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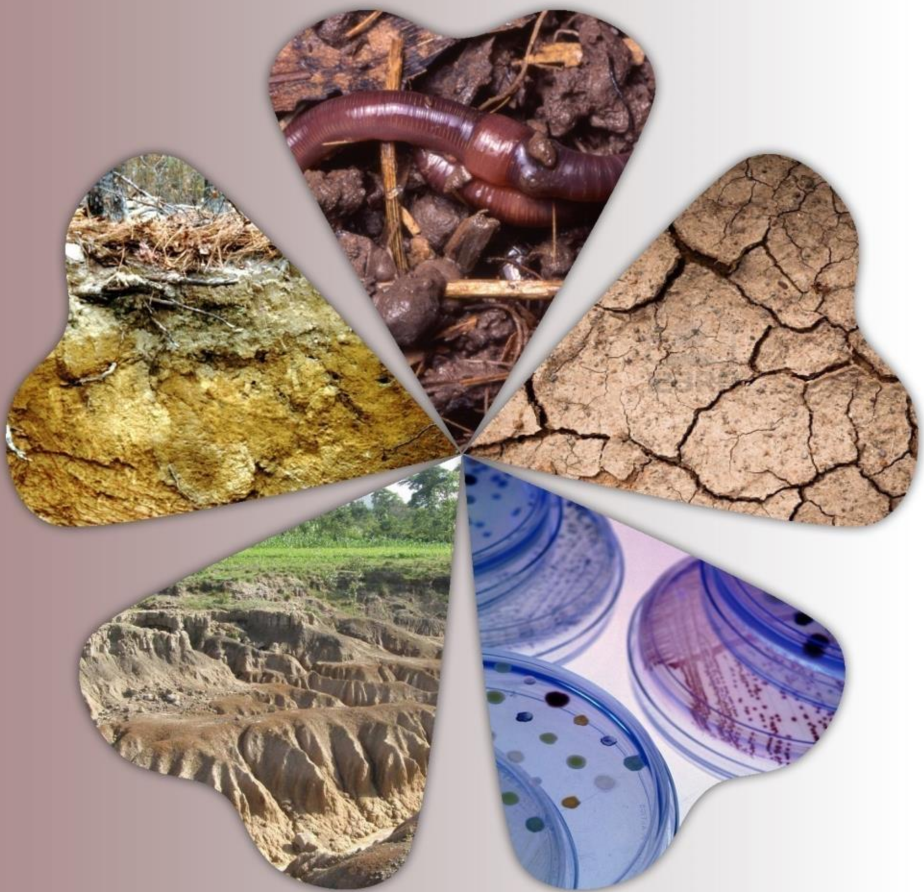

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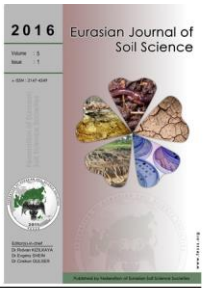

Changes in diversity, biomass and abundance of soil macrofauna, *Parrotio-Carpinetum* forest at organic and semi-organic horizons

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Abstract

Present study evaluates diversity, abundance and biomass of soil macrofauna in organic and semi-organic horizons in P*arrotia persica*-C*arpinus betulus* forest in Shast kola area. Totally 70 sample points were randomly selected from organic and semi-organic horizons then sampling was done by a rectangle 100 cm^2 area. Soil macrofauna were separated from soil samples by hand sorting and using Berlese funnel then dried at 60°C for 72h and weighted in 0.001 gr. With using taxonomic classification key, thirteen macrofauna orders were identified. Most of abundance of soil macrofauna in both soil horizons were allocated to Millipedes order. Changes in diversity, abundance and biomass of macrofauna in both soil horizons were calculated. The results showed Shannon diversity index, Simpson evenness and Margalef richness indices in semi-organic horizon were more than organic horizon. Abundance and biomass of macrofauna in semi-organic horizon were more than organic horizon.

Keywords: Macrofauna, shannon diversity, simpson evenness, Margalef richness, biomass

Introduction

Biodiversity is described as whole plant, animals and microorganisms species which are associated with ecosystems (Altieri, 1999). Soil is one of the most diverse and important habitats in the earth having species richness and includes one of the best combination of living organisms with highest diversity (Khodashenas et al. 2012). Soil fauna have great diversity and according to recent estimates, soil organism might exhibit more than 23 percent of whole living animal's diversity which are defined until now. In some ecosystems, local variation of soil fauns could be very great which could be greatly more than plants and animals group on the earth (Lavelle et al. 2006). Diversity and abundance of soil fauna was used as tension index for soil (Palacios-Vargas et al. 2007). Having regard to importance of invertebrates in litter decomposition and nutrients cycle, invertebrates' diversity is the index which shows the area current status and could be used as the best factor in determination of soil quality (Moghimian and Kooch, 2013). Extreme decrease of soil biodiversity, especially loss of species with unique functioning could have digastric affect which results in long-term soil degradation and loosing agricultural products capacity (Ruiz et al. 2008). Soil fauna are of the main components of ecosystem because they have critical role in accelerating decomposition of organic

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matters and transferring nutrients, though decomposition is the result of microbial activity of soil fauna (fungi and bacteria) (Xin et al. 2012).

Soil fauna affect decomposition process both directly by fragmentation of litter organic matter and indirectly by changing microbial function (Lavelle et al. 2006). Most of soil fauna are existed in top 30 cm of soil surface, although some of them are in lower depth of soil. When soil surface conditions are unfavorable, soil fauna move to lower layers of soil. Most of soil fauna existed in surface layers since these layers includes most of nutrients (Ruiz et al. 2008). Protection of soil macrofauna could help maintenance of earth, because these fauna support nutrients cycle and decomposition processes as well as modification of soil physical properties (Mathieu et al. 2005). Determination of diversity, richness and abundance indices of fauna are required in ecology and habitat management and conservation programs and ecosystem evaluation (Nahmani et al. 2005). These indices are very useful in quantification of soil macrofauna biodiversity pattern (Gonglanski et al. 2008). The objective of this study was to compare the indices of Shannon diversity, Simpson evenness and Margalef richness as well as abundance and biomass of macrofauna in two organic and semi-organic soil horizons in Shast-Kalate forest of Gorgan.

Material and Methods

Study Area

This study was carried out in compartments 7, 8, 15, and 17, district 1 of Shast-Klateh (Bahram Nia) Forest, Experimental and Educational Forest of Gorgan University of Agriculture Sciences and Natural Resources. It is located in northen Iran between 36° 45´ and 36°46´N latitude and 54° 24´ and 54°21´E altitude. Annual precipitation was 649 mm and elevation in studied parcels varied between 300 and 630 m a.s.l. Dominant forest type of these parcels are *Parrotia persica-Carpinus betulus.*

Data Collection Method

In order to determination of diversity and abundance of soil macrofauna, 70 sample points randomly selected from organic and semi-organic horizons. The sampling container was rectangle a 100 cm^2 area. Samples were placed in plastic bags and immediately transferred to laboratory and soil macrofauna were separated manually and using Berlese funnel. Samples were stored in plastic repository with 75% ethanol for next steps (Palacios-Vargas, 2007). Soil macrofauna were identified using taxonomic classification keys up to order level (Borror et al. 1989; Ruiz et al. 2008). Macrofauna biomass was calculated after oven-dried at 60°C for 72h and weighing in 0.001gr (Rahmani and Mayvan, 2004).

Data analysis

Calculation of Shannon diversity index, Simpson uniformity index and Margalef richness index. There are various ways of measuring diversity of soil fauna. In current research, the formulas of Shannon - Wiener (diversity), Simpson (evenness) and Margalef (richness) indices were used as follows (Mbuthia et al., 2012).

1.Shannon - Wiener diversity index.

$$H = -\sum_{i=1}^{s}(P_i \,.\, LnP_i)$$

Where H' is Shannon - Wiener index; S is invertebrate's group's number; Pi is average abundance of per invertebrates groups; Ln is natural logarithm.

2.Simpson evenness (heterogeneity) index.

$$D = \sum_{i=1}^{s} P_i^2$$

Where, D is Simpson evenness index; S is invertebrate's group's number; Pi is average abundance of per invertebrates groups

3.Margalef richness index.

$$R = \frac{S-1}{lnN}$$

Where R is Margalef richness index; S is invertebrate's group's number; *ln* is natural logarithm; N is number of populations.

In order to study the diversity indices of soil macrofauna between two horizons, PAST software was used and then resulted data processed by SPSS v.16 software. Initially, normal being of data assessed using Kolemogroph Smirnof test and if data were not normal, normalized using different methods such as Log10. For comparing changes in biomass and diversity of macrofauna at two organic and semi-organic horizons t-pair test and for comparing changes in abundance at both horizons, non-parametrical Wilcoxon test was used.

Results and Discussion

At 70 collected samples from organic horizon surface to end of semi-organic horizon, 13 macrofauna order were recognized which include Diplopoda, Isopoda, Hymenoptera, Diptera larvae, spiders, Chilopoda, Coleoptera, Haplotuxidae (earthworm Order), Opilionida, Heteroptera, Amphipoda, lepidoptera larvae and stylommatophora snails. Total abeundance of soil macrofauna at two sampling horizon were 10000000 number per ha which Diplopodas by 3557143 number per ha was the most abeundant (Figure 1). Total biomass amount at both horizons was 141.11 kg/ha which greatest amount was associated to mollusks by 64.42 kg/ha (Figure 2). In organic horizon total fauna abundance was 3958714 per ha which the greatest amount was related to Diplopodas and total biomass amount at this horizon was 58.5 kg which the greatest biomass was related to snails (Figure 1). In semi-organic horizon total macrofauna abundance was 6014286 per ha which the greatest amount was related to Diplopodas (Figure 1) and total biomass amount at this horizon was 82.62 kg which the greatest biomass was related to snails (Figure 2).

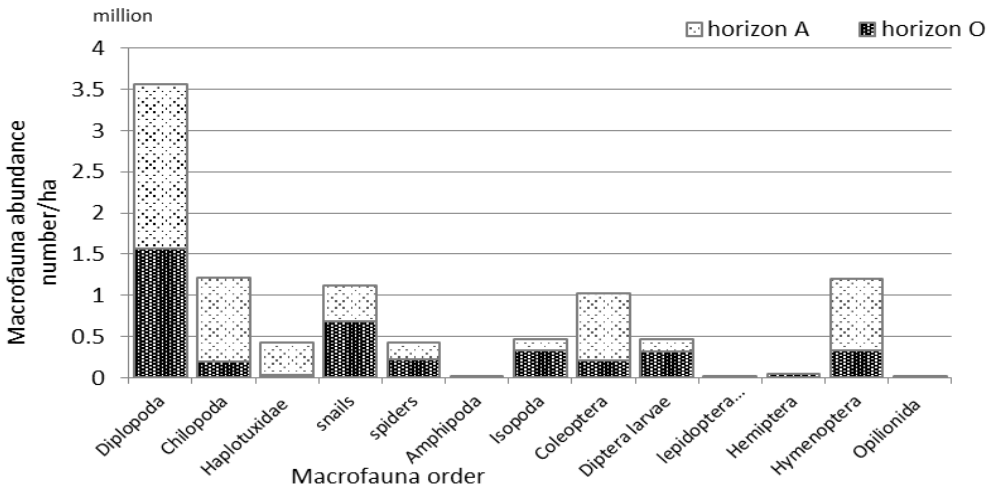

Figure 1. Macrofauna abundance at organic and semi-organic horizons

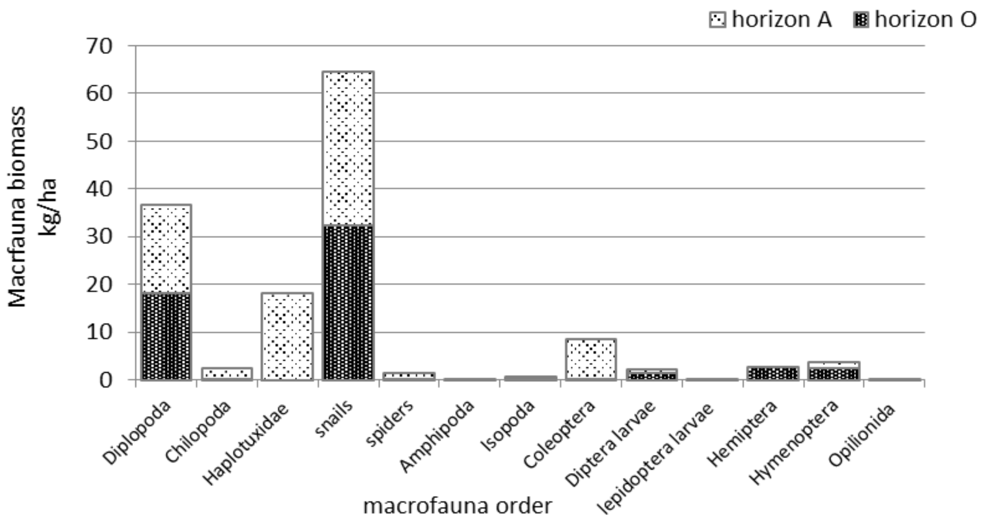

Figure 2. Macrofauna biomass at organic and semi-organic horizons.

