10^{4}

Table 2. Matrix of correlations

Variables	**Pb**	**Cd**	**Zn**	**Cu**	**Ni**	**pH**	**C**	**P**	**N**	**OM**	**CEC**	**Biomass**
Pb	1											
Cd	0,0357	1										
Zn	0,0576	0,9976***	1									
Cu	0,6785*	0,1607	0,1602	1								
Ni	-0,1306	0,5194*	0,5415*	-0,1127	1							
pH	-0,2761	0,2100	0,2203	-0,2538	0,3381	1						
C	0,7089*	0,1145	0,1215	0,6699**	-0,0997	-0,4465*	1					
P	0,0046	0,3100	0,3049	0,0281	-0,1767	0,4111	-0,2035	1				
N	0,4602	0,2149	0,2385	0,2338	0,4273	-0,0885	0,4166	0,0011	1			
OM	0,7159**	0,0990	0,1076	0,6689*	-0,0562	-0,4665*	0,9137	-0,2535	0,4312	1		
CEC	0,3213	0,5891*	0,5794*	0,2958	0,3585	0,1576	0,2249	0,1936	0,1389	0,1505	1	
Biomass	-0,2309	-0,2312	-0,2424	0,0195	-0,4382	0,2657	-0,2614	0,5916*	-0,3382	-0,3368	-0,1864	1

*P<0.05, **P<0.01, ***P<0.001

The PCA analysis of the soil characteristics (Figure 4) led to a reduction in the initial size of the two-component data set, accounting for 58.92% of the variation in the data (F1 33, 47% and F2 25, 44% of the variance). F1 shows that organic matter and CEC affect positively the distribution of metallic trace elements in the soil where we observed a group of metals that preferred to bind to the organic matter (Pb and Cu) and another group that preferred a inorganic ligand (Cd, Zn and Ni). With the PCA analysis, it was possible to obtain a clear separation of the studied zones. Thus, the zone A contains high values of pH and available phosphorus contents , a very important bacterial charge as well as low concentrations of Cd and Ni, Cu, Pb and Zn. Zone B was mainly characterized by its high concentrations of Pb, Cu, Zn, Cd and Ni also its high contents of organic matter and CEC. Whereas, zone C shows intermediate values between the two first zones.

Table 3. Results of identified bacteria populations

Identified species		
Pseudomonas puteda	*Enterobacter intermedus*	*Proteus fenneri*
Pseudomonas alcaligens	*Enterobacter sakazaki*	*Photobacterium damsella*
Pseudomonas luteola	*Enterobacter cloacae*	*Burkhoderia cepacia*
Pseudomonas aerogenosa	*Chrysebacterium meningosepticum*	*Mannheimia haemolytica*
Pseudomonas fluoresens	*Chrysebacterium indologens*	*Hafnia alvei*
Aeoromonas hydrophyla	*Pasteurella sp.*	*Pantoea sp.*
Aeoromonas hydrophyla	*Pasteurella pneumotropica*	*Brecella sp.*
Aeromonas salmonicida	*Providencia alcaligens*	*Vibrio fluviatis*
Seratia plumuthyca	*Klebseilla pneumonia*	*Echerichia coli*
Saratia odorifera	*Acenitobacter lwofii*	*Shewanella putrefaciens*
Seratia laquefaciens		

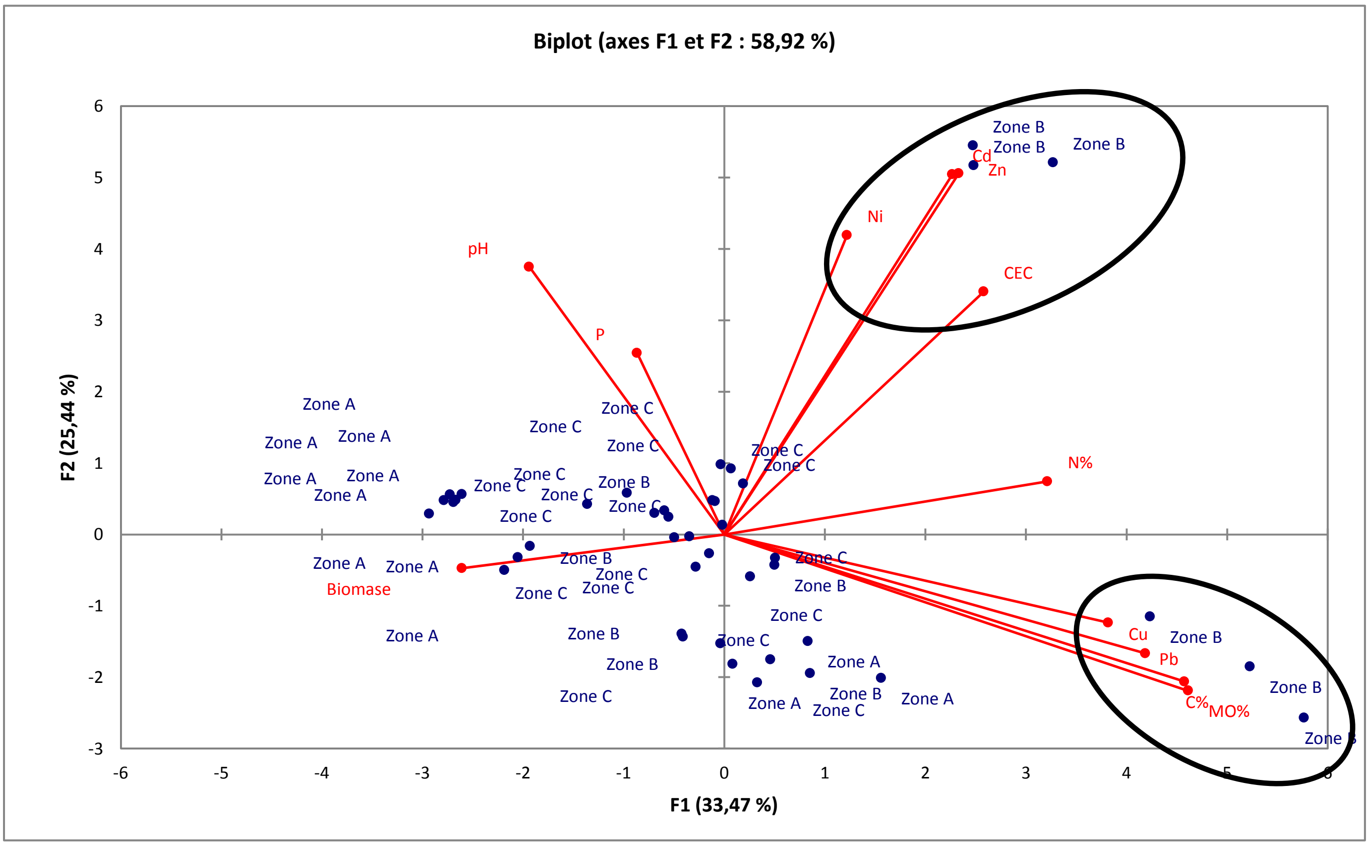

Figure 4. Factorial map applied to the bacterial and physic-chemical variable data conducted by Principal components analysis (PCA)

Discussion

The studied mining area of Sidi Kamber was severely polluted with Cu, Cd, Pb, Ni and Zn far exceeding international standards and maximum permitted levels for the protection of the ecosystem and the human health as well as the commercial and industrial land use which is higher than their respective standard levels set for agricultural soils (Baiz, 2000; CCME, 2007; VROM, 2009). Total metal contents around this site have already shown a high level of heterogeneity, a typical characteristic of many mine tailings (Kock and Schippers 2008; Streten-Joyce et al., 2013; Chodak et al., 2013). However, the high level of metal concentrations found in the soil of this mine could well be responsible of its high values of OM content (4.97, 8.18 and 5.48 respectively) and the low values of TN, P and C/N ratio because heavy metals might affect biological mineralization cycles (Chander and Brookes, 1991). The impairment of the biological activity of the soil due to metal loading leads basically to a reduction in the decomposition and turnover rates of organic matter (Babich and Stotzky, 1985). The relationship between organic matters and heavy metals lies in its important role to the heavy metal binding and complexity (Manskaia and Drozdova, 1968; Rashid and Leonard, 1973). Indeed, organic matter is considered as a preferential support for trace elements especially Pb and Cu (Singer, 1977). Organic matter influence the behavior of the heavy metals in the soil by: i. releasing heavy metals associated with the organic matter; ii. extracting or mobilizing heavy metals from the complexes and; iii. improving soil microbial populations which affect heavy metal mobility and availability to the plant through release of chelating agents, acidification, phosphate solubilization and redox changes. The other elements have preferred to bind to inorganic ions which is the case of Cd, Zn and Ni this explained the correlation between these elements and CEC. Pérez-Esteban et al (2014) have found that the organic matter and the organic carbon could increase the metal mobility through the formation of soluble metal organic complexes facilitating in this way the metal uptake by the plants. In the studied soils, OM amounts differ significantly between the zones. Accordingly, it can be assumed that this soil property would have it due to the variation in the bacterial load, which contributes in the mineralization of this organic matter, where we observed a high bacterial load in zone A compared to the other two zones with a pH close to neutrality favoring the decomposition of this organic matter. Since pH has great effects on the solubility of heavy metals, the difference of pH may have influence on other soil characteristics as well as bacterial community diversity (Giller et al., 1998). Taylor et al (2002) also found that the soil pH would affect microbial community structure and diversity. In the present study an acidic pH (Table I) is observed in the soil samples and there was no significant difference between the three sampling sites. Similar pH values have been reported in other studies (Lin et al., 1987; Tam et al., 1995). The acidic pH was partly resulted from the oxidation of FeS_2 and FeS to H_2SO_4 and partly from active microbial decomposition of litter and hydrolysis of tannin in plants, which released various types of acids. These microbial meditated reactions are important determinants of the acidity generation, metal mobilization and the release of key nutrients (such as phosphate and metal cations) required not only for their own metabolism but for the plants growth and eutrophication potential (Welch et al., 1999; Uroz et al., 2009). Tailing are oxidized in contact with air and water with the help of bacteria and produce acid mine drainage that can contaminate surface and groundwater (Liao, 1990). It has been repeatedly reported that the high values of toxic elements in the soils tend to decrease the soil's pH (Alloway, 2010; Hohl and Varna, 2010). Plate counts have been seen as the most appropriate method to determine the effect of heavy metals on soil bacteria compared to culture-independent approaches (Ellis et al., 2003). The diversity and activity of microbial communities are important indicators of soil's quality. The influence of soil physic-chemical properties (including metal concentrations) on the soil's microbial properties and vice versa is worth mentioning. An increasing piece of evidence suggests that heavy metals have a strong impact on both bacterial and fungal communities (Kozdrój and van Elsas, 2001). In this study, we have observed a strong heavy metals effect on the bacterial biomass. The average bacterial biomass in zone A was 39 times higher compared to zone B and C respectively. This indicates that metal concentrations modified in a strong way the present microbial community in the most contaminated samples (B and C) either ways direct or indirect as verified by the changes in the relative abundance of several plant species. (Epelde et al., 2010; Lehout et al., 2017). The presence of heavy metals decreased the microbial biomass directly, when the heavy metal contents exceeded the threshold of tolerance or by inhibiting some biochemical properties of the soil which are essential for their survival. Moreover, the bacterial biomass can be influenced by many other environmental factors such as pH, temperature, nutrient content and his root exudates nature (Chodak et al., 2013). It is noteworthy that the structure and diversity of bacteria communities in the soil are not only influenced by heavy metal concentrations but also by pH (Kock and Schippers, 2008), organic matter (Bouskill et al., 2010) and the interactions between these factors. The vegetation in this area provides high levels of organic matter. According to Lehout et al. (2017), zone A showed abundance and a vegetal richness compared to the other zones. The natural nutrient content of this soil might therefore play a key role in the evolution of the bacterial diversity. On the other hand, regarding the species richness of bacterial community diversity it appears that it was not strongly influenced by the contamination level in the studied area. Indeed, so many studies have shown a notable decrease in microbial diversity and other animals and plants organisms in the soil contaminated by the metals due to the toxicity of the element (Bamborough and Cummings, 2009; Singh et al., 2014). However, recent studies have shown that after a century of exposure to acid drainage, the bacterial community tends to stabilize and increase its diversity, resulted in an environment with a microbial diversity similar to that of the non-impacted areas (Reis et al., 2013; Bouskill et al., 2010; Pereira et al., 2014). Nonetheless, soil micro-organisms have developed highly efficient systems of metal detoxification which, in bacteria, can be grouped into five categories: intracellular sequestration, export, reduced permeability, extracellular sequestration and extracellular detoxification (Epelde et al., 2010; Pereira et al., 2015). Heavy metal contamination provides a strong pressure selected for the recruitment of multiple resistance phenotypes that encode resistance to the predominant metals in the site (Ryan et al., 2005).

Conclusion

The present study shows that the mining area of Sidi Kamber is characterized by high levels of metals far exceeding international standards. These high levels of heavy metals may have both direct and indirect effect of nutrients content. Negative impact of heavy metals on bacterial communities in the soil is shown by several studies. These were significant differences in the structure of microbial communities depending on the level of heavy metal contamination. However, bacterial biomass can be influenced by other environmental factors such as pH and nutrient content. Moreover, total metal concentration in the soil does not give enough information on metal mobility, bioavailability and phytotoxicity. Instead, assessment of soil contamination can also be determined by estimating the bio-available fraction of toxic metals. Finally, an ecological understanding of how contaminants, ecosystem functions and biological communities interact in the long term is needed for a proper management of these fragile metalliferous ecosystems.