Results from paired t-test stated that there is significant difference between numerical amount of Shannon index at two organic and mineral horizon at 99% probability level. So, Shannon index amount in semi-organic horizon was greater than organic horizon (Figure 3). Also, results of numerical amount of Simpson evenness index in both horizons showed significant differences which greatest amount was related to semi-organic horizon (Figure 3). Comparisons of Margalef richness index mean of showed significantly differences which the greatest amount was related to semi-organic horizon (Figure 3). Also macrofauna biomass in both depths showed significantly differences that the greatest amounts were allocated to semi-organic horizon (Figure 4).

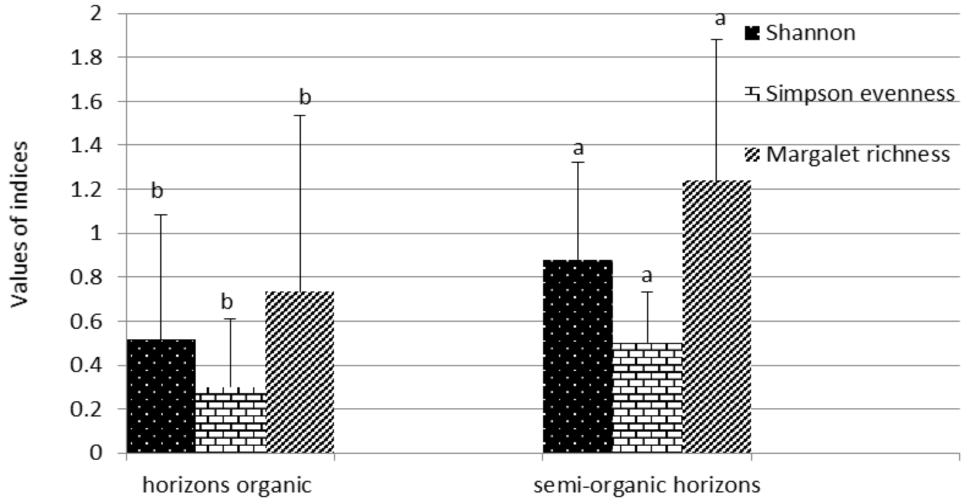

Figure 3. Average amount of Shannon diversity, Simpson evenness and Margalef richness indices at organic and semi-organic horizons.

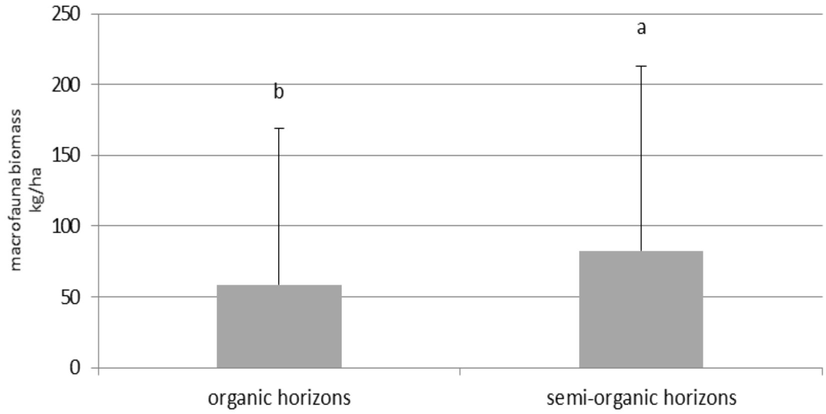

Figure 4. Average amount of macrofauna biomass at organic and semi-organic horizons.

The results of Mann–Whitney U test showed that there was significant difference between macrofauna abundance in two organic and semi-organic horizons while the greatest amount belong to semi-organic horizon (Figure 5).

In the studied area, Diplopoda order has great abundance. Hassall et al. (1987) and David and Handa (2010) stated that Isopoda and Diplopoda are most frequent macrofauna in temperate forests and have considerable contribution in decomposing litter and nitrogen content.

Vegetation cover not only supply considerable fraction of soil organic matter, but indirectly determines soil fauna composition (Frouz et al. 2006). Rahmani and Mohammadnejad Kiasari (2003) noted that tree species is the most important factor in increasing Diplopoda Population. Also, Loranger et al. (2003) stated that Diplopoda due to presence of foliage of Pisonia subcordata enriched by nitrogen at secondary estuary and semi-estuary forests has great abundance. Amini et al. (2011) obtained *Carpinus betulus* nitrogen amount as 1.69 % indicating nitrogen richness of *Carpinus betulus* litter. Warren and Zou (2002) stated that biomass of Diplopoda Class has greatest association with nitrogen concentration and C/N ratio at litter layer O_i. having regard to this point that vegetation type of studied area in terms of volume is *Carpinus betulus -Parrotia persica*, which *Carpinus betulus* share is 58.27 % of volume and 32.75 % of mass abundance, therefore it can be concluded that increasing in litter nitrogen amount caused attraction of Diplopoda.

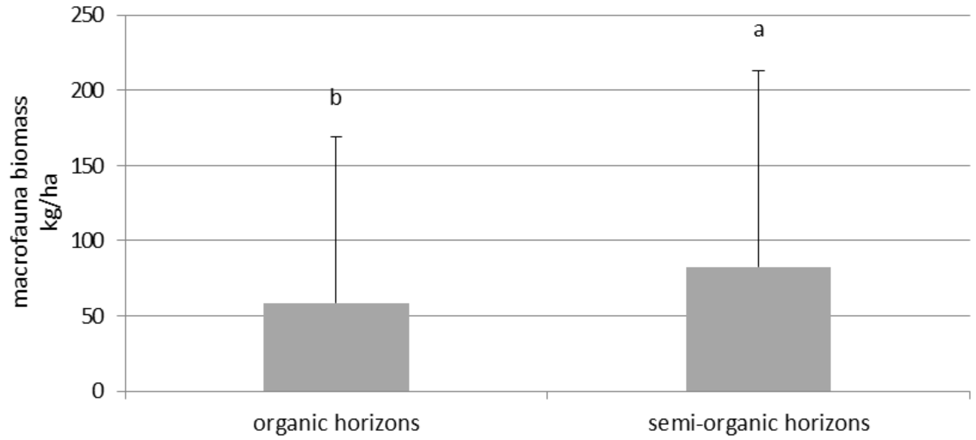

Figure 5. Average amount of macrofauna abundance at organic and semi-organic horizons.

Diversity, evenness and richness indices of macrofauna were calculated by Shannon, Simpson and Margalef equation for both organic and semi-organic soil horizons. Shannon diversity, Simpson evenness and Margalef richness indices amounts in both depths indicated specific pattern. Shannon diversity, Simpson evenness and Margalef richness indices are highest in semi-organic horizon and showed significantly difference with organic horizon (Figure 3). Rahmani and Mayvan (2004) stated that diversity indices in decayed organic layers is more than undecayed organic layers and highest diversity index amount is allocated to decayed organic layer and lowest amount is associated to 20-30 cm layers. Moghimian et al. (2013) stated that all of diversity indices except Margalef richness index have significant difference in 0-10 and 10-20cm introducing organic materials into soil through plant residues affects moisture and temperature of soil invertebrates. Presence of plant residues in soil surface, in addition to supplying soil invertebrates nutrition, caused microclimates changes, since reduces sunlight receiving at soil surface and decrease soil temperature and indirectly will increase soil moisture content which is favorable for growth and development of soil invertebrates. On the other hand, accumulation of soil organic matter results in increasing soil moisture holding capacity and affects soil invertebrates association (Sileshi and Mafongoya, 2006). Considering to that semi organic horizon composed of un-decayed organic matters which don't consumed by macro organisms and/or a little amount is consumed, therefor it can be said that this horizon plays habitat and nest role for soil macrofauna.

It is previously determined that organic matter content and quality, quantity and depth of forest floor affects Colembola spatial distribution (Hasegawa, 2010). Having regard to this point that evenness and richness caused increasing diversity, present study shows that evenness and richness indices amount at semi organic horizon was highest amount which results in greatest amount of diversity index (Figure 3).

Studying the abundance and biomass of soil macrofauna showed that amounts in semi-organic horizon were more than organic horizon and showed significant difference (Figure 4). Irannejad and Rahmani (2009) stated that 82 to 86% of number and biomass of earthworms are presented in 0 to 10 cm layer.

Conclusion

In the present study, the greatest soil macrofauna abundance was related to Diplopoda order. Species vegetation litter type includes *Carpinus betulu* and *Parrotia persica* caused attraction of Diplopodas. Also, the maximum value of Shannon diversity, Simpson evenness and Margalef richness indices among two organic and semi organic horizons, was assigned to semi-organic horizon. The most abundance macrofauna biomass was observed in semi-organic horizon which could be due to composition and depth of organic matter content.

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Geochemical pattern of soils in Bobovdol valley, Bulgaria. Assessment of Cd and Co contents

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Abstract

The chemical composition of soils spread in the Bobov dol valley was studied in order to reveal the natural and anthropogenic patterns of Cd and Co spatial distribution. A sampling procedure based on the irregular grid of points and validated analytical methods were used in the field and laboratory studies. It is found that Cd content varies from 0.21 to 0.90 mg kg^{-1} in studied soils and the average value of 0.55 mg kg^{-1} coincides with concentration demarcating soil pollution (0.5 mg kg^{-1}). Co content ranges from 2.22 to 15.76 mg kg^{-1} and in 70 % of sampled points exceeds the natural background content of 7.8 mg kg^{-1} found in local rocks. Still, Cd enrichment of studied soils is more significant than Co's with coefficient of Clarke concentration of 3.67. Hence, the secondary deposition of studied elements as a result of the Bobov dol Thermal power plant air emissions is verified by results obtained. The spatial distribution of Cd and Co is featured with an altitudinal gradient in deposition and a trend of quantitative depletion in the South of Plant. Soil organic matter and pH have no influence on the content and spatial distribution of studied elements. Elements iron affinity governs their geochemical linkage in soils although cobalt occurs allied with aluminum and titanium.

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Keywords: Soils, trace elements, Bobov dol, spatial distribution, background content

Introduction

The study of distribution of trace elements in soils of Bulgaria is still concentrated in areas affected by industrial activities, such as mining (Marinkina, 1999; Jeleva, 2008; Hristova, 2013) and processing of mineral resources (Misheva et al., 2007; Nikova et al., 2013). Most of these studies are designed to assess soil contamination and include the surface 30-40 cm of soil profile (Petrova, 2009; Dinev, 2012). This approach is used in monitoring programs, but does not provide enough information for three-dimensional (3-D) geochemical characteristics of soils and the cycles of trace elements in pedosphere.

Geochemical maps designed by the grid sampling procedure to reflect the spatial distribution of chemical elements in soils may be used for predicting the extension of soil degradation and its negative impact on the interacting environmental components. Detailed information on the natural variability of background concentrations of chemical elements in soils and the quantitative database allow imaging the geochemical diversity (Jurlik, 2001). In this regard, the elements cadmium and cobalt are of great interest in Bulgaria where there is a limited knowledge of their distribution and availability in the pedosphere.