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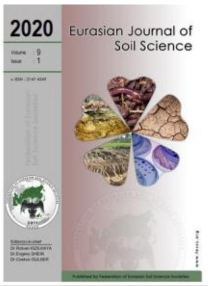

Texture contrast soils (TCS) as indicators of eolian dust inputs in the coastal area of west central Senegal, West Africa

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Abstract

Different hypotheses have been addressed to explain the origin of texture contrast soils (TCS) in coastal regions. Our study investigates the TCS in the coastal region of west central Senegal, West Africa, in order to appreciate the influence of eolian dust inputs on their formation and characteristics. Nine soil profiles, from a levee to backswamp toposequence, three on each landscape position; floodplain, low terrace and middle terrace, were investigated. Soil profiles were described according to the World Reference Base. Particle-size analysis was performed by wet-sieving for the sand fraction and pipette analysis for the silt and clay fractions. Elements were determined in finely powdered soil mixed with cellulose in a ratio of 2:1. In this work, we focused principally on elemental zircon (Zr) and Titan (Ti). Our results show the presence of TCS in the coastal area of west central Senegal. We attribute the formation of these TCS to mainly two different sources of soil material: marine and continental. Marine sediments build the floodplain profiles and the subsoil of low terrace profiles, while the colluvial material derived from continental uplands form the middle terrace profiles and the topsoil of low terrace profiles. The main finding remains the influence of eolian dust inputs on the formation of TCS in this coastal area. Evidence of dust addition was confirmed by the uniformity of soil parent material tested through mS/(cU+mU) and Ti/Zr ratios. Such knowledge enhances understanding of local pedogenesis and may help develop sustainable soil management strategies.

Keywords: Eolian dust inputs, Senegal, texture contrast soil, West Africa.

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Introduction

Early evidenced by Milne (1936), Ahn (1970), and Jones and Wild (1975) in coastal areas of West Africa, soil "catenas" or toposequences are characterized by contrasting pedo-environmental conditions between up- and lowlands; sediments depleted and elements leached uplands accumulate lowlands, giving rise to variable soil properties along the same toposequence. Soil differences in landscapes have their primary origin in topographic position (Birkeland, 1999). Many authors highlighted the influence of topography or landscape position on soil formation and properties (Mulugeta and Sheleme; 2010; Baskan et al. 2016; Tijjani and Hassan, 2017). Understanding of soil forming factors (e.g. parent material, climate, topography, vegetation and time) is crucial to define physical, mineralogical and morphological properties of soils (Tunçay et al., 2020). Topographic positions or physiographical units have important role in soil formation and affects how water and energy were added to and was lost from soil systems (Dengiz and Şenol, 2018).

Abate and Kibret (2016) analysed the "effects of land use, soil depth and topography on soil physicochemical properties along the toposequence at the Wadla Delanta massif, northcentral highlands of Ethiopia" and found that topography of agricultural fields can influence soil physicochemical properties (soil depth, texture, and mineral contents), biomass production, incoming solar radiation, precipitation and affect crop production. As an increased topography/elevation significantly increased soil moisture, precipitation, soil

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organic matter and labile carbon, whereas bulk density, pH and soil temperature were significantly lower at the higher elevations. Topography is, therefore, central to the catena concept of soil development which is characterized by leaching and redistribution of soil material and elements down hill slopes (Baskan et al., 2016).

Another critical factor, which characterizes "catenas" in West Africa, remains the continental and marine influences they permanently face. This most often results in the formation of vertical texture contrast soils (TCS). The development of these TCS, whereby coarser surface material overlies finer subsurface layers, is still debated. Despite common occurrence, a definitive explanation for the formation and development of this characteristic is yet to be elucidated (Bald, 2012). The traditional explanation relies on eluviation and illuviation by downward-moving water. In this model, material removed from upper soil layers in solution is translocated to the illuvial horizon, where some of the solutes precipitate (sometimes termed neoformation). Also, fine-grained particles may be physically washed from upper to lower soil horizons (lessivage or argilluviation). This translocation depletes upper and enriches lower horizons in fines, creating the texture contrast. Erosion and deposition can account for some of these profiles, as can the inheritance of textural contrasts from sedimentary layering or lithological boundaries (Phillips, 2001).

It should also be mentioned that TCS are usually associated with a range of management problems including waterlogging, poor crop establishment, crusting, poor root penetration, desiccation, wind erosion, water erosion, tunnel erosion, salinity, and poor nutritional status. In addition, TCS are naturally very hard setting and suffer low infiltration rates and poor water holding capacity, which is accentuated where excessive cultivation has occurred. The presence of massive, poorly drained subsoils results in regular seasonal waterlogging, which results in poor aeration for roots, nitrogen deficiency, and increased manganese levels where pH is low. Reduced crop yields in TCS result from soil erosion, crusting, limited rooting capacity, poor aeration resulting from the slow movement of soil water through the upper B horizon, and confining of roots to shrinkage cracks and ped faces in the subsoil. In landscapes with sufficient slope, perched water-tables may be exacerbated by lateral movement of water on the upper surface of the B horizon leading to waterlogging and salinity in lower parts of the landscape (Hardie et al., 2012). The interpretation of such textural boundaries is important in a number of geomorphological contexts, such as distinguishing pedological vs. geological layering, the identification of colluvial and other geomorphic processes, and the interpretation of weathering profiles. The formation of TCS is important beyond the study of soil geomorphology and pedogenic processes in specific soils precisely because TCS are so widespread. Explaining the occurrence of TCS in a variety of environmental settings has been an important goal for pedologists and geomorphologists because such an explanation might elucidate general principles of soil development (Phillips, 2007).

This study aims to investigate the formation of TCS in the coastal area of west central Senegal, West Africa in order to appreciate the influence of eolian dust inputs on their development and characteristics. Such knowledge may enhance understanding of pedogenesis in coastal areas of West Africa, and worldwide at large, and will help develop sustainable soil management strategies. Landscapes position influences runoff, drainage, soil temperature, soil erosion, soil depth and hence soil formation. Different soil properties encountered along landscapes will affect the patterns of plant production, litter production and decomposition, which will definitely have effects on carbon (C) and nitrogen (N) contents of the soil (Mulugeta and Sheleme, 2010).

Material and Methods

Presentation of the study area

As a natural region, West Africa may be defined as lying south of the Ahaggar-Tibesti Mountains and west of the watershed separating Lake Chad from the Nile and Zaire drainage basins. West Africa thus lies west of the topographic demarcation of high Africa from low Africa. Geomorphologically, the West African region includes the coastal plain, the Guinea basement shields and the Taoudeni and Chad basins in the Western Sahara (Petters, 1991). The young geological evolution of West Africa is strongly marked by successive transgression and regression phases corresponding to changes in the Atlantic level during the Quaternary. They contribute to the construction of the main sedimentary units which intervene in the evolution of this littoral environment (Barusseau et al., 1995).

The coastal region of west-central Senegal, West Africa, is bounded on the west by the Atlantic Ocean (Figure 1). The climate is of Sudano-Sahelian type with a high mean annual temperature of 29°C and a mean annual precipitation ranging from 600 to 800mm year^{-1}, from north to south. Rainfall is limited to the period July–September (rainy season) (Faye et al., 2003). The geology consists of Holocene marine sediments underlain by Miocene deposits known as the 'Continental Terminal', a thick series of ferruginised and argillaceous sandstones, mudstones and carbonaceous layers, deposited under fluviatile and lacustrine conditions (Wright et al., 1985), which has undergone a profound ferrallitic pedogenesis (Michel, 1975; Kalck, 1978; Lappartient, 1985; Diop, 1986). The hydrologic system is the Saloum River, a tide-influenced 'inverse estuary', fed only by a limited river flow for 2–3 months during the rainy season (Faye et al., 2003). Hydrodynamics are essentially governed by the penetration of the tidal wave and the strong evaporation regime, which develop in the vast system of the interconnected tributaries (locally known as 'bolons') and the mangrove forest within the delta (Barusseau et al., 1995).

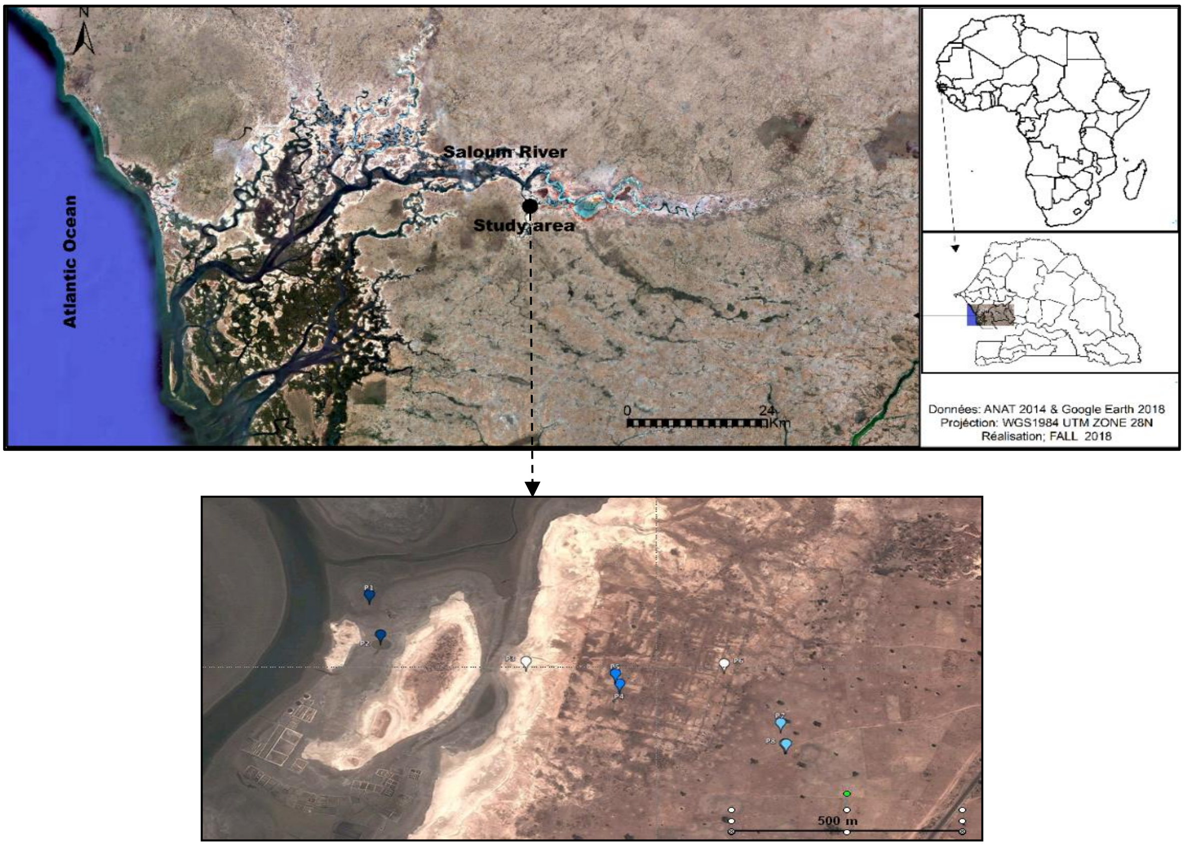

Figure 1. Location map of the study area with indication of soil profiles (from P1, left to P9, right) along the investigated transect (adapted from Google Earth, 2019).

The study site consists on a toposequence of ~1.5 km length with a general slope of 0.5%. The transect is oriented east–west and runs across a tributary of the Saloum River. At the lower part of the toposequence (floodplain), the soils are flooded and entirely devoid of vegetation. This area was formerly occupied by mangrove vegetation, with plant remnants as a prominent landscape feature (Lézine, 1997). Nearby, salt extraction activities using tiny evaporated ponds indicate a high level of salinity in the groundwater system. At the intermediate part (low terrace) we find seasonal salt-tolerant grasses like *Phyloxerus vermicularis* and *Schoenefeldia gracilis*. At the higher part (middle terrace), there is annual and perennial vegetation with the dominance of *Balanites aegyptiaca* (Fall et al., 2014).

Field observations and measurements

The selected toposequence was placed in such a way that influences of the landscape position could be reflected in soil properties. Nine soil profiles were distributed along the toposequence, three on each landscape position, i.e. floodplain, low terrace and middle terrace. All soil profiles were described in the field according to the World Reference Base (IUSS Working Group WRB, 2014). The Munsell soil colours were determined on moist samples. In total, 45 soil samples, one per identified horizon, were collected for laboratory analyses. The topography of the catena was determined using a theodolite.

Particle-size and elemental analysis

Particle-size analysis was performed after removal of carbonates and organic matter by treatment with HCl (pH 4.5) and H_2O_2 (10%), respectively, and of excessive salts by repeated addition of deionised water, centrifugation and decantation until the EC dropped below 40 mS cm^{-1} (Schlichting et al., 1995). After subsequent addition of ammonia (NH_3) for water dispersion, overnight shaking and ultrasonic treatment, the sand fractions (63–2000 µm) were obtained by wet-sieving, and the silt (2–63 µm) and clay (< 2 µm) fractions were separated by pipette analysis after Köhn (Schlichting et al., 1995).

Elements were determined in all samples using Siemens SRS 200 X-ray fluorescence (XRF), (Bruker AXS GmbH, D-76187, Karlsruhe, Germany). In this method the < 2 mm soil samples were ground to fine powder and mixed with cellulose in a ratio of 2:1. Soil-cellulose samples were then analysed against standards to obtain the concentration of the elements which were further converted to their oxide forms using relevant factors. For the purpose of this work, we focused principally on elemental zircon (Zr) and Titan (Ti).

All soil analyses were performed in duplicate on the fine earth fraction (< 2 mm). Results are expressed on the basis of the oven-dry (105°C) soil weight. All graphics were performed using the Excel software, 2013.

Results and Discussion

Soil morphology and particle-size distributions

Detailed particle-size analyses allow a considerable insight into the origin and fate of soil parent materials (Walker et al., 1988). Solonchaks on the floodplain show a fine texture (silt clay loam). The clay content is higher in the central horizons of P1 and P2, but increases continuously with depth within P3 (Figure 2). The silt fraction decreases with depth throughout all profiles, with higher content in the topmost horizons compared to the underlying horizons. The sand fraction shows irregular distribution trend, increasing and decreasing alternately with depth throughout all profiles. This fraction is dominated by the fine sand (fS>mS>cS), while the silt fraction is mostly coarse (cU>mU>fU). The fine texture of the floodplain soils supports less permeability on this lowest landscape position. The low terrace soils, classified as Haplic Gleysol (Thionic), are characterized by sandy loam upper horizons and sandy clay loam lower horizons. The clay fraction increases, thus, downward in these profiles while the sand fraction shows an opposite trend, decreasing downward throughout all profiles. It is dominated by the fine sand fraction (fS>mS>cS). The silt fraction decreases slightly with depth throughout P4 and P5, but increases slightly throughout P6 (Table1, Figure 2). It is also dominated by the coarse silt fraction (cU>mU>fU). Soil material becomes coarser in the Endogleyic Arenosols on the middle terrace, from loamy sand to sand (Figure 2).