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A large number of studies reveal the role of cadmium in geochemical processes (Krauskopf, 1956; Jones, 1973). However, its geochemical fate in pedosphere and especially in soil surface layer is still not well understood (Gong et al., 1977). It is known that cadmium persists only in the +2 valence state in soils and goes readily into solution on weathering. The enrichment of soils with cadmium occurs primarily through the adsorption and/or complexation of cadmium onto organic matter followed by the accumulation of organic debris in the depositional environment. Cd mobility is affected mainly by the pH and oxidation potential (Kabata-Pendias and Pendias, 1984). It is most mobile in acidic soils within the pH range 4.5 - 5.5 and is rather immobile in alkaline soils, wherein $CdHCO_3$ prevails. Studies of cobalt geochemistry in terrestrial environments have also received a lot of attention. However the case studies of cobalt variability and accumulation in soils could contribute to the better knowledge of element global cycling. This article deals with the geochemical patterns of soils located in the vicinity of the "Bobov dol" Thermal power plant, Bulgaria, emphasizing on the amount and spatial distribution of Cd and Co in order to determine their geochemical behavior, associations and origin in soils.

Material and Methods

Site description

The present study is initiated to determine the influence of Bobov dol Thermal power plant (TPP) on Cd and Co contents and distribution in soils surrounding the Plant (Figure 1). The choice of this area is consistent with a few facts:

1) "Bobov dol" TPP is the only major source of trace elements in the researched area, which excludes the cumulative effect of other industrial pollutants. This plant is one of the oldest in Bulgaria (it was working at full capacity since 1975) and its impact on the geochemical status of surrounding soils is of incontestable interest.
2) Soils are mainly developed on sandy clay conglomerates from motley under-coal-bearing formation of Bobov dol graben and thus provide very similar lithology and geochemistry of soil parent materials.
3) The geochemical composition of lignite and brown coal burned in "Bobov dol" TPP is already known as well as the Clarkes – the average element contents in coal (Kortenski, 2011).
4) Almost all agricultural lands there are cultivated, which determines the socio-economic importance of the region.

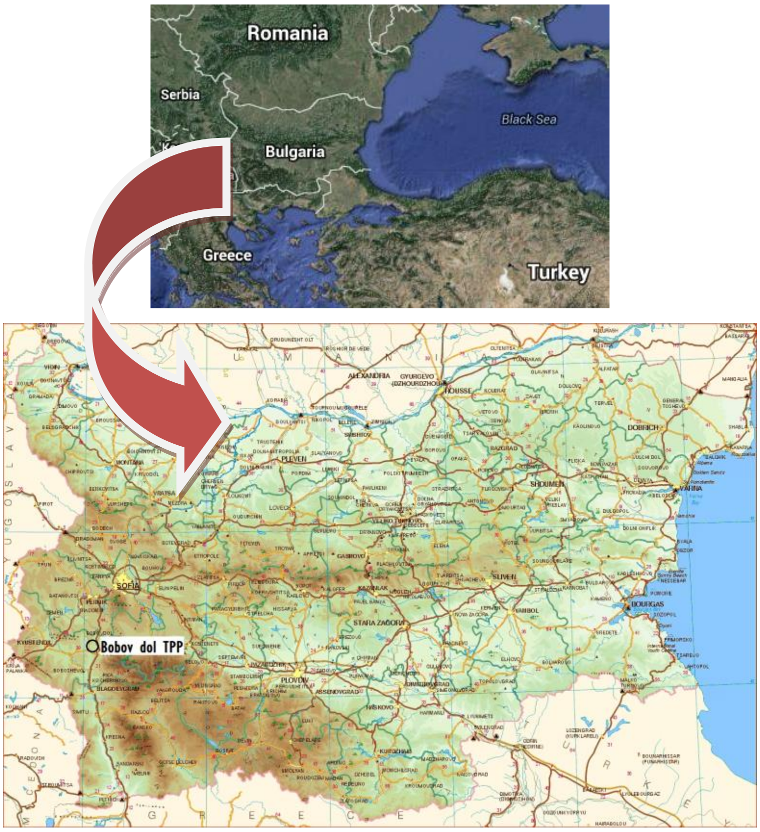

Figure 1. Location of the Bobov dol TPP

Sampling

The spatial distribution of trace elements in soils is studied by irregular network. The grid tightly covers about 5 km zone around the TPP. Soil cover is present by: Endocalcic Chromic Luvisols; Chromic Cambisols, Vertic Luvisols, Umbric Leptosols, Eutric Fluvisols and Colluvic Regosols (WRBSR, 2014).

Samples were taken at a depth of 30 cm, since the surface 30 cm soil layer is the most vulnerable to anthropogenic enrichment with heavy metals (Dinev et al., 2008) and is the most active part of the soil. For the determination of background elements contents in indigenous (soil parent rocks) three samples located in the southern part of the region, near by a conveyor belt to a tailings pond "Kamenik" were taken.

Analyses

The concentration of Cd and Co was determined by the method described in ISO 22036:2012. Analysis of samples pre-treated according to the ISO 11466:2010 was carried out in the laboratory of "Geochemistry" (University of Mining Geology "St. Ivan Rilski", Sofia). 30 soil samples were set for full silicate analysis implemented in agreement with the inter-laboratory method CNILG BM-2: 2013. The content of macroelements was determined by the ICP 720-ES (Agilent Technologies) after lithium metaborate fusion sample preparation..

The pH was determined potentiometrically in compliance with ISO 10390:2005. Organic carbon content was analyzed by the modified method of Turin (Kononova, 1963; Filcheva and Tsadilas, 2002).

Assessment of trace elements content

The concentration coefficients of Cd and Co are calculated with the average elements content in local rocks (local geochemical background) marked as Ccl, as well as in Bulgarian rocks using data of Kuikin et al. (2001) - marked as Ccr, in Bulgarian soils - Ccs with data of Atanasov (2003), and in the Earth's crust - Cc - data from www.webelements.com.

Geoinformation

Remote sensing data: 11 ASTER (Advanced Space borne Thermal Emission and Reflection Radiometer) Level 1B scenes obtained on 2002/07/04 is used. The calibrated ASTER Level 1B data represent the TOA (top of atmosphere) radiance with radiometric and geometric corrections. ASTER scene ID is ASTL1B 0207040929070208060920. The image is georeferenced in projection UTC North Zone 35, WGS-1984. Digital model of the relief created by the space shuttle Terra in February 2000, known as SRTM (shuttle radar topographic mission) with a resolution of 90 m is applied for 3D visualization of terrain.

Geostatistical methods are used for spatial interpolation of points with unknown contents. Preliminary assessments of the difference between the values were made with variogram analyses using directional semivariograms. The ordinary kriging and spherical model of variogram function are used for interpolation. For each element were investigated the statistical properties of dataset and the global trend (second order) in data was removed. It was also made assessments of the output surfaces. Soil geochemical maps were made with ArcGIS.

Statistics

Microsoft Excel software is used for statistical analysis of data. Standard error of the mean (SEM) is calculated by commonly accepted formula.

$$SEM = \frac{\sigma}{\sqrt{n}}$$

Where, σ is the standard deviation of the population; n – a population size.

Results and Discussion

The levels of cadmium in soil widely vary from 0.06 mg kg^{-1} in weak developed soils to hundreds of mg/kg in the vicinity of pyro metallurgical Plants (Waldron, 1980). The highest amount with average level of 7.5 mg kg^{-1} are found in soils developed on shale (Lund et al., 1981) contrary to soils on sandstone and basalt, which have the lowest average concentration of 0.84 mg kg^{-1}. McBride (1994) announced the concentrations of soil Cd exceeding 0.5 mg kg^{-1} suggest a soil contamination.

The average cadmium content in studied soils slightly differs from the lithogenic threshold of 0.5 mg kg^{-1}, but in all grid points is higher than its average value in Bulgarian soils (Table 1, 3).

Table 1. Statistic data of studied parameters

	C org	Al_2O_3	CaO	Fe_2O_3	K_2O	MgO	MnO	Na_2O	P_2O_5	SO_3	SiO_2	TiO_2	Cd	Co	Zn	pH
Mın	0.42	8.86	1.18	3.01	1.41	0.80	0.04	0.97	0.07	0.03	36.58	0.36	0.24	2.22	21.6	4.0
Max	4.41	16.46	3.48	7.32	2.74	11.38	0.11	2.50	0.22	0.88	70.46	0.86	0.90	15.76	96.5	7.9
Average	1.21	14.28	3.01	4.62	2.26	2.08	0.07	1.77	0.12	0.06	63.44	0.65	0.55	8.71	54.0	6.0
Medıan	1.15	14.74	1.66	4.64	2.29	1.70	0.07	1.80	0.11	0.03	65.49	0.67	0.51	8.90	52.7	5.9
SD	0.54	0.96	2.36	0.59	0.22	0.89	0.02	0.39	0.03	0.06	4.80	0.08	0.13	2.14	13.60	1.04
SEM	0.06	0.17	5.48	0.11	0.17	0.16	0.00	0.07	0.01	0.01	0.88	0.01	0.01	0.23	1.45	0.11

The spatial distribution of Cd is characterized by frequently alternating zones with different element content (Figure 2). The highest accumulation of Cd goes off on the hillsides of Konyavska Mountain showing a altitudinal gradient in deposition. The last trend is also documented by (Van de Velde et al., 2000; Planchon et al., 2002; Barbante et al., 2003; Lee et al., 2008). It is reasonable to assume that the areas of higher content (> 0.5 mg kg^{-1}) reflect a technological abundance of Cd while lower in concentrations areas are its lithogenic zones. In the nearest to the Plant area with lowest cadmium content is situated the old reclaimed ash-pond (Bd 72-1, Bd 72-2 and Bd 72-3). Zinc content is also low in reclaimed zone (21.60-30.70 mg kg^{-1}) and reveals the lithological pattern of soils existed in the valley prior to ash-pond construction. Furthermore, due to the similarity in their geochemistry, Cd^{2+} usually replaced Zn^{2+} in zinc minerals which are the most important host for cadmium (Adriano, 1986; Alloway, 1995; Minkina et al., 2014). This process catalyzed by the additional supplement of Cd (as a result of Plant emissions) could explain the established spatial fluctuations in Cd content (Figure 2).

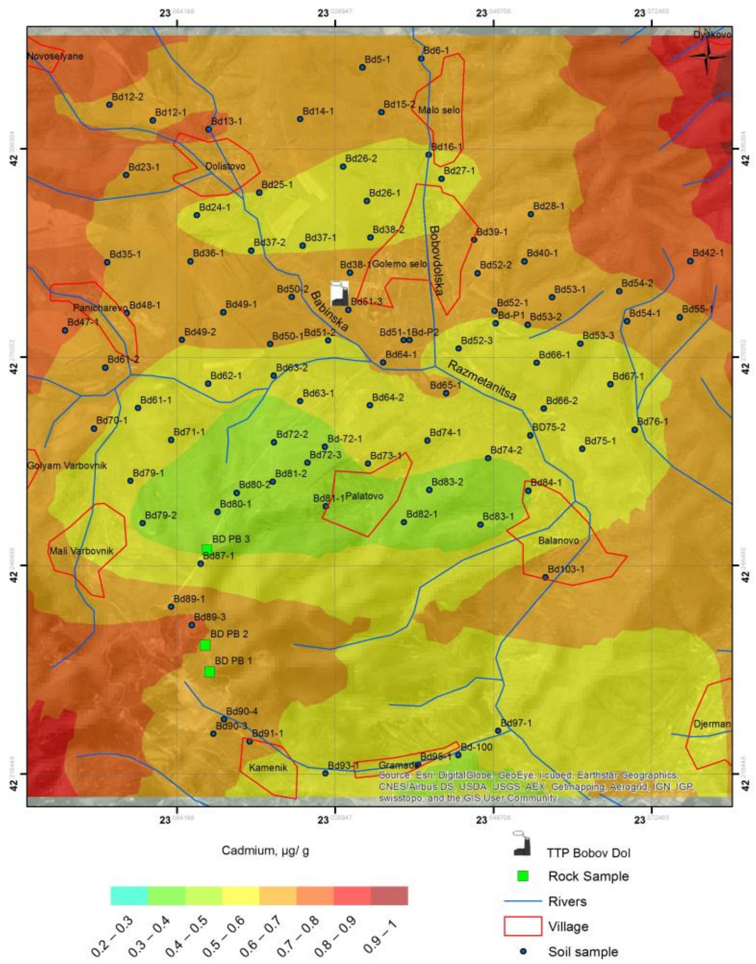

Figure 2. Spatial variability of Cd content in the surface layer of studied soils

Although, the correlation between Cd and Zn is not high ($R^2=0.34$), probably due to the technogenic origin of Cd accumulation established in surface layers of studied soils (Figure 3).

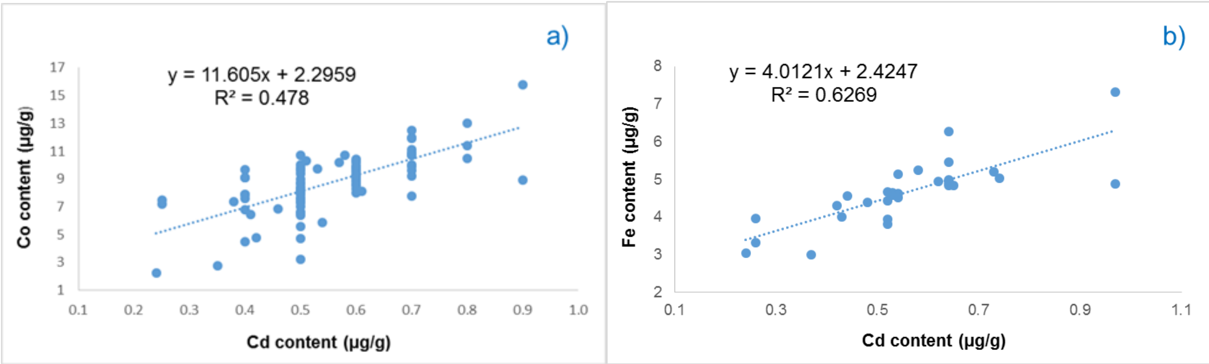

Figure 3. Correlation between the content of Cd and Co (a), Cd and Fe (b)

Cadmium shows slight affinity to Al (expressed as Al_2O_3 content, $R^2=0.44$) and Ti (TiO_2, $R^2=0.46$) but well correlates with Fe (Figure 3). With Co whose distribution is managed by secondary processes Cd does not significantly correlated (Figure 3).

In order to evaluate the content of trace elements in studied soils various concentration coefficients are calculated (Table 2). Data obtained in this study were compared with the average content of these elements in Bulgarian soils and in the Earth's crust - Clarke values (Table 2).

Table 2. Average contents of trace elements in various objects (mg/kg) and concentration coefficients in studied soils

	Content in studied soils	Local background	Ccl	Content in rocks	Ccr	Content in soils	Ccs	Content in the Earth's crust	Cc
Cd	0.55	0.0	-	2.3	0.24	0.21	2.62	0.15	3.67
Co	8.71	7.8	1.12	10.0	0.87	13.0	0.71	30.0	0.29

Legend: CcI – coefficient of concentration accounting local rocks content; Ccr – coefficient of concentration accounting average content in main rock types; Ccs- concentration coefficient accounting soil content; Cc- Clarke coefficient

The results in Table 2 show that Cd is not found in local rocks presuming that soil cadmium content has not lithogenic origin and can be consider as a result of technogenic deposition. According to Ccs the accumulation of Cd in studied soil varieties is well pronounced and exceeded the background values in Bulgarian soils. These data confirms the findings of Lalor et al. (1995) that cadmium is predominantly accumulated in soil surface layers and the processes responsible for its accumulation are: adsorption by soil humus and deposition of atmospheric sediments, fertilizers and plant debris.

Regardless of Cd low average content in the Earth's crust it is extensively distributed in the lithosphere - in shale, igneous rocks, coal, sandstone, limestone, lake and ocean sediments and soils (Waldron, 1980). Its levels in igneous and metamorphic rocks widely vary but rarely exceed 0.5 mg kg^{-1}. However, in sedimentary rocks, carbonate and sandstone it occurs in the lowest values (0.035 mg kg^{-1}). The accumulation of Cd to potentially hazardous concentrations in some coal and crude oil deposits may also occur (Ketris and Yudovich, 2009; Kortenski, 2011).

Table 3. Average content of trace elements in coal burned in TPP "Bobov Dol" (in mg kg^{-1})

Parameter	Beli breg coalfield[1]	Stanyantsi coalfield[1]	Chukurovo coalfield[1]	Pernik coalfield[1]	Bobov dol coalfield[2]	Average	Clarke[3]
Cd	1.4	2.2	1.6	3.2	nd	2.1	0.24
Co	0.8	2.2	12.4	43.1	8.0	13.3	4.2

Legend: [1] – Kortenski (2011) data; [2] - Vassilev (1994) data; [3] - Ketris and Yudovich (2009); nd – no data

The cadmium content in coal is cursory studied. This is a consequence of low Cd content which requires sophisticated techniques for determining the parts per billion levels of concentration especially as routine analysis (Lee and Tebo, 1994). The average Cd content in coal varies between 0.2 and 0.24 mg kg^{-1}, depending on the degree of coalification (Ketris and Yudovich, 2009). In coal incinerated in TPP Bobov dol, cadmium content exceeds Clarke value 6 to 13 times and could provoke a danger to the environment (Table 3). During the high temperature combustion of coal cadmium forms volatile compounds and is released into the atmosphere, either in the form of gas (metallic Cd and oxides), or condensed onto the submicron dust particles (Yudovich and Ketris, 2005; Kabata-Pendias and Mukherjee, 2007). In soils technogenic cadmium forms simple inorganic compounds (oxides, hydroxides, chlorides, sulfates depending on the soil pH and Eh) or organo-metallic complexes which are accessible to plants (Kabata-Pendias and Mukherjee, 2007). Some plants, such as lettuce, carrots, tobacco accumulate significant quantities of Cd and became a serious threat to human's health.

Sources of cadmium in the atmosphere are quite diverse and can be either natural (volcanic activity, forest fires, wind transported soil or rock particles) or anthropogenic (mining and processing of lead and zinc ores, combustion of coal, oil and waste products, the production of batteries and pigments, Hutton, 1982; Kabata-Pendias and Mukherjee, 2007). Among them, metallurgical and thermal power plants utilizing solid or liquid fuel are the most significant Cd emitters in the atmosphere (Kabata-Pendias and Mukherjee, 2007).

With relation to Co geochemistry a few features should be noted.

Cobalt displays chalcophile and siderophile properties and associates with the iron sulphides, arsenopyrite and pyrrhotite. It is also present as an accessory component in olivine, pyroxene, amphibole, mica, garnet and sphalerite and in oxide accessory minerals, such as magnetite (Ure and Berrow, 1982). It forms several rare minerals: smaltite $(Co,Ni)As_{2-2.5}$, cobaltite (Co,Fe)AsS and linnaeite $(Co,Ni)_3S_4$.

Cobalt, together with Cr and Ni, is indicative of mafic rocks. It replaces Fe^{2+} and Mg^{2+}, which are similar in charge and ionic radius, and hence accumulates in mafic rocks (Wedepohl, 1978). Generally, the average abundances of Co in different rocks types cited by Mielke (1979) is: ultramafic - 150 mg kg^{-1}; basaltic - 48 mg kg^{-1}; granitic 1-7 mg kg^{-1}; syenite 1 mg kg^{-1}; an average bulk continental crust abundance of 29 mg kg^{-1}, and 10 mg kg^{-1} in the upper continental crust (McLennan and Murray, 1999).

Cobalt is immobile during metamorphism (Condie, 1976; Nicollet and Andriambololona, 1980). Minerals such as quartz, feldspar and pure calcium carbonate contain low amounts of Co (<2 mg kg^{-1}), as well as sandstone and limestone. In sedimentary rocks, Co is concentrated in the fine-grained fractions and tends to vary with the Fe and Mn content (Vine and Tourtelot, 1970). Formation of organo-metallic complexes is not an important enrichment mechanism for Co in sedimentary processes.

Cobalt is most mobile in the surface environment under acidic and reducing conditions (Qian et al., 1998). Cobalt does not form residual silicate minerals in soil. The average concentration of Co in European soils is quoted as 8.91 mg kg^{-1} (extracted with aqua regia) with a range from <1 to 255 mg kg^{-1} (Salminen et al., 2005). Koljonen (1992) announced 10 mg kg^{-1} as a total content in soil worldwide.

Human induced sources of cobalt include coal combustion, special steels production, fertilizers, lead, iron and silver mining and processing (Reimann and Caritat, 1998). Ecological problems arose from pollution with technogenic Co are generally less significant than those associated with some other heavy metals (Cole and Carson, 1981). Atmospheric cobalt, derived from combustion sources is assumed that exists primarily in oxide form (Schroeder, 1987). Cobalt and its inorganic compounds are not volatile.

Yudovich and Ketris (2005) adduced data of coal with high amount of Co. The influence of rich in Co coal on the element spatial distribution strongly depends on the form of its presence in the coal and the temperature in the combustion chamber. Cobalt as a siderophilic metal has high affinity to sulfur and in coal often participates as isomorphic alloy of sulphide minerals (mainly pyrite). In some coal cobalt is also present in association with organic matter (Kortenski, 2011). Yudovich and Ketris (2005) noted that in low temperature combustion (1000-1200°C) the element is separated from organic matter and partly from sulfides, and then usually condensed in the form of Co-containing spinel in cinder-slag portion of the ashes. At high temperature combustion (1200-1600°C) cobalt goes into the gaseous phase, where either partially condenses on fine ash particles, or is emitted into the atmosphere. Vassilev and Vassileva (1997) found that Co amount in the volatile fraction ranges between 10 and 20 % of the concentration of cobalt in the coal.

Data summarized in Table 3 show that some of coal burned in the Plant are characterized with high concentrations of cobalt and its average contents exceeds over 3 times Clarke, determined by Ketris and Yudovich (2009).

In studied soils Co content ranges from 2.22 to 15.76 mg kg^{-1} and the extension of values is greater than 4.5 to 12 mg kg^{-1} issued by Kabata-Pendias and Mukherjee (2007) for soil surface horizons. Soils containing

<0.5–3 mg kg^{-1} are considered cobalt-deficient and risky to ruminants breeding (Becker and Smith, 1951; Boikat et al., 1985; Keener et al., 1949)

The content of cobalt is higher than in local rocks and in 70 % of points exceeds 7.8 mg kg^{-1} (Table 1). Compared to the average content in Bulgarian soils it is somewhat lower reflecting the negligible impact of TTP "Bobov dol" emissions (Table 2).

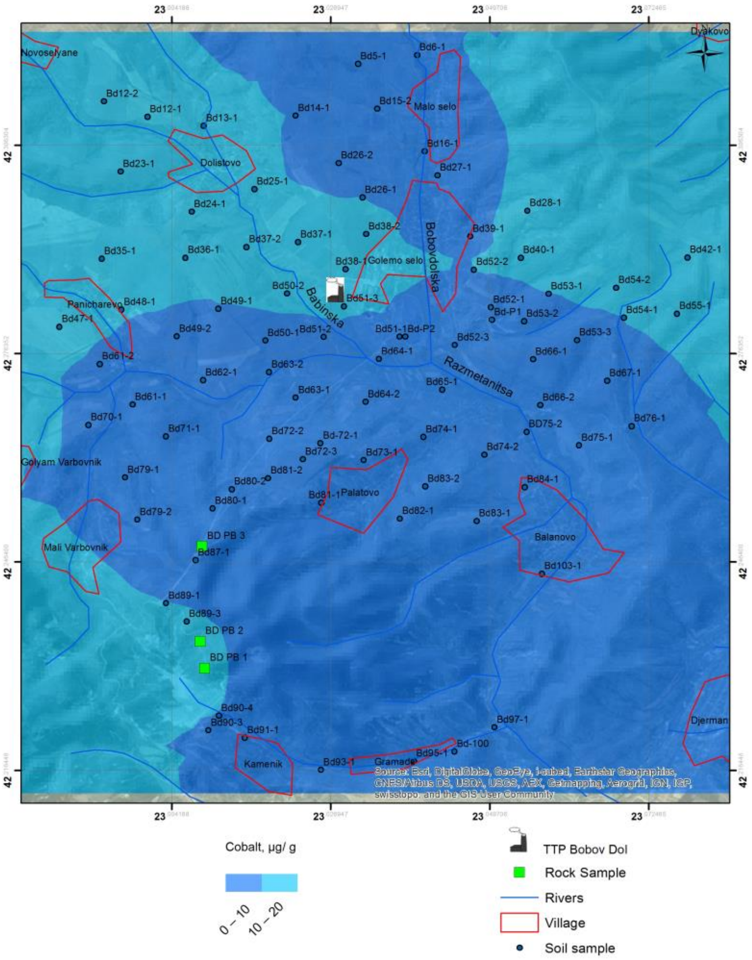

Figure 4. Spatial variability of Co content in the surface layer of studied soils

Cobalt slightly accumulates in studied soils (Ccl, Table 2), and this reflected in its quite uniform spatial distribution along the studied area. In general, cobalt content decreases from North to South and likewise Cd its lowest amount is found in the reclaimed area.