First interpretation of particle size distribution within soil profiles along the investigated toposequence suggests homogenous soil material on the floodplain and the middle terrace sites, and texture-contrasted soil on the low terrace site. This trend suggests the presence of TCS on this intermediate landscape position (Figure 2). The origin and formation of such TCS remains controversial. Some workers (Retallack, 1990; Birkeland, 1999) linked it to *in situ* weathering processes, from which, eluviation/illuviation has typically been perceived as the major. It is based on field evidence of cutans; coatings or accumulations on ped faces, in pores, or on particle surfaces (Phillips, 2001). Another evidence of eluviation-illuviation includes increases in fine clay/total clay ratios with depth in the absence of lithologic discontinuities, and oriented clay in thin sections (Langley-Turnbaugh and Bockheim, 1997). Other workers (Paton et al., 1995) maintain however that illuviation is inadequate as a general explanation for the development of TCS. The most fundamental line of their argument is evidence that in many soils there is simply too much clay in the subsoil to have been derived from the overlying horizons. They support the existence of processes other than eluviation-illuviation that may be of comparable or greater importance in creating TCS. Phillips (2001) identified five general explanations for the development of TCS: (1) vertical contrasts inherited from parent material, (2) deposition of coarse surface layers, (3) erosional winnowing, (4) eluviation-illuviation, and (5) bioturbation. The second explanation supports TCS inherited from sedimentary layering. It seems more consistent with our findings and may help explain the nature and stratification of sediments in the low terrace profiles. The three (3) criteria retained by Phillips (2001) to identify TCS in the Nacogdoches County, Texas: (1) A and/or E horizon texture of sandy loam or coarser, (2) B horizon texture of sandy clay loam or finer, and (3) a difference of at least three textural classes between the surficial and B horizon; are thoroughly met on this transitional landscape position. These profiles read from the bottom upwards, record a sequence of soil material with silt clay loam sublayers overlain by sandy loam upper layers (Figure 2). Confirmation of this hypothesis requires however uniformity tests of soil parent material.

Uniformity tests

Uniformity tests were performed to confirm the textural contrasts suspected in the low terrace profiles on the basis of particle-size distribution, whereby inferring the origin and nature of sediments in the study area. The main reason why parent-material uniformity is important is that it provides clues as to the original particle size distribution of the soil in question (Birkeland, 1999). Uniformity of the parent material was tested through sedimentologic (mS/cU+mU) and geochemical (Ti/ Zr) parameters.

Table 1. Selected soil characteristics along the investigated toposequence

Depth (cm)	Horizon	Colour (moist)	Texture (%)			Ti	Zr	TiO_2	ZrO_2
			Sand	Silt	Clay	ppm		%	
Floodplain (P1), Gleyic Hyposalic Solonchak (Sulphatic), 14° 04' 27.4'' N / 016. 11' 17.4'' W									
0-1	Az	7.5Y4/1	24.5	56.7	18.8	3636	397	0,6	0.05
1-4	Bjz	7.5YR5/6	25.6	37.4	37.0	5064	544	0,8	0.10
4-23	Bjv	7,5YR4/2	17.9	34.8	47.2	5864	550	1,0	0.10
23-60	Bv	10YR3/3	29.4	31.4	39.2	5612	465	0,9	0.10
Floodplain (P2), Gleyic Hypersalic Solonchak (Sulphatic), 14° 04' 23.8''N / 016. 11' 16.6''W									
0-1	Azm	5Y3/1, 5YR5/8	22.8	45.7	31.5	1780	168	0,3	0.02
1-8	Az	2.5Y4/1	27.6	32.9	39.4	5225	477	0,9	0.10
8-30	Cjr	2.5YR3/1 10YR7/6	21.3	30.7	47.7	6450	594	1,1	0.10
30-60	Cr	7.5YR2/2	42.3	30.5	27.2	4151	440	0,7	0.10
Floodplain (P3), Gleyic Hyposalic Solonchak (Sulphatic), 14° 04' 21.4'' N / 016. 11' 06.0''W									
0-5	Ahz	7.5YR5/4	75.2	21.6	3.2	2814	957	0,5	0.10
5-11	Cz	10YR7/2	69.8	25.9	4.3	2978	826	0,5	0.10
11-17	C	10YR6/4	79.5	17.8	3.1	2132	660	0,4	0.10
17-26	Bl1	7.5YR5/8	83.6	10.7	6.1	1238	278	0,2	0.04
26-34	Bl2	mottled	78.9	12.2	8.8	1584	328	0,3	0.04
34-70	Bhr	7.5YR2/1	64.3	11.0	24.7	2911	295	0,5	0.04
Low Terrace (P4), Haplic Gleysol (Thionic), 14° 04' 19.4'' N / 016. 10' 59.2''W									
0-2	Ah1	7.5YR3/3	57.2	26.4	16.3	3490	421	0,6	0,1
2-16	Ah2	10YR4/4	57.9	18.3	23.7	3706	439	0,6	0,1
16-38	Bl1	7.5YR4/3	61.8	18.3	20	3559	476	0,6	0,1
38-62	2Bl2	10R4/3	45.8	23.3	30.9	5052	467	0,8	0,1
62-90	2Bl3	5Y6/1	46.0	21.3	32.7	4863	441	0,8	0,1
90-100	2Brl	2.5Y5/2	41.8	23.6	34.6	4989	436	0,8	0,1
Low Terrace (P5), Haplic Gleysol (Thionic), 14° 04' 20.3''N / 16. 10' 59.5''W									
0-2	Ahz1	10YR3/3	70.4	19.7	9.7	2567	456	0,4	0,1
2-11	Ahz2	10YR3/4	62.5	18.3	19.1	2847	307	0,5	0,04
11-29	Bwz	10YR4/6	46.7	22.4	31	3944	386	0,7	0,05
29-51	Bl1	2.5YR4/6	50.0	21.4	28.6	4103	408	0,7	0,1
51-81	Bl2	2.5Y6/2	48.7	21.3	30	4393	403	0,7	0,05
81-100	Brl	2.5Y6/2	38.6	22.9	38.3	5097	434	0,8	0,1
Low Terrace (P6), Haplic Gleysol (Thionic), 14° 04' 21.2''N / 016. 10' 51.6''W									
0-4	Ahz	10YR2/3	82.1	12.8	5.1	2495	511	0,4	0,1
4-32	Ahlz	10YR2/2	67.6	21.8	10.5	2936	409	0,5	0,1
32-62	Bl1	10YR4/6	71.0	20.0	8.8	2608	368	0,4	0,05
62-82	Bl2	10YR5/6	68.6	17.2	14.3	2610	320	0,4	0,04
82-110	Brl	10YR4/6	61.7	12.4	26	3089	320	0,5	0,04
Middle Terrace (P7), Endogleyic Arenosol, 14° 04' 15.9''N / 016. 10' 47.5''W									
0-26	Ap	10YR3/4	85.1	9.7	5.1	2114	300	0,3	0,04
26-60	Ah	10YR3/4	79.8	1.0	6.2	2534	355	0,4	0,05
60-108	Cw	7.5R8/3	90.4	8.6	0.8	1066	204	0,2	0,03
108-160	Bl	7.5YR7/4	88.4	9.7	1.8	1368	241	0,2	0,03
Middle Terrace (P8), Endogleyic Arenosol, N 14° 04' 14.0'' / W 016. 10' 47.1''									
0-31	Ap	10YR2/2	77.6	15.3	7.1	2376	367	0,4	0,05
31-62	AhC	10YR3/2	78.8	18.3	3	2447	456	0,4	0,1
62-107	1Cw	7.5YR7/3	82.1	15.9	2	1384	301	0,2	0,04
107-138	2Bl	10YR6/3	72.9	10.7	16.4	2698	409	0,4	0,1
138-160	1Bl	10YR8/2	83.5	12.5	4.3	1239	265	0,2	0,04
Middle Terrace (P9), Endogleyic Arenosol, 14° 04' 14.0''N / 016. 10' 47.2''W									
0-36	Ap	10YR2/3	67.5	18.2	14.3	3558	684	0,6	0,1
36-67	Ah	10YR3/3	64.6	20.3	15.1	3575	670	0,6	0,1
67-88	Cv	10YR4/3	70.1	17.4	12.5	3452	582	0,6	0,1
88-126	Bc	10YR5/4	64.9	20.5	14.6	3667	641	0,6	0,1
126-160	Bl	2.5Y3/2	61.4	14.5	24.1	3626	517	0,6	0,1

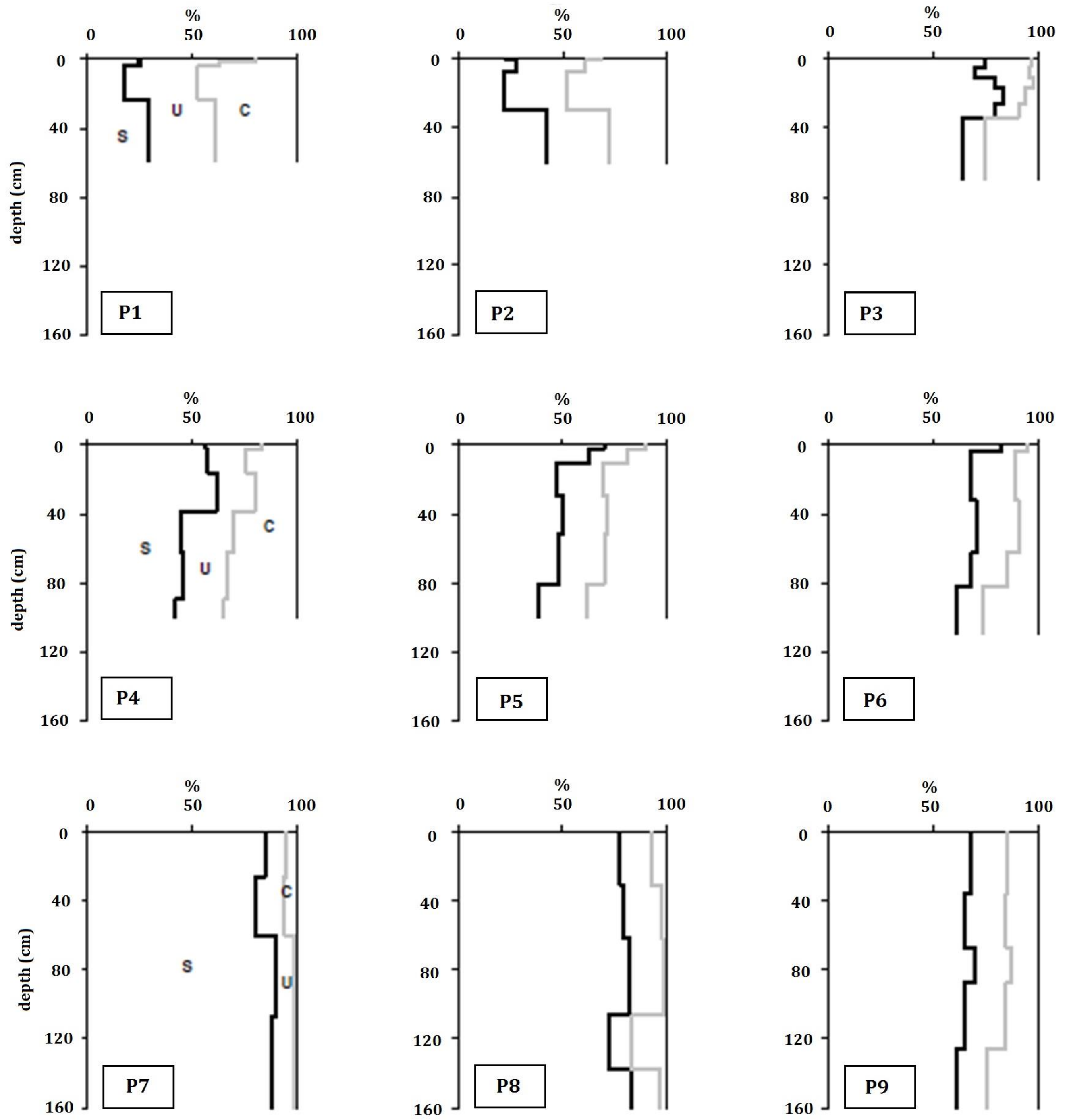

Figure 2. Particle size distribution with depth in the floodplain (P1-P3), low terrace (P4-P6), and middle terrace (P7-P9) profiles. S = sand; U = silt; C = clay

Ratio of mS/(cU+mU)

"Dust" is a term that refers to silt-size or smaller sediment that has an eolian origin (Simonson, 1995). The depth distributions, particularly of silt and clay, provide good clues as to the soil-forming processes and factors operating at the site, and thus help ensure that pedogenesis, rather than some parent-material layering that can mimic pedogenesis, has taken place (Birkeland, 1999). Particle-size distribution can help to identify the contribution of eolian fines to the soil surface. Simonson (1995) demonstrated that many soils carry the imprint of a dust contribution. In many localities, even those far removed from eolian source areas, parent-material layering might be due to eolian deposition (Birkeland, 1999).

The mS/(cU+mU) ratio was applied to estimate the importance of silt-size material in soil profiles. Floodplain profiles show lower mS/(cU+mU) values in the topmost horizons (Figure 3). Values decrease generally with depth in the low terrace profiles, The highest values of mS/(cU+mU) were obtained in the middle terrace profiles, mainly in the subsoil of P7 and P8 (Table 1, Figure 3).