Several significant correlations of Co are found with: aluminum (R^2=0.63), titanium (R^2=0.65) and iron (Figure 5). Its association with zinc (Figure 5) presumably originates from the secondary processes or diagenesis.

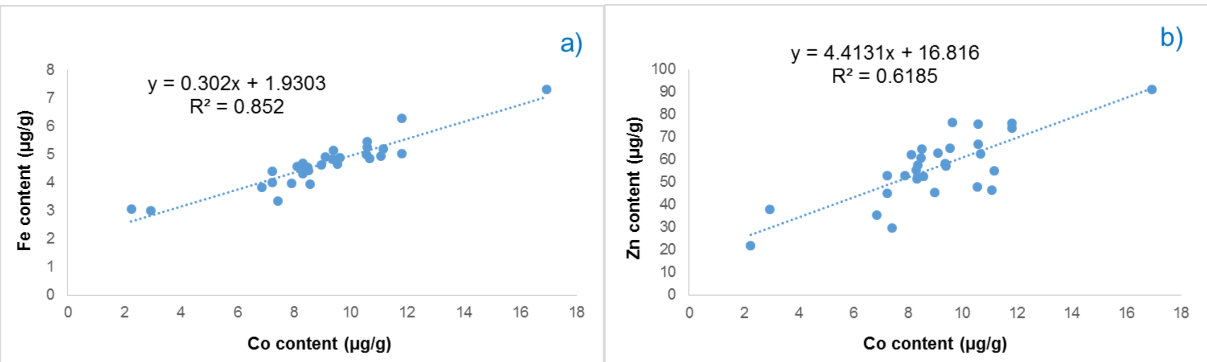

Figure 5. Correlation between the content of Co and Fe (a), Co and Zn (b)

Soil chemical factors, such as pH and organic matter have no influence on the content and spatial distribution of cobalt.

Conclusion

Cadmium is exogenous element in studied soil varieties supplied by anthropogenic activities. It is distinctly accumulates in soil surface layers regardless of the soils organic carbon content and pH. The element iron affinity governs its geochemical linkage in soils although it occurs allied with aluminum, titanium and cobalt.

Cobalt, likewise cadmium forms the same geochemical associations, but the correlations between elements are much stronger. Cobalt more slightly accumulates in studied soils but its content is somewhat lower than Clarke and background value for Bulgarian soils. It prevails in the northern part of the area occupied by Konyavska Mountain and gradually disperses to the South.

Great differences between the content of cadmium and cobalt in soil parent rocks from researched area and the summarized average values of the rocks in Bulgaria does not allow a firm conclusion about elements typomorphic accumulation to be made.

Acknowledgement

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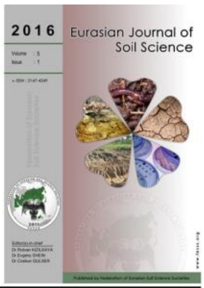

Screening for *Pseudomonas* and *Bacillus* antagonistic rhizobacteria strains for the biocontrol of *Fusarium* wilt of chickpea

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Abstract

The aim of this work is to study the ability of several isolates belonging to Rhizobacteria (*Pseudomonas* and *Bacillus*) collected from several chickpea growing areas in Algeria, to control the mycelium growth of *Fusarium oxysporum f. sp. ciceris.* Interesting isolates were characterized for their morphological characteristics, physiological and biochemical activities as potential bio-control agent. Fungal inhibition tests were performed using plate assay and each isolate were tested for the production of protease, cyanide hydrogen, indole acetic acid, antifungal volatile and extracellular compound. According to API 50 CH, we are able to identify six *Bacillus* species (*B. subtilis*, *B. circulans*, *B. lentus*, *B. aneurinilyticus*, *B. firmus*, *B. licheniformis*; and with API 20NE test we have identified three Pseudomonas species (*P. aeruginosa*, *P. luteola*, *P. fluorescens*). The ability of bacterial isolates was varied in production of Protease, Gelatinase, Amylase, Cellulase, Acid Indole acetic, Lipase, Catalase and Cyanid Hydrogen. This is traduced in different rate of inhibition growth due to various extracellular compounds, where B61 (*Bacillus aneurinilyticus)* and P39 (*Pseudomonas luteola*) and P70 (*Pseudomonas fluorescens)* were the most efficient with 77 and 55.5% respectively, while B39 (*Bacillus firmus*) and P41 (*Pseudomonas luteola*) were the most efficient by volatile compounds with 70.5 and 77.5% respectively. Our results indicate that these bacteria isolates can be used in the biocontrol of *Fusarium oxysporum f. sp. ciceris.*

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Introduction

Chickpea (*Cicer arietinum* L.) is an important pulse crop grown and consumed all over the world, especially in the Afro-Asian countries (Jukanti et al., 2012). Fungal plant pathogens are among the most important factors that cause serious losses to agricultural products annually (Ekundayo et al., 2011). Chickpea production is severely limited by Fusarium wilt which is caused by *F. oxysporum* Schlechtend. Fr. f. sp. *ciceris* (Padwick) Matuo and K. Sato. (Jalali and Chand, 1992). *Fusarium* wilt is a serious disease threat, especially in low rainfall areas, where weather conditions are favourable for disease development. From 33 countries of

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the world has been reported (Nene et al., 1996) causing 10–15% yield losses annually (Singh and Dahiya, 1973) depending upon the environmental conditions. The disease is more prevalent in the Indian subcontinent, United States, Tunisia, Algeria, Turkey, Ethiopia, Spain, and Mexico (Halila and Strange, 1996; Labdi, 1990; Nene et al., 1989; Westerlund et al., 1974). In severe cases, yield losses riches 100% under favourable conditions in chickpea (Landa et al., 2004). Other species and formae specialis of *Fusarium* also cause wilt in chickpea and produce mycotoxins (Gopalakrishnan et al., 2005), which markedly reduce the potential of crop rotation as a disease management strategy. *Fusarium oxysporum* f. sp. *ciceris* (FOC) may survive in soil and on crop residues as chlamydospores for up to six years in the absence of susceptible host, and spread by means of both soil and infected seed (Haware et al., 1978).

It is difficult to manage the disease either through crop rotation or application of chemicals because of soil nature persistence and its capacity to survive for long time even in the absence of host (Haware et al., 1996). Efficacy of wilt management was improved when bio-control agents were combined with cultural practices such as sowing date (Landa et al., 2004). Biological control provides an alternative to the use of synthetic pesticides with the advantages of greater public acceptance and reduced environmental impact (Reino et al., 2008). However, management of fungal diseases using antagonistic microorganisms, known as biological control, has been the focus of intense research worldwide (Killani et al., 2011). The use of bacteria as biocontrol agents of soil borne plant pathogens, as an alternative or complementary strategy to physical and chemical disease management, has been investigated for over 70 years (Weller, 1988). The lack of consistency in performance of beneficial bacteria such as *Pseudomonas* spp. or *Bacillus* spp. under field conditions has limited their use in commercial agriculture (Raaijmakers et al., 2002). Much of that inconsistency has been attributed to variability in physical and chemical properties within niches occupied by biocontrol agents that affect both colonization and expression of biocontrol mechanisms such as antibiosis, parasitism, competition and induced resistance.

Various mechanisms are involved in the biological control of fungal pathogens by Plant Growth Promoting *Rhizobacteria* (PGPR). These mechanisms include the production of secondary metabolites such as antibiotics, siderophores, hydrolytic enzymes, volatile extracellular metabolites, hydrogen cyanide and competition for nutrients, promotion of plant growth and, finally, induced resistance within the plants (Moeinzadeh et al., 2010; Kloepper et al., 1992)

Therefore, biological control offers potential for suppression of *Fusarium* wilt under field conditions, particularly when used in combination with cultivars with partial resistance to the disease and choice of sowing date. Species of *Bacillus* have also been known to produce compounds which promote plant growth directly or indirectly, hydrogen cyanide (HCN), siderophores, indole acetic acid (IAA), solubilize phosphorous and antifungal activity (Saharan and Nehra, 2011; Wahyudi et al., 2011; Godinho et al., 2010). The objectives of this research were: (1) to characterize and select *Pseudomonas* and *Bacillus* isolates from rhizospheric and rhizoplanic soils infested with chickpea wilt, and (2) to determine their antagonistic activity *in vitro* in dual cultures against *Fusarium oxysporum ciceris*.

Material and Methods

Preliminary screening

One hundred and forty for bacterial isolates were tested for their ability to produce antifungal substances against *Fusarium oxysporum* f. sp. *ciceris* using a dual-culture *in vitro* assay on PDA plates. Twenty µl of each bacterial suspension (10^8 cfu/ml) was placed on the plate. After 48h incubation at 28°C, a single 6 mm diameter mycelial disc was placed at the extremity of plates. Then, plates were incubated at 27-29 °C in darkness and after 5 days the growth diameter of the pathogen (distance between the point of placement of fungal disk and actively growing edges of the fungus) was measured. The percentage of growth inhibition was calculated using the method described by Erdogan and Benlioglu (2010). This experiment was conducted twice. Bacteria with inhibitory potential were selected for further experiments.

Identification of bacterial antagonist

Initially, the selected isolates were identified based on gram positive, spore forming, and fluorescent pigment production, aerobic or anaerobic growth. To identify *Pseudomonas* species, oxidase, catalase, amylase, protease, cellulase, indole acetic acid, lipase and gelatinase, growth at 41°C, growth at 4°C tests were further performed. To identify *Bacillus,* motility, growth at 45˚C, anaerobic growth in glucose broth, were assessed (Shaad, 1988). The Analytical Profile Index (API), particularly API 20E and API 50CHB were used as supplementary tests, for the identification and differentiation of *Pseudomonas, Bacillus* and related species, respectively (Logan and Berkeley, 1984).

Protease production

Bacterial isolates were tested for protease production by growing them on skim milk agar (SKM) (Chantawannakul et al., 2002). The ability to clear the skim milk suspension in the agar was taken as evidence for the secretion of protease. Non- bacteria inoculated plates were used as control.

Hydrogen cyanide production

Production of hydrogen cyanide was determined on nutrient agar medium+ 4.4g of glycine. 100 µl of bacterial culture (48 h) were streaked on the surface of medium, and then sterilized filter papers were soaked in 2.0% Na_2CO_3 in 5.0% (w/v) picric acid and placed in the upper lid of the Petri dish. The Petri dishes were sealed with parafilm and incubated at 30 °C for 4 days. A change in the colour of the filter paper from yellow to reddish brown was accepted as an index for cyanogenic activity. Non - inoculated plates with bacteria was used as control (Alstrom, 1987).

Indole acetic acid production (IAA)

The production of IAA was determined as described by Bric et al. (1991). Bacterial strains were inoculated into nutrient broth (peptone, 5 g; yeast extract, 1.5 g; beef extract, 1.5 g; and NaCl, 5 g; each per liter) and incubated at 30 °C for 5 days. A 5 ml culture was removed from each tube and centrifuged at 10,000 rpm for 15 min. An aliquot of 2 ml supernatant was transferred to a fresh tube to which 100 µl of 10 mM orthophosphoric acid and 4 ml of reagent (1 ml of 0.5 M $FeCl_3$ in 50 ml of 35% $HClO_4$) were added. The mixture was incubated at room temperature for 25 min, and the absorbance of pink colour developed was read at 530 nm using a spectrophotometer.