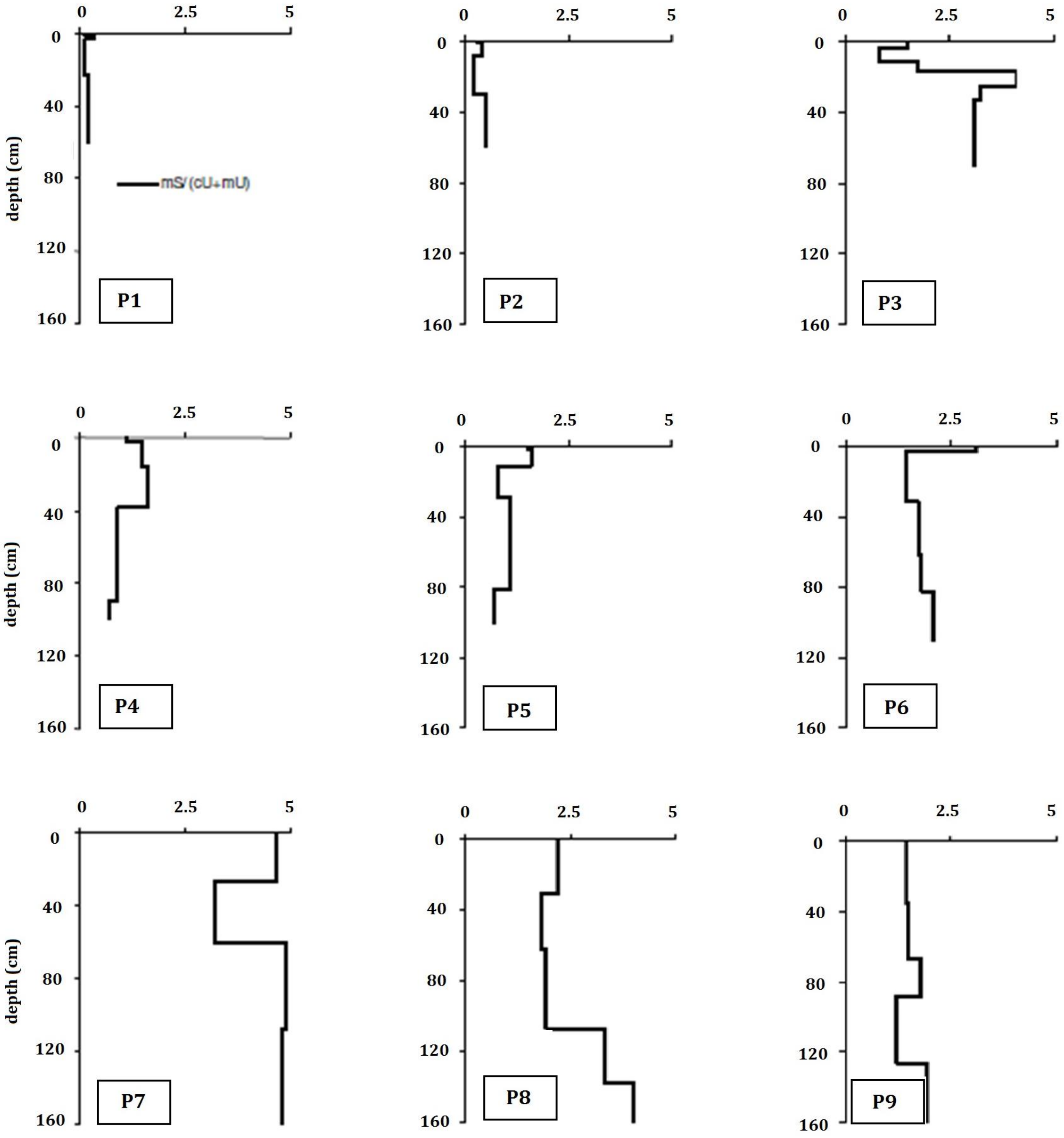

Figure 3. Depth function of mS/(cU+mU) ratio in the floodplain (P1-P3); low terrace (P4-P6); and middle terrace (P7-P9) profiles

The topmost horizons of the floodplain profiles show a silt-rich mantle (lowest mS/(cU+mU) ratios); supporting addition of allochtonous material. This silty mantle is also identifiable in the topsoil of low terrace profiles. Values of mS/(cU+mU) vary however within this latter landscape position; with the highest values yielded in P6 and the lowest values in P4 (Figure 3). This suggests variable intensity of colluvium addition in soil profiles on this landscape position. So the sandy mantle decreases seaward, while the silty mantle increases in the same direction. Eolian dust inputs seem the likeliest accountable factor for such distribution pattern. This suggests that thickness and fineness of the eolian mantle is a function of distance from the source site. Globally the incidence of dust additions in soil profiles is determined by the distance between source area and accumulation site (Schaetzl and Anderson, 2005). McTainsh et al. (1997) investigated the contribution of eolian accessions in soils of Mali, West-Africa. They found dust deposition being function of particle size with long-distance deposition producing fine deposits (mainly < 5 µm); while dust from regional sources produce deposits mainly in the size range 20-40 µm, and local dusts produce relatively coarse material in the size range 50-70 µm. The transported material concerns, thus, mainly the silt-fraction (2-63 µm), and to a lesser extent, the clay (<2 µm) and very fine sand (63-200 µm). Based on this argumentation (finding) we assume that regional dust sources (from the surrounding Continental Terminal plateau) play the determining role regarding the supply of allochtonous material in the study area, whereas local (Saloum region) and long-distance (Sahara) dusts appear less critical. Herrmann (1996), Middleton and Goudie (2001) and Goudie and Middleton (2001) identified two source areas for major dust events which could interest West Africa region: the Bodele depression and an area covering eastern Mauritania, western Mali and southern Algeria. Dust is transported from the southern Sahara Desert over the West African savanna and across the tropical Atlantic Ocean (Prospero and Carlson, 1972; Jaenicke and Schutz, 1978; Prospero et al., 1981). These long-range transports of dust are supposed to permanently feed soil material in our study area. Their nature as well as their individual contribution in local soil development remains to be investigated. Resolution of such questions is, however, outside the scope of the present study. It requires detailed regional studies.

The Ti/ Zr ratio

Soil uniformity was secondly tested using the Ti/Zr ratio. Many studies (Raeside, 1959; Alexander et al., 1962; Reheis, 1990) demonstrated ZrO2 to be more resistant to weathering than any other commonly occurring oxide in soils. Likewise, Harden (1988) found zirconium to be the most stable element, since zircon grains remained remarkably unchanged with no increased etching with time. She used zirconium as reference to show changes in soil composition, and found indications for eolian deposition on fluvial terraces in central California by an increase of the ratio over time.

Also, due its low mobility during chemical weathering, Ti is commonly associated with Zr to evaluate parent-material uniformity within soil profiles presenting no evidence of translocation of silicate clay, i.e. without Bt horizons, as those being studied; because the titanium oxides are observed to partially co-illuviate with the clay fraction towards the Bt-horizons (Chapman and Horn, 1968; Smeck and Wilding, 1980; Wagner, 2009). The Ti/Zr ratio figured out two main trends in the present study: (1) nearly constant values within the floodplain and middle terrace profiles and (2) gradual downward increase in the low terrace profiles. Similar values are obtained in the floodplain (except for the topsoil of P3) and subsoil of low terrace profiles (9 < Ti/Zr < 13), while the middle terrace soils share approximately the same values with the topsoil of low terrace profiles (4 < Ti/Zr < 10) (Table 1, Figure 4). This gives insight into the nature and origin of soil material in our study area.

We attributed the textural features at landscape scale and within soil profiles to mainly two different sources of soil material: marine and continental. Marine sediments compose the floodplain profiles (soil material) and the subsoil of low terrace profiles, while the colluvial material derived from continental uplands form the middle terrace profiles and the topsoil of low terrace profiles. The TCS in the low terrace profiles accounts for this colluvio-alluvial origin of sediments on this intermediate landscape position. Assess the contribution of each source appears however complex due to the possible mixing between allochtonous and materials derived *in situ* in soil profiles. This alters the normal pathway of soil development, making homogeneity tests of parent material in coastal areas more intricate. Determining the homogeneity of soils developed from unconsolidated materials in semiarid and arid regions poses serious problems because of different source areas and the pronounced episodic nature of deposition (Allen and Hajek, 1989).

Application of the Ti/Zr ratio gives nevertheless additional insights regarding the texture contrasts analysed above. It mainly confirms the presence of discontinuities in soil parent material on the low terrace site. These discontinuities may be, according to the available sedimentologic and geochemical data, located at ca. 38 cm depth in P4, 11 cm and 51 cm in P5, 4 cm and 82 cm in P6. They remain less pronounced in the floodplain profiles (23 cm in P1, 30 cm in P2, and 34 cm in P3); and were not identified in the middle terrace profiles (Figure 2, 3, and 4). This represents however a general distribution trend of soil material, because colluvial sediments may be identified on all landscape positions; their influence decreases seaward, being less significant on the floodplain location.

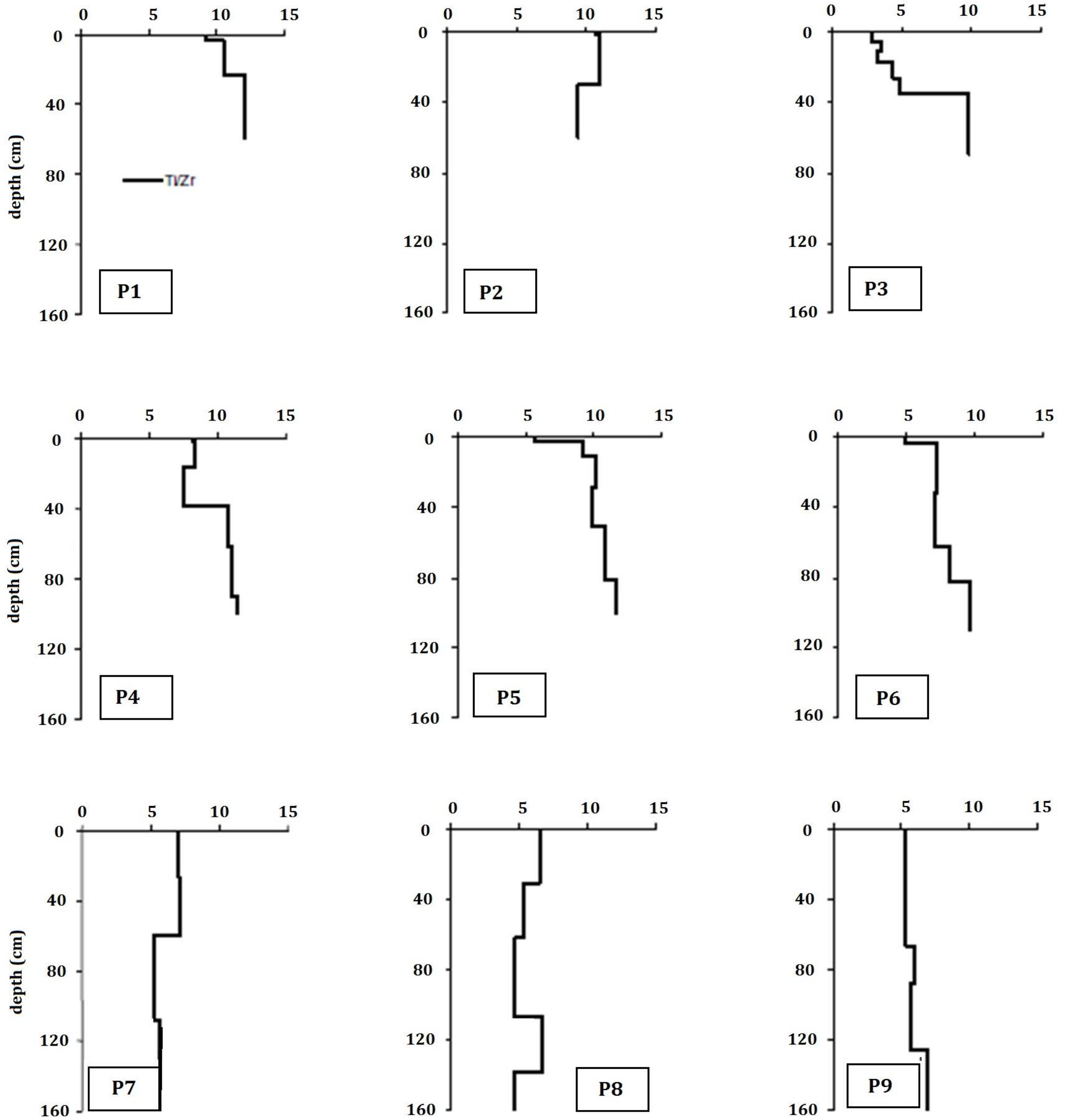

Figure 4. Depth function of Ti/Zr ratio in the floodplain (P1-P3); low terrace (P4-P6); and middle terrace (P7-P9) profiles

Conclusion

The present study demonstrates the presence of TCS in the coastal area of the Saloum river basin, west central Senegal, West Africa. We attributed the formation of these TCS to mainly two different sources of soil material: marine and continental. Marine sediments build the floodplain profiles and the subsoil of low terrace profiles, while the colluvial material derived from continental uplands form the middle terrace profiles and the topsoil of low terrace profiles. The TCS of the low terrace profiles accounts for this colluvio-alluvial origin of sediments on this intermediate landscape position. The main finding remains the influence of eolian dust inputs on the formation of TCS in this coastal area. Evidence of airborne addition suggested by particle size distribution was confirmed by the uniformity of soil parent material tested through mS/(cU+mU) and Ti/Zr ratios. They give insights into the origin and nature of sediments in the study area. Such knowledge enhances understanding of pedogenesis in coastal areas worldwide and may help develop sustainable soil management strategies.