Production of volatile antibiotics

Firstly, 100 µl of bacterial suspension (1×10^7 cfu/ml) from each isolate were sprayed on the surface of a Petri plate containing nutrient agar medium and incubated at 27-30 °C for two days. In another Petri plate containing PDA medium, a 5 mm disk of a 7 days-old pure culture of *Fusarium oxysporum* f. sp. *ciceris* was placed at the centre. Then both half plates were placed face to face preventing any physical contact between the pathogen and the bacterial suspension. Plates were sealed with parafilm. In the control plates, bacterial suspension was replaced with sterile water. Plates were incubated at 27-29 °C for 5 days and the percentage of inhibition zone was calculated for each isolates (Fiddaman and Rossall, 1993). For each treatment, there were four replicates and the experiment was repeated twice.

Data analysis

The results obtained were statistically processed through the analysis of variance ANOVA and B Tukey test at *P<0.05 and **P<0.01 to evaluate the significance between treatments. Correlation matrices have also been developed to define the interactions between the various parameters studied using SPSS version 18.0 and Microsoft Excel software 2010.

Results

Bacterial isolates Screening and characterization

Sixteen isolates for each genus (*Pseudomonas* and *Bacillus*) out of one hundred and forty for bacteria strains isolated from chickpea rhizosphere have shown substantial inhibition zones against and revealed a high antifungal activity against *Fusarium oxysporum* f. sp *ciceris* in *in vitro* tests (Figure 1). Based on biochemical, physiological and morphological properties, selected isolates were identified as *Bacillus subtilis (*B40, B45, B48, B62 and B65), *Bacillus circulans* (B72 and B73), *Bacillus lentus* (B79, B69, B53 and B41), *Bacillus aneurinilyticus* (B64, B61 and B47), *Bacillus firmus* (B39) and *Bacillus licheniformis* (B59) (Table 1). Three species of *Pseudomonas* were identified: *Pseudomonas aeruginosa* (P29, P37, P44 and P50), *Pseudomonas luteola* (P31, P36, P39, P41, P53, P59, P61, P64 and P65) and *Pseudomonas fluorescens* (P66, P70 and P45) (Table 2).

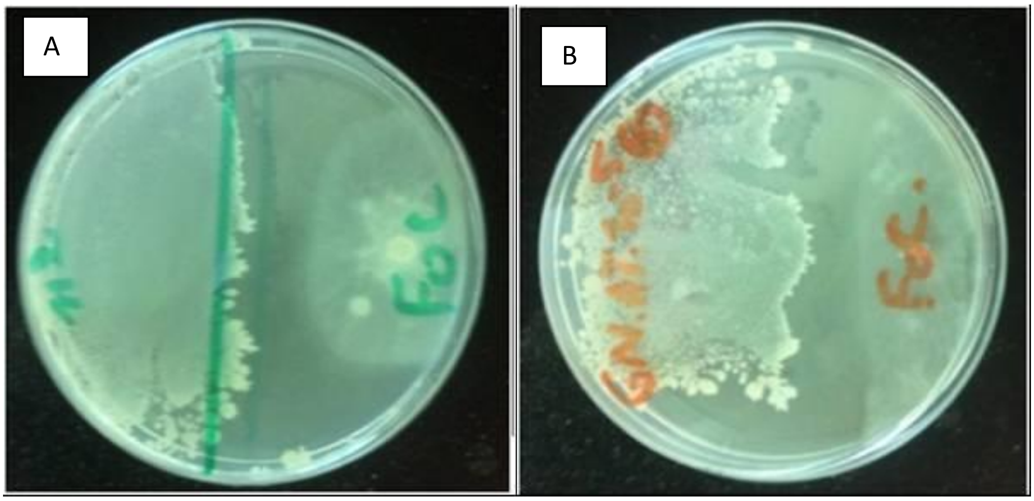

Figure 1. Inhibition of mycelium growth of *Fusarium oxysporum* f. sp. *ciceris* by bacterial isolates on PDA media by dual-culture assay (A *Pseudomonas*; B *Bacillus*).

Table 1. Antimicrobial metabolites activities of *Bacillus* isolate against *Fusarium oxysporum* f. sp. *ciceris*

Isolates	DC (%)	IZ (mm)	VC (%)	HCN	Prot	Gel	Amy	Cel	IAA	Lip	Cat
B39	70,5	9	70.5	++	+++	-	+	-	+	+	+
B40	69,5	5	60.5	++	+++	+	+++	+	+	-	+
B41	59,5	7	30	++	+++	-	+	++	+	+	+
B45	64	12	60.5	++	+++	+	+++	+	+	-	+
B47	55	10	35	+	+++	+	+++	++	-	-	+
B48	48	7	57	++	+++	+	++	++	+	-	+
B53	40	10	50	++	++	-	++	-	++	+	+
B59	54,5	5	14.5	++	+	-	+++	+	+	+++	+
B61	77[c]	3	9	-	++	+	+++	++	+	-	+
B62	45	1	20	+	+++	+	+++	+	+++	++	+
B64	68,5	7	46.5	+	+	-	+++	-	+++	+	+
B65	61	10	55	+	++	-	+++	-	+	+	+
B69	52,5	5	15.5	++	+++	+	+++	+	+	++	+
B72	60	5	50	+	+	+	++	++	+	++	+
B73	64,5	7	40.5	+	+	+	+++	+	+	+	+
B79	64,5	4	34	-	+++	+	++	++	++	-	+
Average	59,63	6,69	37,78								
Gaptype	9,99	2,96	16,55								

DC = Percent growth inhibition in dual culture method, IZ : diameter of Inhibition Zone in dual culture, VC= Percent growth inhibition in volatile compound, HCN : Cyanid Hydrogen, Prot : Protease, Gel : Gelatinase, Amy : Amylase, Cel : Cellulase, IAA : Indole acetic Acid, Lip : Lipase, Cat : Catalase.

In vitro antifungal activity

Extracellular and volatiles compounds

In dual culture test, *Bacillus* strains showed to have more inhibition of the pathogen growth in the PDA medium than *Pseudomona*s isolates. Indeed, with extracellular compounds diffused in the PDA medium, *Bacillus* strains (Table 1) gave an average of inhibition equal to 59.63 ± 9.99% vastly superior to 47.16 ± 9.32% recorded with Pseudomonas strains (Table 2). While, with volatiles compounds, *Pseudomonas* appears to be more effective in inhibition of the pathogen mycelium development. Thus, we obtained 42.15 ± 22.77% of mycelial growth inhibition by *Pseudomonas* isolates (Table 2) compared to 37.78 ± 16.55% recorded by *Bacillus* isolates (Table 1). If we look to gap-type calculated (22,77% and 16,55%), we must note here the wide intraspecific variability in both genera studied, particularly with volatile compounds it is more pronounced.

Table 2. Antimicrobial metabolites activities of *Pseudomonas* isolate against *Fusarium oxysporum* f. sp. *ciceris*

Isolates	DC (%)	IZ (mm)	VC (%)	HCN	Prot	Gel	Amy	Cel	IAA	Lip	Cat
P29	52	2	45	+	+++	+	-	-	++++	+	+
P31	55	18	21	+	+++	-	-	-	+	-	+
P36	41	10	37	+	-	-	+	+	++	+	+
P37	49	7	39.5	+	+	-	-	-	++++	-	+
P39	55,5	18	68	+	-	+	+	++	+	-	+
P41	45	7	77.5	++		-	+++	-	++++	+	+
P44	40,5	7	66	++	-	-	-	+	+	-	+
P45	46,5	0	66	++	+++	+	+++	+	+	+	+
P50	49	7	17	+	-	-	-	++	-	-	+
P53	71	8	52	+	+	+	+++	-	+	+	+
P59	34,5	1	4	+	++	-	-	++	-	-	+
P61	40,5	4	13	+	+	-	++	+	++++	++	+
P64	45,5	8	72	+	+	-	++	+	++	-	+
P65	38	15	9.5	++	+	+	-	+	+++	++	+
P66	36	10	42	++	++	+	+++	-	+++	+	+
P70	55,5	6	45	+	++	+	+++	-	+++	+	+
Average	47,16	8,00	42,15								
Ecartype	9,32	5,35	22,77								

DC = Percent growth inhibition in dual culture method, IZ : diameter of Inhibition Zone in dual culture, VC= Percent growth inhibition in volatile compound, HCN : Cyanid Hydrogen, Prot : Protease, Gel : Gelatinase, Amy : Amylase, Cel : Cellulase, IAA : Indole acetic Acid, Lip : Lipase, Cat : Catalase.

As regards the connection between the percentage inhibition of mycelial growth and the inhibition zone, the results obtained do not show a direct bond because the largest zone of inhibition was not observed with the strains giving the higher level of inhibition either with *Bacillus* or *Pseudomonas* strains. Indeed, it was the strain B45, P31 and P39 that have gave the high read inhibition zones with 12 and 18mm respectively (Table 1 and 2). We can conclude, the general inhibition is due to the combined effects of several extracellular and volatile compounds.

Interspecific inhibition efficiency

In the case of *Bacillus* genera, the most efficient strains in the inhibition of mycelial growth were B61 with 77% of efficiency in extracellular compounds and B39 with 70.5% of efficiency in volatiles compounds (Figure 2). In the case of Pseudomonas, P53 was the most efficient in extracellular compounds (71%) and P41 (77.5%) in volatiles compounds tests (Figure 3). Regarding the interspecific efficiency, strain identified as *Bacillus firmus* appears as the more efficient (70,5%) in the inhibition of mycelia growth of *Fusarium oxysporum* f. sp. *ciceris*, both in extracellular and volatiles compounds tests. But the failure to identify a single strain, these results should be confirmed by interspecific comparison tests. With regard to Pseudomonas, there is very little difference between the three species identified by studying the effect of extracellular compounds, while in the case of volatile compounds, *Pseudomonas fluorescens* isolates seem to be more efficient with an average of 51 % of efficiency.

Both *Bacillus* and *Pseudomonas* isolates has gave a significant effect at 1% level according Tukey's test in inhibiting mycelia growth by extracellular compounds. These results were traduced by the appearance of three homogeneous groups and the distinction of B53, P31, P39, P53 and P70 isolates.

Production of antifungal metabolites

In vitro test using 32 bacterial strains against *Fusarium oxusporum fs ciceris* showed that different strains exhibited different combinations of antimicrobial metabolites. If all *Bacillus* strains have produced protease (Figure 4), amylase, catalase and Indole acetic acid excepting B47 isolate, however, they showed a great variability in the production of gelatinase, cellulase and lipase as an enzyme products and HCN as a volatile compound (Table 1). With regard to Pseudomonas strains, except catalase and HCN which all strains gave a positive reaction, we note a high qualitative and quantitative variability between species and strains of Pseudomonas, in protease production (Figure 4), gelatinase amylase, lipase and IAA products (Table 2).

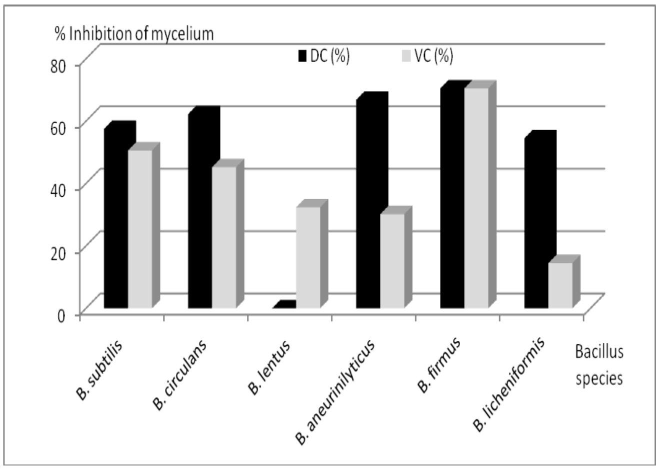

Figure 2. Inhibition of mycelium growth of *Fusarium oxysporum* f. sp. *ciceris*, by extracellular and volatiles compounds of *Bacillus* species.