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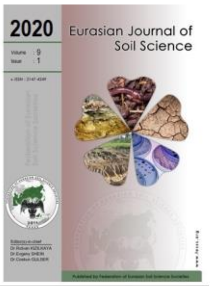

Quality and quantity of soil organic matter as affected by the period of organic farming in Sekem farm, Egypt

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Abstract

Sekem commercial organic farm was chosen for the present work; it is located at Belbeis 20 Km northeast of Cairo city which represented sandy soils. Five plots in Sekem farm were chosen to represent different periods of organic farming application, i.e. 0, 12, 15, 18 and 23 yrs. Surface (0-20 cm) and subsurface (20-40 cm) soil samples were collected in both winter and summer season. The collected soil samples were subjected to the dry sieve analysis to determine and separate the dry aggregate size of <0.25, 0.25-0.50, 0.50-1.00 and 1.00-2.00 mm diameter. The distributions of total organic carbon were studied in the whole soil and its aggregate fractions. The data showed that total organic carbon significantly increased by increasing the period of organic farming in the surface samples especially in the longest period of organic farming. Total organic carbon was concentrated in the finest aggregate fraction (<0.25 mm) for both summer and winter seasons and it was also increased by increasing the period of organic farming. The investigation of the humic and fulvic separates using infrared (IR) spectrophotometry, showed the dominance of carboxylic bands in fulvic especially in the subsurface soil samples which indicated its acidic function. Humic separates showed a relative increased in the intensity of aromatic bands as compared to fulvic separates with increasing the period of organic farming.

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Introduction

Egypt has a well-developed and still rapidly growing organic farming sector. About 26000 hectares are organically cultivated, accounting for 1.0% of total of agricultural area of Egypt. Organic production concentrates on about 500 farming enterprises. Most of these farms are desert farms irrigated from the Nile (IFOAM and FIBL, 2006).

Organic farming relies on building up soil organic carbon through crop rotation, green manure, compost to maintain soil productivity, excluding synthetic agrichemical. Organic farming protect the environment by building soil organic carbon and increase carbon sequestration (Bellows, 2002). Field experiments have shown that soil organic carbon content is controlled by change in management via the annual input of organic manures and wastes and the rate at which it decays (Mikha and Rice, 2004). Soil organic carbon contained a15% liable form while 85% is in the stable humified form (Pettit, 2000). The relative distribution of the soil humified organic forms i.e. humic and fulvic is mainly controlled by the period of organic farming. Consequently, the present in situ work aimed to investigate the effect of the long term organic farming on the distribution of soil organic carbon and their humic and fulvic characteristics using infrared technique in an organic enterprises at Belbies (SEKEM), Egypt.

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Material and Methods

Sekem farm started organic agriculture in 1977. The farm is located in Bilbeis (20 km north east of Cairo, 30^{o}09’09N and 31^{o}25’46E elev. 70 m). This farm was used as a model farm of the sandy soils in Egypt (the clay range was 3.1-10.2 %). The site of Sekem farms were presented in Figure 1. This farm contains 4 regions with different periods of organic farming practices. Organic farming started since 12, 15, 18, and 23 years for S_1, S_2, S_3, and S_4, respectively, with S_0 used as control (where no farming was initiated in this sector of the farm). The 12yr treatment, S_1, is planted with olive trees, for that, samples in this sector were collected from near trees (S_n) and between trees (S_b).Compost was applied to the soil at rate of 20 $m^3\,yr^{-1}Fd^{-1}$.

Sampling was carried out at the end of each growing seasons, in May and October, which represent the end of winter and summer seasons, respectively. Soil samples were collected from both surface soil layer (0–20 cm) and subsurface soil layer (20-40 cm). Each soil sample was a composite of five sub-samples. The soil samples were air-dried at room temperature, grounded to pass through a 2 mm sieve and stored prior to analysis.

Figure 1. Map of Northern Egypt showing the site of Sekem Farm

The collected soil samples were subjected to the determination of dry aggregate distribution, namely < 0.25mm, 0.25-0.50 mm, 0.50-1.00 mm and 1.00-2.00 mm, using mechanical shaker sieves based on the method of Whalen and Chang (2002).

Total organic carbon was determined in the whole soil samples as well as their dry aggregate separates as described by Walkely-Black method (Nelson and Sommers, 1982).

The classical method of fractionating soil humified organic carbon (HOM) is based on the extraction of organic carbon from soil with caustic alkali and the further subdivision of the extracted material by partial precipitation with mineral acids, and by partial solubility in alcohol. The following groups were obtained: the "humin fraction," insoluble in alkali; the "humic acid fraction," soluble in alkali, insoluble in acid; the "fulvic acid fraction," soluble in alkali, soluble in acid (Hayes, 2006).

After the purification, dried humic and fulvic acid were subjected to the FT-IR examinations by a JesCo FTS 460+ single beam using the KBr technique (2 mg sample in 150 mg KBr) (Celi et al., 1997; Eleerbrock et al., 1999) to obtain absorption spectra of both acids extracts (range of wave number 3900-400 cm^{-1}). All spectra were obtained under the same resolution ($1cm^{-1}$) and measurement conditions.

Results and Discussion

Distribution of soil organic carbon as affected by organic farming periods

Table 1 showed an increase in total organic carbon (TOM) with the increase in cultivation period in Sekem farm for both surface and subsurface layers in winter and summer seasons. The magnitude of TOM increase was much higher in the surface layer, as it reached after 23 yrs of cultivation almost 18 times that in the beginning, while it was only 5 times in the subsurface layer after 23 yrs of cultivation, due to the surface application of organic compost and plant residues. The application of organic manures for ten years caused superior increses in total SOC as compare to the applications of n and P fertilizers (Mikha and Rice, 2004).

Table 1. Total organic carbon changes in surface and subsurface with different periods of organic farming in winter and summer seasons in Sekem farm.

Season	Depth (cm)	S_0	S_1	S_2	S_3	S_4
				mg/kg		
Winter	0-20	560	6132	7203	8119	10789
	20-40	734	1162	1630	1348	3956
Summer	0-20	579	5978	7122	5885	10380
	20-40	767	1032	2516	2482	3894

S_0= control; S_1= 12 yrs; S_2= 15 yrs; S_3= 18 yrs and S_4= 23 yrs.

Rochette and Gregorich (1998) stated that at the end of the growing season @ 50% of carbon of the applied organic manure was remained in soil. Soil Organic carbon could be doubled by annual application rate of 2.0 tons carbon from organic amendments (Gerzabek et al., 2001).

Buildup of soil carbon could only be attained when Carbon input is larger than decomposition rate. Soil management processes such as continuous cropping, cover crop and or rotation includes perennial leguminous crops as well as continuous addition of manures certainly guarantee carbon buildup in the soil. Grant et al. (2001) stated that two year rotation was not sufficient to raise SOC in the uppermost topsoil (15 cm depth) in the absence of organic manuring.

Relationship between long term organic farming and TOM was illustrated in Figure 2. Total organic carbon (TOM) was highly significant relationship related with time for Sekem soil (R^2=0.996 and 0.908 for winter and summer seasons, respectively). High R^2 was also observed for the subsurface layer (0.782 and 0.851 under winter and summer seasons, respectively. This relative decrease in R^2 was due to the lower amount of organic compost and plant residual that could reached the subsurface layer.

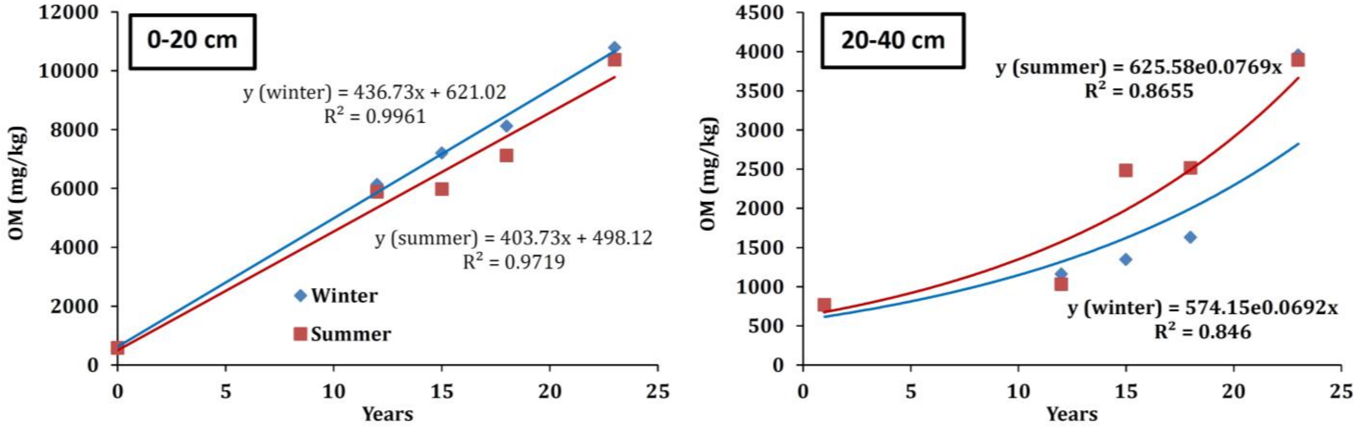

Figure 2. Relationship of OM with time in Sekem farm for winter and summer seasons in surface (0-20 cm) and subsurface (20-40 cm) layers

Distribution of TOM in dry sieve separates with time

Figure 3 represents TOM distribution in dry sieve separates for Sekem farm which indicated that the highest TOM content was recorded in the finest fraction (<0.25 mm) and it increased with increasing period of organic farming. On the other hand, the coarse separates (1.0-2.0 mm) contained the lowest TOM %. This trend was consistently found in winter and summer seasons for the surface and subsurface layers of Sekem farm. Desjardins et al. (1994) confirmed that C_{org} concentrations increased with decreasing soil particle size. In addition, Aoyama et al. (1999) stated that particulate and mineral-associated organic carbon in water-stable aggregates were increased by increasing the rate of organic manure applications.

This could be due to that the smallest size separate has the largest quantity of clay size particles which indicated that organic compounds had been stabilized through the formation of strong clay-organic complexes (Gonzalez and Laird, 2003). In addition, the complexation of light molecular weight (rapid decomposable) organic compounds by clay and silt soil particles also maintain its stability in the soil (Sørensen, 1972). Gerzabek *et al.* (2001) stated that siltsoil particles had medium capacity to retain soil organic carbon while clay ones slowed down OC turnover rates as it shows the maximum capacity for fixing organic compounds (Buyanovsky et al. 1994).

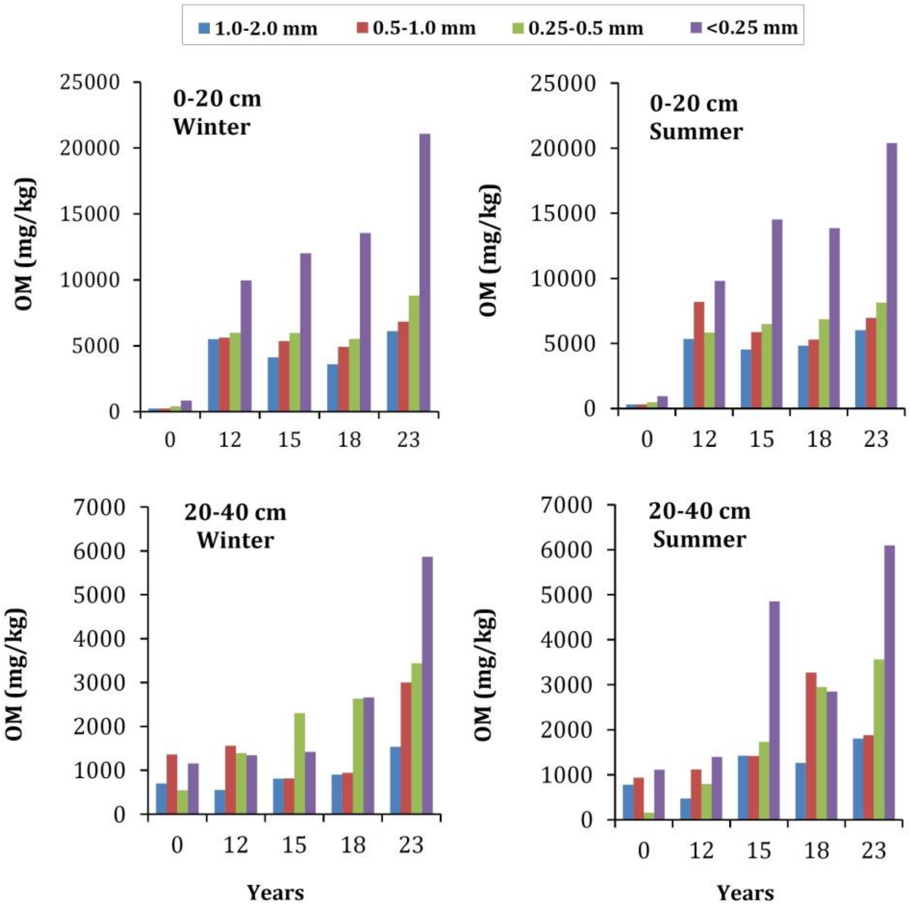

Figure 3. Distribution of OM through dry sieve separates for Sekem in surface and subsurface samples under winter and summer seasons.

Infrared (IR) investigation of humus fractions in Sekem organic farm as affected by organic farming periods

Humic and fulvic acids were separated from the surface (0-20 cm) and subsurface (20-40 cm) soil samples collected from Sekem (23 yrs) and the compost that continuously used as an organic fertilizer. The obtained humic and fulvic acids separates were subjected to IR examination which could be discussed as follows:

IR-characteristics of fulvic acid separate from Sekem farm

Figure 4 represents IR-spectra of fulvic acid separates and the main absorption bands are presented in Table 2. IR-bands were assigned according to Sánchez-Monedero et al. (2002). IR-spectra of fulvic acid exhibit a broad band at 3459-3437 cm^{-1} (stretching of O-H bonds), medium band around 2361-2315 cm^{-1} (stretching of H-N bonds). A well defined band around 1640 cm^{-1} was assigned to aromatic C=C stretching, C=O of ketones and carboxylate ions. A couple of peaks around 1430 cm^{-1} due to aliphatic C-H stretching and peaks around 1030 cm^{-1} due to C-O stretching bond of polysaccharides were also assigned.

Table 2. Corrected relative transmission (T%) for the main IR bands of fulvic acid separates from Sekem organic farm

Bond	compost		Surface (0-20 cm)		Subsurface (20-40 cm)	
	band	T% $_{Corr.}$	band	T% $_{Corr.}$	band	T% $_{Corr.}$
			Sekem FA 23 years			
OH st.*	3455	7	3437	2	3439	1
H-N st.	2361	96	2317	90	2318	75
C=C st.	2066	95	2069	85	2087	77
	1868	98	-	-	-	-
C=C	1639	48	1638	25	1638	17
	1517	87	1520	90	-	-
C-H aliph.**	1458	91	1460	85	1459	89
COO st.	1420	92	1400	86	1400	88
KBr	574	55	552	32	572	26

*st.= Stretching band and **aliph. = Aliphatic band.

Relative intensities (transmission T %) of the obtained IR-bands had been corrected to 100% for all IR-spectra in order to make comparisons among them. Table 3 represents the corrected transmission (T %) of IR-bands for compost, surface and subsurface fulvic acid separates. It is well known that the absorbance (A) not transmittance (T %) which expressing the intensity of a specific bond in the examined complex according to Beer-Lambert law (Robincon, 1996). This means that the higher the T% the lower the absorbance and the lower the intensity of the relevant band. Therefore, the obtained intensity differences showed that fulvic acid separates had higher contents of aliphatic and carboxylic H-bonded C=O and amides. The higher content of aliphatic and carboxylic groups in compost fulvic acid separates was widely reported in the literature (Gigliotti et al., 1990), which indicated the higher acidity nature of fulvic acid separates. With respect to the relative transmission (T%) of the IR-bands around 2069 and 1640cm^{-1} (aliphatic C=C and carboxylic C=O stretching), it was decreased from 96 and 48 % in Sekem compost to 90 and 25 % in the surface layer then reached 75 and 17 %, respectively, in the subsurface layer. This indicated that aliphatic C=C and carboxylic C=O bonds had the lowest absorption intensity in Sekem compost-FA then increased in the surface-FA while the highest intensity of these groups was found in FA separates from subsurface layer. This could be attributed to the progress of FA polymerization which increased double bond intensity through the advancement of the compost decomposition process (Plaza et al., 2007; Abouelwafa et al., 2008). In addition, absorption intensity of carboxylic band was much higher than that of aliphatic C=C bond which indicated the acidic nature of fulvic acid. On the other hand, the intensity around 1520 cm^{-1} (aromatic C=C), 1460 cm^{-1} (aliphatic C-H) and 1420 cm^{-1} (COOH stretching) were slightly changed which indicated that the proportions of these bonds didn't show major differences in Sekem compost and amended soil.

IR-characteristics of humic acid separated from Sekem farm

Figure 5 represents IR-spectra of humic acid separates from Sekem compost, soil surface and subsurface soil layers. IR-spectra of humic acid separates were significantly different from these of FA separates. Hydroxyl O-H stretching was shifted to around 3405 cm^{-1}, new aliphatic COOH group was developed at 2927 cm^{-1}, and new aromatic C-C and C-O-C bonds were developed at 1227 cm^{-1} and 1033 cm^{-1} bands. Additional group of aromatic bands were developed at 1628 cm^{-1} (C=C aromatic), and 1513 and 1415 cm^{-1} (C-H aromatic).

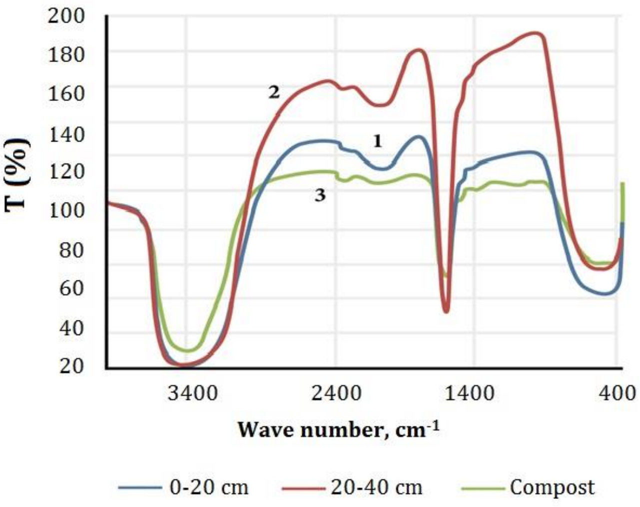

Figure 4. IR-spectra of fulvic acid separates of compost, surface (0-20 cm) and subsurface (20-40 cm) layers of Sekem organic farm cultivated for 23 yrs.

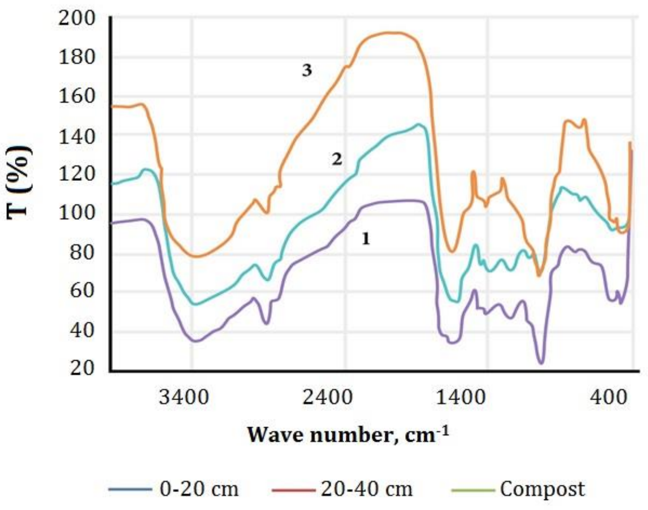

Figure 5. IR-spectra of humic acid separates of compost, surface (0-20 cm) and subsurface (20-40 cm) layers of Sekem organic farm cultivated for 23 yrs.

Regarding the relative intensities of the HA IR-spectra, Table 3 showed a relative increase in the intensity of the aromatic bands (from 16 to 50%) which indicated the abundance of aromatic compounds in humic acid separates as compared to fulvic acid separates which is in agreement with finding of Lguirati et al. (2005) and Guignard et al. (2005).

The relative intensity of OH bond (phenyl and alcohol) was the highest in Sekem compost then decreased in the surface and subsurface soil layer which indicated the polymerization to form humic acid. The relative intensities of HA separates was significantly higher in Sekem compost compared which may confirmed by the high content of stable OM% in Sekem by increasing the period of organic farming.

Table 3. Corrected relative transmission (T%) for the main IR bands of humic acid separates from Sekem organic farm

bond	compost		Surface (0-20 cm)		Subsurface (20-40 cm)	
	band	T% Corr.	band	T% Corr.	band	T% Corr.
			Sekem HA 23 years			
OH st.*	3404	14	3406	34	3405	28
Ali. C-H st.	2929	31	2927	42	2927	38
N-H_2 st.	2367	86	2368	88	-	-
C=N, C=O	-	-	1706	36	-	-
C=C aro.**	1648	16	1628	32	1626	28
	-	-	1513	50	-	-
CH_2 aliph.	1456	36	1456	48	1455	44
C-H aro.***	1386	34	1415	47	-	-
	-	-	1227	45	1227	42
C-O-C aro.	1031	8	1033	23	1033	42
KBr	522	27	525	52	531	58

* st.= Stretching band; **aliph. = Aliphatic band and *** aro. = Aromatic band

In conclusion, total organic carbon TOM significantly increased by increasing the period of organic farming in the surface samples especially in the longest period of organic farming. Total OM was concentrated in the finest aggregate fraction (<0.25 mm) for both summer and winter seasons and it was also increased by increasing the period of organic farming. The investigation of the humic and fulvic separates using infrared (IR) spectrophotometery, showed the dominance of carboxylic bands in fulvic especially in the subsurface soil samples which indicated its acidic function. Humic separates showed a relative increased in the intensity of aromatic bands as compared to fulvic separates with increasing the period of organic farming.

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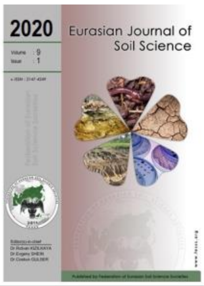

Estimating the long term leaching potential of PCBs in soil

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Abstract

Polychlorinated Biphenyls (PCBs) are a group of man-made organic chemicals known as chlorinated hydrocarbons. PCBs were banned in 1979 due to its toxicity and persistence in the environment. Variations of PCBs were determined in soils and plants. Degradation of PCB Aroclors and congener patterns were investigated from known contaminated site at a former Military Air Base. PCB concentrations ranged between 17 - 1,040 µg L^{-1} at 0 - 0.5 m depth, between 36 - 898 µg L^{-1} at 1.0 - 1.5 m depth, between 20 - 73 µg L^{-1} at 4 m depth and 21 µg L^{-1} at 8 m depth. There is also a negative correlation between the mean of the Toxicity Characteristic Leaching Procedure (TCLP) of PCBs versus the sampling depth. Linear regression analyses were used to determine the correlation of the factors such as depth, Octanol/Water Partition Coefficient (K_{ow}) and solubility affected Soil/Water Distribution Coefficient (K_d). Effects were found only when the soil depth is at 0.3 - 0.5 m (at 95% confidence level). K_d is positively correlated with K_{ow} while K_d has some level of negative correlation with solubility. No effect was found at 1.0 - 1.5 m, 4.0 m, and 8.0 m. Organic Carbon/Water Partition Coefficient (K_{oc}) showed high results exceeding the log K_{oc} criteria of 4.5; as a result, this is now a matter of concern on the potential adverse effects of the substance on terrestrial organisms within its vicinity.

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Introduction

Polychlorinated biphenyls (PCB) are a class of 209 congeners with isomers of chlorinated biphenyls exhibiting varying degrees of chlorination. These organic chlorine compounds have been widely used worldwide since the 1930s in capacitors, transformers, flame retardants, inks, and hydraulic fluids due to their thermal and chemical stability, resistance to alkalis and acids, low flammability, and low electrical conductance (Harrad et al., 1994; Hansen, 1999; Bozlaker et al., 2008; Zhang et al., 2011).

PCBs used for industrial and commercial purposes are known by different names in several countries, e.g., Kanechlor (Japan), Sovol (USSR), Fenchlor (France), Chlophen (Germany), and Aroclor (United States). In particular, Aroclor had a PCB composition with chlorine content comprised of penta- to octa- chlorinated congeners (Hutzinger et al., 1974; US EPA, 1996; ATSDR, 2000). Specifically, Aroclor contains congeners comprising of 12% penta-chloro biphenyls (CBs), 42% of hexa-CBs and 38% hepta-CBs, 7% octa-CBs, and 1% nona-CBs (US EPA, 1996; ATSDR, 2000).

Concerns on PCB contamination in the environment and on human health surfaced during the 1960s, which led to countries scaling down their PCB production and its eventual discontinuation worldwide in the late 1970s (Hansen, 1999; Fang et al., 2017). Eventually, PCBs were considered and listed as one of the 12 Persistent Organic Pollutants (POP) under the Stockholm Convention in 2001 due to its persistence and ease

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in being spread across environments and ecosystems, as well its potential for bioaccumulation and biomagnification (IARC, 2016; Nguyen et al., 2016; Adeyinka and Moodley, 2018).

Due to the lipophilicity and hydrophobicity of these compounds, PCBs are notably stable, persistent, and have long half-lives that promote accumulation in the environment, preferentially in organic matter and lipids (Notarianni et al., 1998; Malina and Mazlova, 2017; Neira et al., 2018). Despite being out of production for several decades, PCBs are still being detected in various environmental media in high concentrations such as in soils (Girvin and Scoot, 1997; Armitage et al., 2006; Fu et al., 2009; Zhang et al., 2011; Melnyk et al., 2015), marine sediments (Vane et al., 2007; Wang et al., 2010; Neira et al., 2018), atmosphere (Choi et al., 2008; Fu et al., 2009; Baek et al., 2010), and water (Harrad et al., 1994). PCBs released and stored in the soil and water media may eventually be distributed and transported widely across environments through local and long-range atmospheric processes, ultimately entering into the food chain (Wania and Mackay, 1996; Nguyen et al., 2016; Malina and Mazlova, 2017).

Although PCBs partition in soil, water, and air media, numerous studies (Cetin et al., 2007; Cetin and Odabasi, 2007; Bozlaker et al., 2008; Glüge et al., 2016) suggest that atmospheric processes remain the main driver in the mobility and deposition of these persistent organic pollutants; especially in soils and sediments. Volatile biphenyls, particularly highly-chlorinated congeners with chlorine atoms > 4 released through air-soil exchange, are easily adsorbed onto organic matter (Hansen, 1999; Cetin et al., 2007; Malina and Mazlova, 2017). Hites and Eisenreich (1987); Steuer et al., (1999) and Malina and Mazlova (2017) reported that these highly-chlorinated biphenyls were sorbed to dust particles in the atmosphere, clay and oxide surfaces, and on suspended solids in water (Wang et al., 2008; Badea et al., 2014).

More specifically, PCBs are known to have very low water solubility and high Octanol/Water Partition Coefficient (K_{ow}), causing strong sorption in soils (Badea et al., 2013). Its solubility decreases as chlorine content becomes higher or chlorinated biphenyls increase. The physical and chemical properties of soil, prevailing weather conditions, and age of contamination affect the varying degrees of PCB contamination in soils (Badea et al., 2013; 2014).

The mobile fractions of PCBs in soil can be evaluated by determining various leaching tests. Toxicity Characteristic Leaching Procedure (TCLP), developed by the United States Environmental Protection Agency (US EPA), estimates the inorganic and organic contaminants of solid wastes that may pose potential hazard to health and environment (US EPA, 1992). Toxicity assessments from these hazardous contaminants recommend the use of TCLP. According to US EPA (1992), this method identifies contaminants present in leachate and their concentrations as TCLP simulates the conditions in landfill over time. This correlates to contaminants present in soil in a period of time with slow movement through external influence of rainwater and slow degradation from sunlight. For a solid or hazardous waste that is 100% physically solid, the maximum theoretical leachate concentration is 5% of the total concentration in the waste (US EPA, 1992).

Elutriate test measures and predicts the release of contaminants in water column (Vicinie et al., 2011). The Soil/Water Distribution Coefficient (K_d) is used to calculate the distribution of contaminants between the soil and leachates. (Badea et al., 2013; 2014) and therefore also used to estimate risks of contamination in groundwater and surface water.