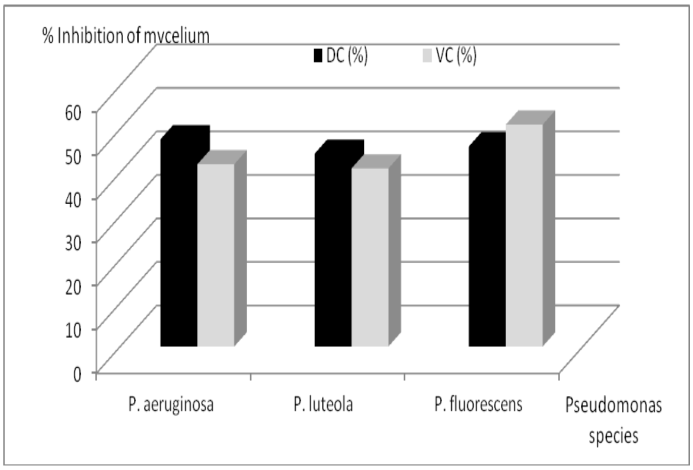

Figure 3. Inhibition of mycelium growth of *Fusarium oxysporum* f. sp. *ciceris*, by extracellular and volatiles compounds of *Pseudomonas* species.

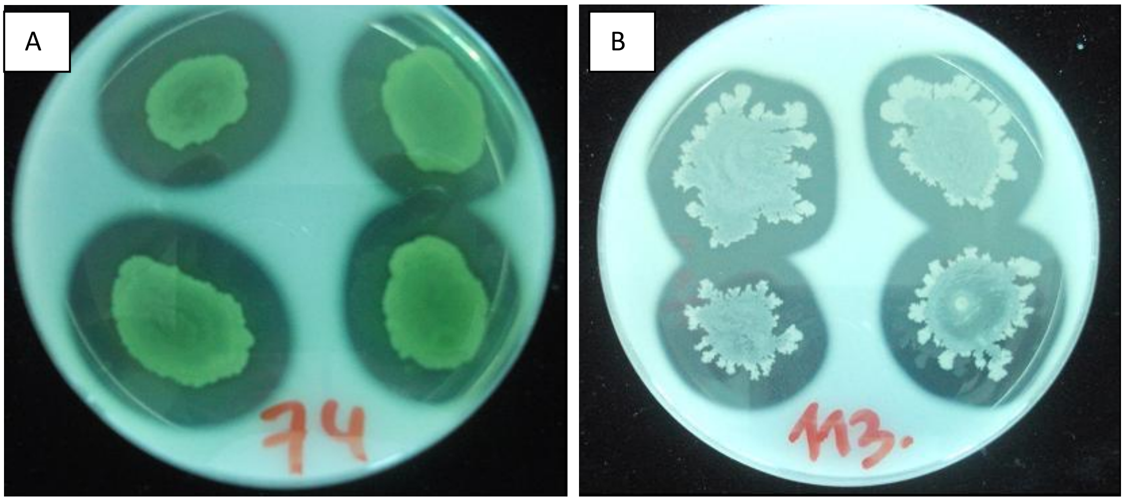

Figure 4. Enzyme activities: Protease production by bacterial isolates on PDA media (A *Pseudomonas* isolate; B: *Bacillus* isolate).

In the case of Bacillus strains, ANOVA analysis gave is a positive correlation between mycelia growth inhibition and inhibition zone, as well as for enzymatic activity between amylase and gelatinase. While, in the case of *Pseudomonas* strains, it has registered several positive correlations among different biochemical activities, example: the amylase is positively correlated with gelatinase and lipase, whereas the production of IAA is correlated with the cellulase and lipase production. From the principal components analysis (PCA), it emerges an entirely consistent correlation between biochemical tests and identification of bacterial species by API 50 CH and API 20NE.

Bacterial antagonism towards Fusarium species and strains

The results obtained show a great variability in inhibiting mycelial growth of the three FOC strains tested. Indeed, the results of direct confrontation in dual cultivation give the isolate FOC1 of the pathogen as the most sensitive fungal strain to antagonism of Bacillus isolates with an average reduction of mycelial growth equal to 58.16%. In addition, the rest of the Bacillus isolates do not exhibit a great difference in the effectiveness of the antagonism because the standard deviation is equal to 10.26 between the four deviations calculated. In contrast, the FOC2 behaves very differently when it is confronted with the Bacillus isolates, giving an average of reducing in mycelial growth equal to 40.87%. We have obtained zero inhibition with three strains of *Bacillus* ie B48, B65 and B69. This has resulted in a standard deviation of about 23.26. The FOC3 and *Fusarium solani* isolate gave intermediate sensitivity resulted in average reduction of mycelial growth of about 43.81% and 49.47%, respectively (Figure 5, 6).

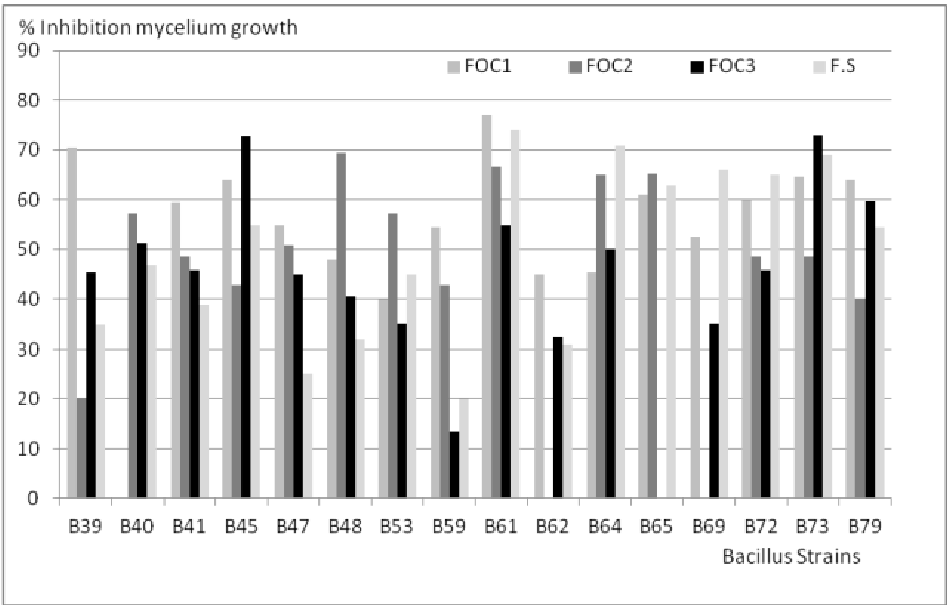

Figure 5. *Bacillus* strains antagonistic effect on the hyphal growth in dual culture of *Fusarium oxysporum* f. sp. *ciceris* *s*trains (FOC1, FOC2 and FOC3) and *Fusarium solani*.

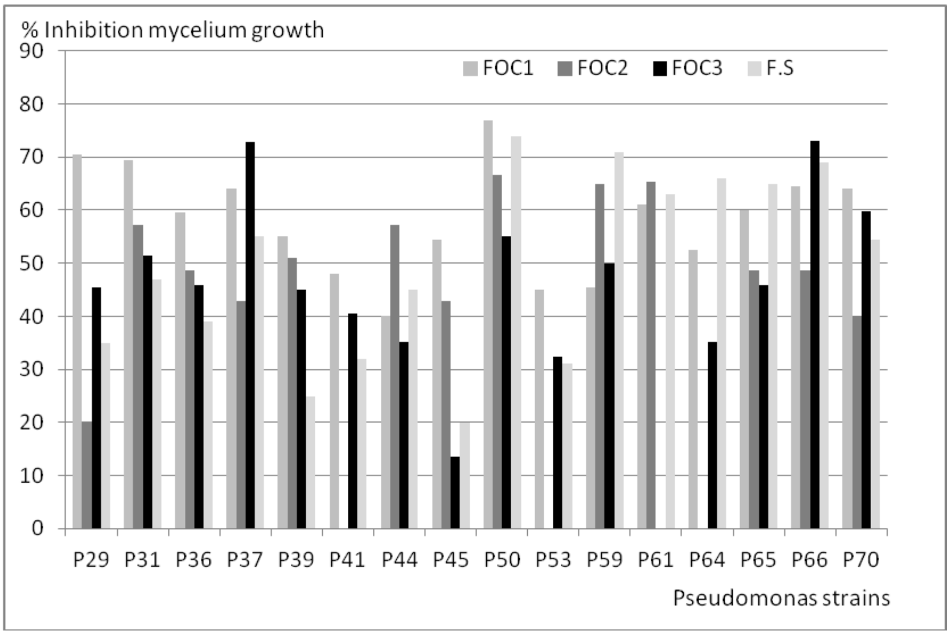

Figure 6. *Pseudomonas* strains antagonistic effect on the hyphal growth in dual culture of *Fusarium oxysporum* f. sp. *ciceris s*trains (FOC1, FOC2 and FOC3) and *Fusarium solani*.

Tukey's test provides a significant effect at 1% and 5% level in the inhibition of mycelia growth with *Pseudomonas* and *Bacillus* isolates, respectively; thus resulting in the emergence of three distinct groups for each bacterial genus. While the effect of volatiles compounds was not significant at 5% level, that's mean, there is little quit diversity between strains tested.

Discussion

Fusarium oxysporum f. sp. *ciceris* is economically significant disease agent on chickpea. Due to the soil-borne nature of the disease, chemical applications for controlling the disease are rarely successful. Inconsistencies in biocontrol under varying environmental conditions have been a common limitation of soil borne pathogens. The present research was conducted to evaluate the efficacy of *Pseudomonas* and *Bacillus* species and strains against *Fusarium oxysporum* f. sp. *ciceris*. The bacterial strains are in most cases correlated and more efficient in the inhibition of the mycelail growth of Fusarium oxysporum f. sp.ciceris isolates, if they have the same agro-ecological origin; this makes them very effective in the biocontrol. Our results corroborate with those of Weller (1988) which states that the use of rhizobacteria in the control of plant diseases is most effective when rhizobacteria were isolated from the same rhizosphere of host plant. In this study, all bacterial isolates were selected from rhizosphere of chickpeas fields. Some bacterial isolates showed high inhibition activity on pathogen, whereas others showed only mild or no activity at all. About 39% (57 of 144) of isolates showed antagonistic activity against *Fusarium oxysporum* f. sp. *ciceris* in *in vitro* tests and giving more than 30% of inhibition of mycelia growth. The rest of isolates tested exhibited no or weak antagonistic activity against the pathogen on PDA plates assay. Similar results have previously been reported (Erdogan and Benlioglu, 2010; Tjamos et al., 2004; Khot et al., 1996).

Reduction of fungal growth by certain PGPR and formation of inhibition zones were presumably due to the antifungal substances and/or cell wall degrading enzymes; released by the bacteria into the culture medium (Fatima et al., 2009). Also, Sarhan et al. (2001) and Montealegre et al. (2005) pointed that the cell free culture filtrate of *B. subtilis* inhibited the mycelial growth, radial growth, and spore germination and germ-tubes length of *F. oxysporum* f.sp. *ciceris*. Many strains of *Bacillus* strains have been found to be potential biocontrol agents against fungal pathogens. This antifungal action involves the production of antibiotics, especially within soil microsites (Fravel, 2005).

Cyanide is a toxic and dreaded chemical produced by many rhizobacteria. Some bacteria synthesis it, others excrete it and yet others metabolize it in other to avoid predation and competition (Zeller et al., 2007). All *Bacillus* species and strains efficiency of inhibition of mycelium growth is due to the extracellular products than volatiles compounds. It has been reported that *Bacillus* strains have been able to inhibit the growth of a variety of fungal pathogens because of their ability to produce a vast array of antibiotics such as Zwittermicin, Bacillomycin, Fengycin, Bacilysin and Difficidin (Athukorala et al., 2009; Chen et al., 2009). While with *Pseudomonas* species and strains, *Pseudomonas aeruginosa* and *Pseudomonas luteola* join *Bacillus* in their action mode, whereas *Pseudomonas fluorescens* inhibits the development of the mycelium much more with volatile compounds. These results are in agreement with the research previously carried out by Romanenko and Alimov (2000). Production of Hydrogen cyanide in *Bacillus* is about 50% in both rhizospheric soils and nodules compared to *Pseudomonas* that is over 80% (Ahmad et al., 2005). This result demonstrated that cyanide hydrogen was one of the most important volatile compounds of *Pseudomonas* spp. Plant growth was enhanced in vitro by most of the rhizospheric isolated that produced HCN (Wani et al., 2007).