This paper presents the leachate concentrations of PCBs from soil collected in August 2013, 2014, and 2017 in the study area. Partitioning and hydrophobicity of leachate properties of PCBs in soil at different depths were analyzed and studied. The purpose was to examine the patterns of leachability of PCBs as to horizontal and vertical migration and trends including the PCB weathering of Aroclor and congener profiles in the natural environment with respect to time. Furthermore, this research seeks to affirm low leaching rates of highly chlorinated Aroclors and congeners in relation to low water solubility and high octanol/water coefficient in the immediate environment.

Material and Methods

Study Site

The study area is located at the Wagner Aviation, Clark Freeport Zone, Pampanga, Philippines (15°10'14.44"N, 120°32'39.18"E) (Figure 1), which was part of the former Clark Air Base ran by the United States military until 1991. There were 20 PCB transformer units stored in Wagner Aviation in 1997 (CDC-EPMD, 1997). Each transformer unit at Wagner Aviation had an oil capacity of 757 L and a rating of 225 kVA (Weston International, 1997). One unit tested had a PCB Aroclor 1260 concentration of 62,540 mg kg^{-1} based on records of the US Air Force on March 18, 1991.

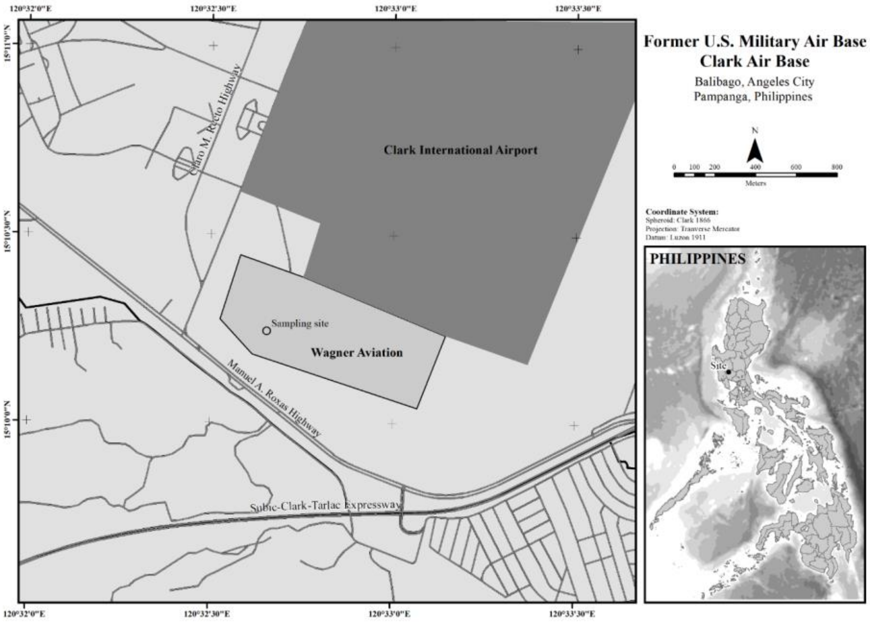

Figure 1. PCB Transformer sampling site at former US Military Air Base, Angeles City, Philippines

In 2002, it was reported that some of the transformer units at the Wagner Aviation site were missing. Moreover, PCB-laced oils have spilled into the underlying soil. Several tests and surveys were done on the soil and surface material of the site. Results of PCB concentrations at Wagner Aviation were 7,800 mg kg^{-1} (Weston International, 1997), 7,200 mg kg^{-1} (Harvard University-School of Public Health cited in CDC-EPMD (1997) and 4,400 mg kg^{-1} (ARRPET-De La Salle University cited in CDC-EPMD (1997) exceeding the US EPA Risk Based Concentration for industrial areas of 10 mg kg^{-1}.

Sample Collection

Samples were collected in the site with an area of approximately 9 sq. m. Surface and sub-surface soil sampling were done in accordance with standard protocols. Soils were collected at sampling depths of 0.3m, 0.5m, 1.0m, 1.5m, 2.0m, 4.0m, and 8.0m. on August 2013, August 2014, and August 2017. Physico-chemical parameters (Table 1), measured were pH, moisture, organic carbon, and particle size for collected surface soils. Grain size distribution within the contaminated area was found to be 25% gravel, 60% sand, and 15% silt with negligible clay fractions (Figure 2, sample 1).

Table 1. Moisture content and pH of soils

Sampling Location	Sampling Depth	Date	pH					% Moisture					Average moisture (%)
			Test 1	Test 2	Test 3	Test 4	Test 5	Test 1	Test 2	Test 3	Test 4	Test 5	
Within the contaminated area	0.5 meters	08.2013	6.85	7.15	-	-	-	14.75	17.99	-	-	-	16.37
		08.2014	6.96	5.02	4.73	4.24	5.12	15.68	21.40	16.67	20.05	16.31	18.02
		08.2017	6.63	6.62	6.33	6.27	-	29.31	22.57	23.19	21.93	-	24.25
	1.0- 1.5 meters	08.2013	7.89	6.92	-	-	-	23.61	24.29	-	-	-	23.95
		08.2014	4.90	5.04	-	-	-	21.11	29.88	-	-	-	25.50
		08.2017	6.69	6.58	6.59	-	-	17.28	18.16	14.69	-	-	16.71
	4.0 meters	08.2013	7.16	7.08	7.16	-	-	12.65	17.49	16.51	-	-	15.55
	8.0 meters	08.2013	6.83	-	-	-	-	12.68	-	-	-	-	12.68

Toxicity Characteristic Leaching Procedure (TCLP) (US EPA Method 1311) tests were done by mixing the soil thoroughly and 50 - 70 g subsamples for each test were collected. A rotary agitation instrument was used and samples were rotated at 30 ± 2 rpm for 18 ± 2 hours. Samples were filtered and extracts were collected using US EPA Method 3510C (Separatory Funnel Liquid/liquid extraction method). The average weight of each sample used was 60 g filled to a total 1 L filled with an extraction fluid. The extraction fluid was made by mixing 5.7 mL glacial CH_3CH_2OOH, added to 600 mL distilled water, and finally added with 64.3 mL of 1N NaOH and diluted to a volume of 1 L. Surrogate recovery standards containing Decachlorobiphenyl and Tetrachloro-m-xylene were spiked on each sample prior to extraction. Extracts were concentrated into 5 mL using a Zymark Concentrator. Clean-up using US EPA Method 3630 Silica Gel method was employed after every sample extraction procedure.

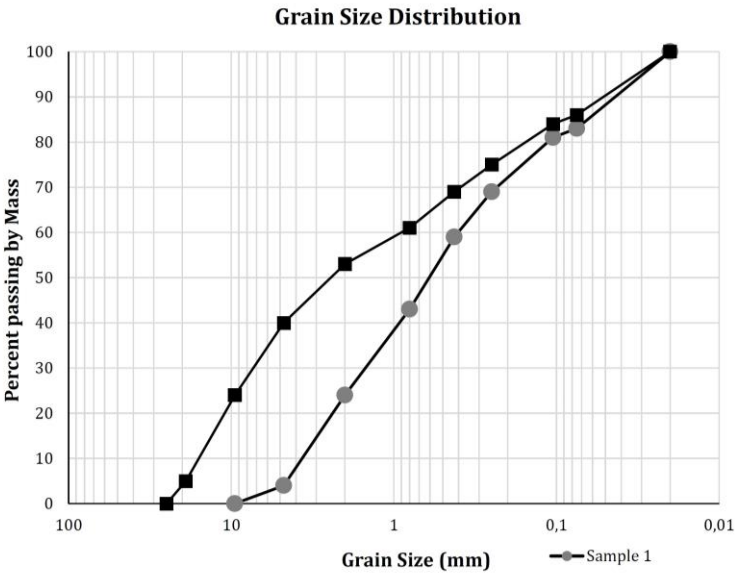

Figure 2. Grain size distribution of soils in Wagner Aviation, Angeles City, Philippines

Gas Chromatography coupled with dual Electron Capture Detector (GC-ECD) - Hewlett Packard 5890 Series II/Agilent 7890B was used in the quantitation of PCB Aroclors and PCB Congeners. Nine Aroclors were selected and analyzed. Around 19 PCB congeners were quantitated based on the US EPA list.

An elutriate test was performed by mixing one part of soil with four parts of water from the area allowing the soil/water mixture to settle, filtering the supernatant and measuring the release of contaminants in the elutriate or filtered supernatant. The filtered supernatant was extracted using US EPA method 351^{0}C and cleaned up using US EPA method 3630. Detection limit using GC-ECD for PCBs in TCLP and elutriate aqueous extracts were 1.0 µg L^{-1} for Aroclors and 0.25 µg L^{-1} for total congeners.

Computational methods and statistical analyses

Descriptive statistics such as mean, range, and standard deviation were computed for each sample collected at different depths. Calculation of soil-water distribution coefficients used the following equation:

$$K_d = C_s / C_w$$

where, C_s and C_w are the concentrations of a given compound in the soil and aqueous phase, respectively.

Calculation of soil-water distribution coefficients used the following equation:

$$K_{ow} = C_o / C_w$$

where, C_o and C_w are the concentrations of a given compound in octanol and aqueous phase, respectively.

Log10 K_d was then used to normalize its distribution. The relationships between K_d to K_{ow} and solubility of the samples were analyzed through two-factor, simple linear regression between Log K_d vs Log K_{ow} and Log K_d vs Solubility; grouped for each soil depth.

Results and Discussion

Characterization of PCBs in surface soils

Total organic carbon was <0.0001% in all soil samples collected. The low Total Organic Carbon present was a result of the dominantly sand-silt grain composition of the mid to upper horizon soils deposited during the June 1991 Mt. Pinatubo volcanic eruption (Paladio-Melosantos et al., 1996). The eruption of Mt. Pinatubo, located 20 km southwest of the study area, deposited a blanket of silt to fine-grained sand sized ashfall, derived chiefly from andesitic to dacitic magma, over extensive areas surrounding the volcano. Thick beds of volcaniclastic sediments were deposited over the area as heavy rains brought by Typhoon Yunya caused the mobilization of ash and other volcanic products as lahar and mudflows tens of kilometers around the volcano (Paladio-Melosantos et al., 1996).

Figure 3 shows the historical trends of total PCBs from 2013 to 2017. Not all PCB Aroclors were detected except for Aroclor 1260, which was positively detected in all soil depths sampled. Table 3 shows the results of TCLP in soils. The degree of PCB concentrations is expectedly inversely correlated to depth. PCB concentrations range between 17 and 1,040 µg L^{-1} at 0 - 0.5 m depth, between 36 and 898 µg L^{-1} at 1.0 - 1.5 m depth, between 20 and 73 µg L^{-1} at 4.0 m depth, and 21 µg L^{-1} at 8.0 m depth. This suggests that the transport of PCBs to soil is fractionated and mobility is poor (Girvin and Scott, 1997).

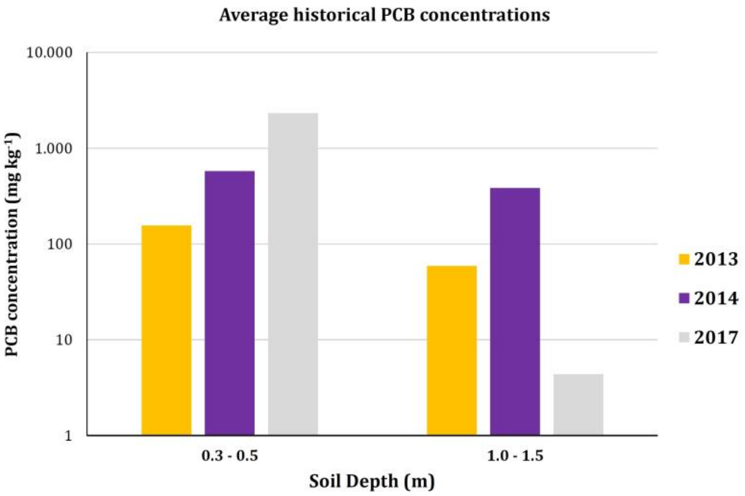

Figure 3. Historical trends of average PCBs from 2013 to 2017 at Wagner Aviation, Angeles City, Philippines

The PCB screening value of the Philippine Department of Environment and Natural Resources (DENR) for soil is 1 mg kg^{-1} and 0.74 mg kg^{-1} for Dutch Intervention Value (DIV) and US EPA Regional Screening Values (Industrial), respectively. PCBs in soil were assessed because they exceeded these screening values. The risk for ingestion from groundwater through leachates was evaluated based on groundwater level. According to groundwater guidelines of DENR Tier 1 Screening Values and Dutch Standard Intervention, total PCBs should not exceed above 0.017 μg L^{-1} and 0.01 μg L^{-1}, respectively. PCBs leachates in all surface and subsurface soils significantly exceed the required screening and intervention values. Based on this screening and intervention standards, the area is heavily polluted with PCBs. Since the site was contaminated with PCBs for almost three decades, this strongly suggests that PCBs are persistent and have poor degradation to sunlight and organisms. It is also noted that Aroclors with larger chlorine composition, particularly Aroclor 1260, are present in the soil and have leached out significantly even if it is naturally liphophilic. As a result, PCB leachates indicate a large risk for groundwater contamination. Unfortunately, there has not been a detailed study on PCB contamination of the groundwater in the area.

Different leaching tests were done to confirm the leaching potentials of PCBs using the congener approach. This test has a short exposure to water from contaminated soil compared to TCLP exposure at 18 hours. Table 3 shows the results of elutriate tests. Hexa-CBs and Hepta-CBs predominantly showed the highest concentrations among all other CBs.

PCB concentrations in the soil profile succession as illustrated in Figure 4, show a distinct distribution pattern. The topsoil is dominated by highly chlorinated biphenyls: 6Cl, 7Cl, and 9Cl, accounting for more than 75% of the total concentration of PCBs. As depth increases however, less chlorinated biphenyls such as 4Cl and 5Cl begin to increase in proportion relative to the total amount of PCB. At 0.3 to 0.5 m depth, K_d is positively correlated with K_{ow} while K_d is inversely correlated with solubility as shown in Figure 5. The same trend was not observed in all other sampling depths.

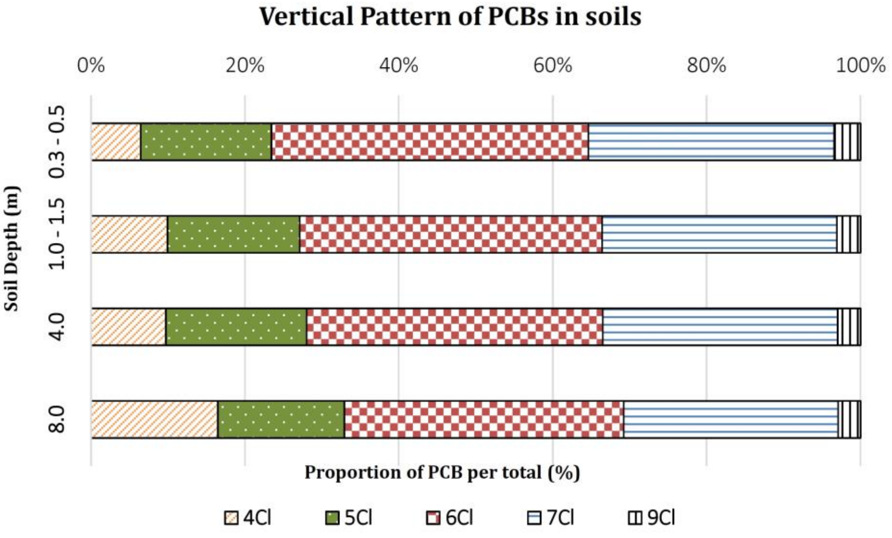

Figure 4. Depth distribution of PCB concentrations in the soils at the Wagner Aviation site

The graphical regression graphs of K_{ow} and Solubility with K_d are shown in Figure 5 and the tabulated statistical results in Table 4. Regression analyses show that the near surface layer (0.3 - 0.5 m layer) is the most susceptible soil profile to contamination as expected. Take note that although there are changes in the pattern, the relationship was not significant, likely because of lack of data points or other factors that changed the concentration of the chemicals being analyzed.

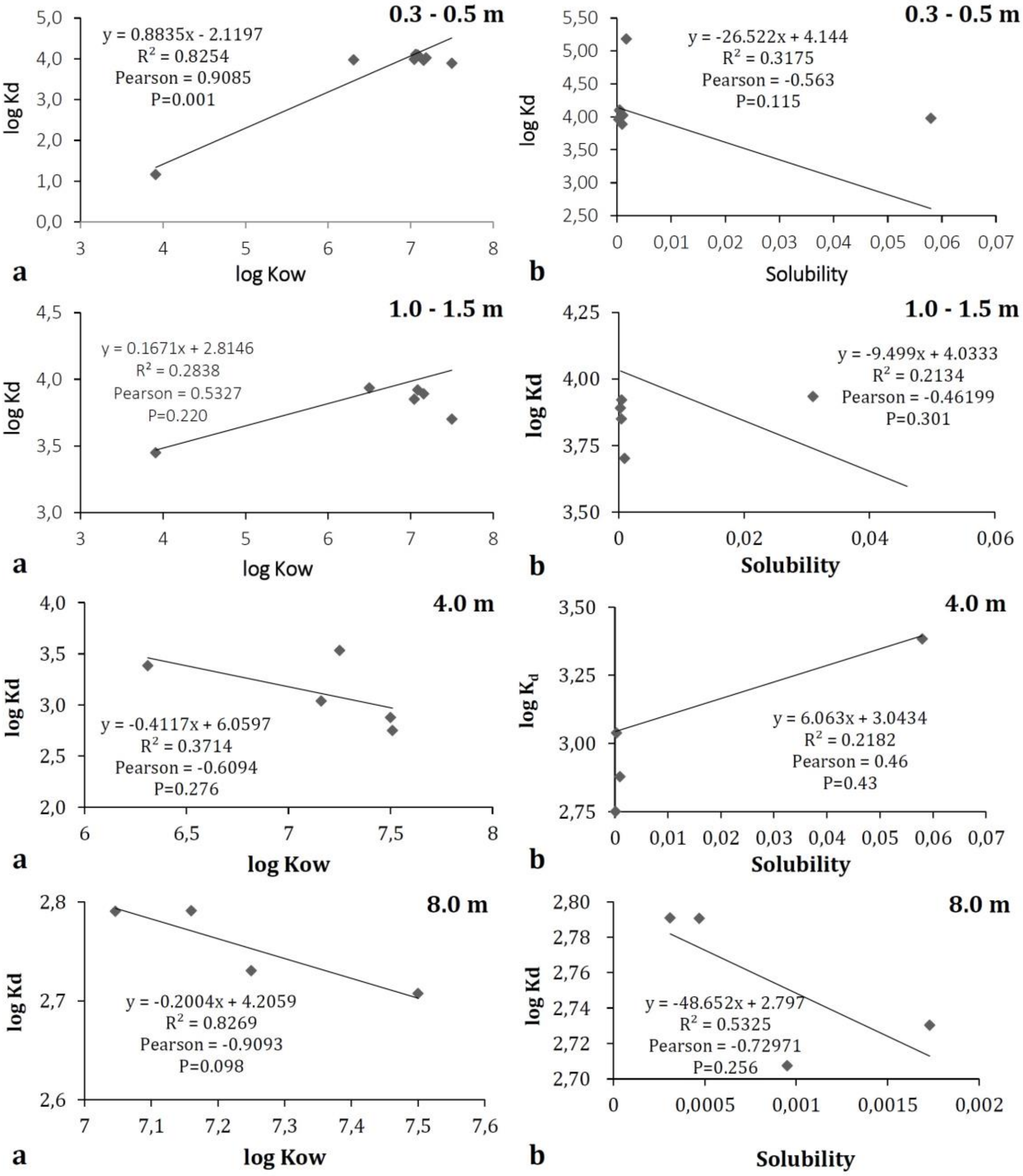

Figure 5. Linear relationship between (a) Kow vs Kd and (b) solubility vs Kd at different soil depths.

This pattern reflects the expected behavior of these PCBs in the soil media. Highly chlorinated biphenyls are lost due to eventual degradation and volatilization in deeper soil profiles and are transported upwards to more surficial soil layers; confirming the same observations found by other researchers (Backe et al., 2004; Wang et al., 2010; Zhang et al., 2011).

Relationships of Organic Carbon, K_d and K_{oc} in soil leachates

The relationship among Organic Carbon/Water Partition Coefficient (K_{oc}), K_d, and Organic Carbon was statistically determined based on the following:

$K_{oc} = (K_d * 100) / \%$ Organic carbon

where the % Organic carbon is = 0.0001

Figure 6 shows the relationship of the two variables based on the equation. All readings show very high K_{oc} of a substance and exceed the log K_{oc} criteria of 4.5. If log K_{oc} is greater than 4.5, this means that there should be significant concerns on the potential adverse effects of the substance on terrestrial organisms.

Table 2. Elutriate Results of PCB concentration as Congeners, μg L^{-1}

2013 PCB Congeners	0.3-0.5 meters		1.0-1.5 meters		4.0 meters		8.0 meters	
	Average	Range	Average	Range	Average	Range	Average	Range
2,5,2',5'-Tetrachlorobiphenyl (BZ # 52)	0.03	0 ~ 0.08	0.09	0 ~ 0.20	0.04	0 ~ 0.12	0.08	0 ~ 0.14
2,3',4,4'-Tetrachlorobiphenyl (BZ # 66)	1.06	0~ 3.2	0.00	-	0.28	0 ~ 0.85	-	-
2,2',4,5,5'-Pentachlorobiphenyl (BZ # 101)	1.18	0 ~ 3.5	0.43	0 ~ 1.3	-	-	-	-
2,2',3,4,4',5'-Hexachlorobiphenyl (BZ # 138)	0.28	0 ~ 0.83	0.21	0 ~ 0.64	0.50	0 ~ 1.2	0.78	0 ~ 2.3
2,2',3,4,5,5'-Hexachlorobiphenyl (BZ # 141)	1.45	0 ~ 4.3	0.00	-	-	-	-	-
2,2',4,4',5,5'-Hexachlorobiphenyl (BZ # 153)	5.12	0 ~ 14	2.10	0.06 ~ 5.6	2.26	0 ~ 5.5	0.85	0 ~ 2.2
2,2',3,3',4,4',5-Heptachlorobiphenyl (BZ # 170)	1.39	0 ~ 4.2	0.00	-	-	-	-	-
2,2',3,4,4',5,5'-Heptachlorobiphenyl (BZ # 180)	4.97	0 ~ 14	1.62	0 ~ 4.8	1.88	0 ~ 4.5	0.73	0 ~ 2.2
2,2',3,4,4',5',6-Heptachlorobiphenyl (BZ # 183)	0.99	0 ~ 3.0	0.39	0 ~ 1.2	-	-	-	-
2,2',3,4',5,5',6-Heptachlorobiphenyl (BZ # 187)	2.52	0.13 ~ 7.0	0.91	0 ~ 2.7	-	-	0.44	0 ~ 1.2
2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl (BZ # 206)	-	-	-	-	0.19	0 ~ 0.56	-	-
Total PCBs	18.99	0.13~54.11	5.75	0.06~16.44	5.15	0.0-12.73	2.88	0.0-8.04

Table 3. Average log K_d (L kg^{-1}) values obtained for target PCBs from surface to subsurface soils.

PCBs	Elutriate Results of PCBs, μg L^{-1} (CW)				Concentrations in Soil, μg kg^{-1} (CS)				K_d, L kg^{-1}				(Mackay et al., 2006)	
	0.3-0.5 m	1.0-1.5 m	4.0 m	8.0 m	0.3-0.5 m	1.0-1.5 m	4.0 m	8.0 m	0.3-0.5 m	1.0-1.5 m	4.0 m	8.0 m	log K_{ow}	Solubility, μg L^{-1}
PCB 52	0.03	0.09	0.04	0.08	0.4	256	-	-	14.52	2,808	-	-	3.910	0.046000
PCB 66	1.06	0.00	0.28	-	10,145	4,076	686	283	9,530	-	2,422	-	6.310	0.058000
PCB 101	-	0.43	-	-	13,328	3,733	643	141	-	8,611	-	-	6.500	0.031000
PCB 138	0.28	0.21	0.50	0.78	41,887	11,061	1,718	419	151,962	51,816	3,419	537.64	7.250	0.001730
PCB 141	1.45	0.00	-	-	15,309	4,004	635	141	10,553	-	-	-	7.190	0.001090
PCB 153	5.12	2.10	2.26	0.85	39,836	10,574	1,711	374	7,778	5036	757	510.02	7.500	0.000950
PCB 170	1.39	0.00	-	-	17,862	4,263	638	141	12,825	-	-	-	7.060	0.000504
PCB 180	4.97	1.62	1.88	0.73	45,441	12,580	2,054	450	9,152	7,784	1,094	618.08	7.160	0.000310
PCB 183	0.99	0.39	-	-	12,343	3,264	545	122	12,423	8,358	-	-	7.087	0.000498
PCB 187	2.52	0.91	-	0.44	24,651	6,418	1,063	243	9,775	7,090	-	617.57	7.046	0.000470
PCB 206	-	-	0.19	-	2,674	667	104	25	-	-	564	-	7.510	0.000112
Total PCBs	17.81	5.75	5.15	2.88	223,476.4	60,896	9,797	2,339	224,012.52	91,503	8256	2283.31	74.523	17.81

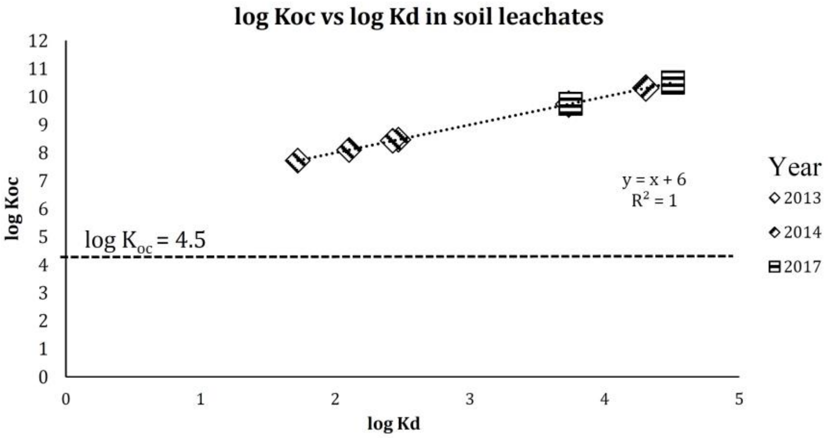

Figure 6. Relationship of log Kd and log Koc in soil leachates

Table 4. Results of K_d, K_{ow}, and Solubility at different depths

Depth	Log K_d vs Log K_{ow}			Log K_d vs Solubility			Log K_{ow} and Solubility Interaction
	R^2	Pearson's R	P	R^2	Pearson	P	
0.3 - 0.5 m	0.8254	0.9085	0.001	0.3175	-0.5630	0.115	not significant (P=0.455)
1.0 - 1.5 m	0.2838	0.5327	0.220	0.2134	-0.4620	0.301	not significant (P=0.827)
4.0 m	0.3714	-0.6094	0.276	0.2182	0.4600	0.430	not significant (P=0.258)
8.0 m	0.8269	-0.9093	0.098	0.5325	-0.7297	0.256	not significant (P not computed)

Conclusion

The data shows the relationship between K_d and predictors K_{ow}, solubility and the interaction between K_{ow} and solubility. Significant effects were found only when the soil depth is near the surface at 0.3 - 0.5 m. K_d is positively correlated with K_{ow} while K_d is weakly inversely correlated with solubility.

Pertaining to the mobility of the substance through the soil profile, the obtained K_d showed high values which indicated a strong adsorption onto soil resulting to lower mobility towards the deeper layers of the soil profile. Still, after 15 years since the PCBs entered the environment, migration of some of its congeners are observed at 8 meters depth and beyond. K_{oc} showed high results which exceeded the log K_{oc} criteria of 4.5. This indicates the potential adverse effects of PCBs on terrestrial organisms. K_{oc} is an important indicator for estimating environmental distribution and environmental exposure level of PCBs. It is recommended to examine the transport of PCBs to terrestrial fauna, groundwater table, and its potential risk to humans.

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