The majority of selected *Pseudomonas* and *Bacillus* strains showed amylase and protease activity pronounced comparatively to other metabolites productions. The protease is known that has interference in wall degrading of fungal pathogen (Ahmadzadeh and Sharifi-Tehrani, 2009). The *Pseudomonas* isolates had shown a high level of production of indole acetic acid comparatively to *Bacillus* isolates. Xie et al. (1996) indicated that compared to other strains, *Pseudomonas* strains had higher levels of IAA production. According to Joseph et al. (2007), while working with chickpea, all *Bacillus* isolates produced IAA. Even though some microorganisms produce high concentration of auxin, that is, IAA and this helps to increase plant growth and yield in wheat crop, others producing low concentration of IAA also improve plant growth (Tsavkelova et al., 2007).

Regarding bacterial antagonism towards *Fusarium* species and strains, our results agree with Adebayo and Ekpo (2005), because *B. subtilis* inhibited fungal growth and also promoted the growth of tomato plant in screen house trial. *B. subtilis* has been shown to have a broad spectrum of antimicrobial activities over diverse fungal and bacteria pathogen (Grover et al., 2009). This may be as a result of production of antibiotic, competition with pathogen for nutrients and direct antagonism (Akhtar et al., 2010). *Bacillus* spp. are known to reduce wilting index in *F. udum*, increase plant growth and cause rapid colonization of tomato tissue in order to induce systemic resistance against *F. oxysporum* (Kloepper et al., 2004).

As regards the behavior of FOC isolates and *Fusarium solani* in direct confrontation with *Pseudomonas* isolates in dual culture, it appears that the *F. solani* was the most sensitive with 53.06% of mycelia growth reduction followed by FOC1 isolate. The FOC2 is confirmed as the isolate which has the most variable behavior when confronted with different *Pseudomonas* isolates. Indeed, we noted that three isolates (P37, P53 and P65) did not any impact on mycelia growth. Besides that, we found that *Bacillus* and *Pseudomonas* strains are more effective in inhibiting mycelia growth if they came from the same geographical area with *Fusarium* strains. In general, *F.oxysporum* f.sp *ciceris* was more sensitive to bacterial metabolites and antagonisms than *F. solani* evidenced by the inhibition percentages recorded (Mudawi and Idris, 2014).

In conclusion, this study shows that some species and strains of *Bacillus* and *Pseudomonas* are very beneficial and effective as agents of biocontrol *in vitro* tests. The most interesting bacterial strains are being evaluated *in vivo* tests in the presence of the host plant for the study of their antagonistic faculty. Research must continue to be able to produce industrially as microbial biocontrol agents enjoying the characteristic of being respectful of human health of the environment in general.

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Spatial variability of soil physical properties in a cultivated field

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Abstract

Spatial variability of soil physical properties in a cultivated field such as; bulk density (BD), penetration resistance (PNT), saturated hydraulic conductivity (Ks), field capacity (FC) and permanent wilting point (PWP), were determined by geostatistical method. While BD values varied between 1.12 and 1.41 g cm^{-3}, PNT resistance (0.66 to 1.88 MPa), clay content (31.48 to 43.97%), Ks (1.46 to 3.37 mm h^{-1}), FC (30.40 to 39.66%) and PWP (19.22 to 24.42%) values showed variations with soil cultivation. In kriging interpolation for the spatial variability of soil properties, the biggest r^2 and cross validation r^2 values were determined with spherical model for PNT, Ks, FC values, and exponential model for clay, BD and PWP. Spatial dependences of the properties, except BD, were found to be strong in the field. Ks values significantly increased with increasing BD (0.340*), and decreasing clay content (-0.905**) and PNT (-0.288*) values in the field. Spatial variations of soil physical properties in the field are generally controlled by the particle size distribution as a fundamental factor. Heterogeneity and variation of soil physical parameters in a field due to soil plowing should be taken into consideration for a successful agricultural management.

Keywords: Tillage, spatial variability, soil physical properties, kriging

Introduction

There are several spatially variable factors influencing crop yields. These are usually soil related, anthropogenic, topographic, biological, and meteorological factors (Tanji, 1990; Corwin, 2012). Knowledge of the spatial variation of soil properties is important for crop production in precision agricultural management systems. It has been known that most soil properties are spatially variable in a field (Burrough, 1993). Iqbal et al. (2005) reported that spatial variability of soil properties in any field position is inherent in nature due to geologic and pedologic soil forming factors, but some of the variability may be induced by tillage and other management practices. Benefits from soil tillage are known as i) improvement of soil-air-water relations in seedbeds, ii) control of undesired vegetation, and iii) reduction of the mechanical impedance to root growth (Gardner et al., 1999). Skuodiene et al. (2013) determined that the shallow ploughing and shallow ploughless tillage treatments contained more weed seed species in the soil compared with the deep ploughing treatment. Soil tillage practices causes changes to soil structure and hydraulic properties dynamically in space and time (Mueller et al., 2003; Strudley et al., 2008). The ordinary kriging is one of the most common methods in spatial interpolation of soil properties after estimating semivariogram parameters of soil properties using geostatistical tools (Goovaerts, 1998; Utset and Cid, 2001; Castrignanò et

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al., 2003; Zhao et al., 2009; Zhao et al., 2015). Tsegaye et al. (1998) studied the intensive tillage effects on spatial variability of soil physical properties such as; particle size, bulk density, soil strength, mean pore size and saturated hydraulic conductivity. They reported that all soil physical properties, except saturated hydraulic conductivity, were weakly spatially dependent for the 6 to 9 cm depth, and moderately spatially dependent for 27 to 30 cm soil depth. Although the major purposes of tillage are to reduce bulk density and soil strength and to control pests and diseases, soil cultivation may lead to the formation of a hard pan below the plough layer that restricts root penetration and downward movement of water, therefore zero or minimum till practices must be carried out in these areas. (Singh and Singh, 1996). Özsoy and Aksoy (2007) reported that soils, especially having vertic soil properties, must have a good and right soil management for a long term productivity. Inappropriate soil tilling and using unsuitable instruments, firstly cannot manage healthy plant growth and cause soil degradation in long time periods.

Strudley et al. (2008) reviewed tillage effects on soil hydraulic properties in space and time, and stated that zero tillage practices generally increase macropore connectivity and saturated hydraulic conductivity while generating inconsistent responses in total porosity and soil bulk density compared with conventional tillage practices. Specific management effects are often overshadowed by spatial and temporal variability, and differences in temporal variability depend on spatial locations between rows, within fields at different landscape positions, and between sites with different climates and dominant soil types. They reported that soil hydraulic properties are influenced by most tillage practices immediately, but these effects can diminish rapidly. Hangen et al. (2002) watched the infiltration of dye tracer (methylene blue) on small plots in sandy and silty loams under conventional tillage and minimum tillage. They found that dye stains were much deeper under minimum tillage than conventional tillage, indicating greater vertical connectivity of the macropore network.

The objective of this study was to determine changes in spatial variability of some physical properties of Vertic Haplustoll on a small-scale part of cultivated field by geostatistical method. Haplustolls are the great group of Ustoll suborders of Mollisols which are naturally fertile soils, because they are rich in humus that stores mineral nutrients, water and have a strong structured surface layer including high organic carbon content (Soil Survey Staff, 1999). Ustolls are common throughout the Middle and Eastern parts of Black Sea Region and Eastern Anatolia of Turkey. Soil fertility of Mollisols in Turkey are restricted by their shallow soil depth and erosion problem due to moist climate properties of the region and slope factor. Therefore, if these lands are used in agriculture, much more attention should be paid for the erosion, cultivation, fertilization and irrigation by the decision makers, planners and farmers in order to produce the new management plans economically regarding the soil type.

Material and Methods

This study was carried out on Vertic Haplustoll in the Experimental Field having a 4% slope north to south (41º21´ N, 36º10´ E) direction in Ondokuz Mayis University, Samsun-Turkey. Conventional tillage was used with a mouldboard plough at a depth of 15 cm in 4 ha size field in November 2010. Soil properties were measured in a randomly selected small-scale plot near the center of the field 20 days after soil plowing. The measurements in 49 different soil sampling points were made in a square grid at 5 m spacing in the 30 x 30 m^2 plot. A hand-pushing penetrometer (Eijkelkamp) was used for the measurements of penetration resistance with a cone diameter of 15.96 mm and the cone base area of 2 cm^2. The PNT measurements in each soil sampling point were made pushing vertically the penetrometer to the soil at an approximated speed of 3 cm/s up to 15 cm with five replicates (Bradford, 1986). After determining the bulk density (BD) by undisturbed soil core method (Demiralay, 1993), total porosity (F) was calculated using the equation; F=1-(BD/2.65). Saturated hydraulic conductivity (Ks) values of the soils were measured with the constant head method (Richards, 1954). Moisture contents at the field capacity (FC) and the permanent wilting point (PWP) were determined equilibrating soil moisture of the saturated samples on the ceramic pressured plates at 33 kPa for 24 hours and 1500 kPA for 96 hours, respectively (Tüzüner, 1990). Particle size distribution of the surface soil samples (0-15 cm depth) was determined by hydrometer method (Demiralay, 1993). Organic matter contents of the samples were analyzed by Walkley-Black method (Kacar, 1994).

The geostatistical analyses were performed with the GS+ version 9, and the correlations among the soil properties were calculated using SPSS program. The semivarince describing degree of spatial dependence of random variable Z(xi) over a certain distance was estimated from (Trangmar et al., 1985): ($\gamma(h)$)

$$\gamma(h) = \frac{1}{2N(h)} \sum_{i=1}^{n} \left[Z(x_i) - Z(x_i + h) \right]^2$$

Where γ(h) is the semivariance for the interval distance class h, N(h) is the number of pairs, Z(xi) and Z(xi + h) are the measured sample values at position i and (i + h), respectively. Models used in the study to estimate semivariograms are given in below:

Exponential model: $$\gamma(h) = \begin{cases} C_0 + C\left[1 - \exp\left(-\frac{h}{a}\right)\right] & h \le a \\ C_0 + C & h > a \end{cases}$$

Spherical model: $$\gamma(h) = \begin{cases} C_0 + C\left[\frac{3}{2}\left(\frac{h}{a}\right) - \frac{1}{2}\left(\frac{h}{a}\right)^3\right] & h \le a \\ C_0 + C & h > a \end{cases}$$

Where, C_0: nugget variance, C: structural variance, (C_0+C): sill value of semivariogram, a: range of spatial correlation (Samra et al., 1988).

Results

Descriptive statistics for the soil physical properties in 15 cm soil depth of the cultivated field are given in Table 1. In 15 cm soil depth of the cultivated field, while bulk density values varied between 1.12 and 1.41 g cm^{-3}, PNT resistance varied between 0.66 and 1.88 MPa (Table 1). Also, clay (31.48 to 43.97%), silt (14.49 to 36.38%), sand (30.11 to 47.57%), organic matter (2.03 to 2.98%), Ks (1.46 to 3.37 mm h^{-1}), FC (30.40 to 39.66%), PWP (19.22 to 24.42%), AWC (8.67 to 15.65%) and gravimetric water content (15.19 to 32.56%) values showed variations among the sampling points in the field.

Table 1. Descriptive statistics for the soil properties.

	Minimum	Maximum	Mean	Std. Dev.	CV, %	Skewness	Kurtosis
Clay, %	31.48	43.97	38.31	2.92	7.62	0.030	-0.785
Silt, %	14.49	36.38	22.54	3.42	15.17	1.266	4.907
Sand, %	30.11	47.57	39.15	3.74	9.55	0.209	-0.463
OM, %	2.03	2.98	2.52	0.23	9.13	-0.254	-0.419
BD, g cm^{-3}	1.12	1.41	1.27	0.067	5.28	0.016	-0.109
PNT, MPa	0.66	1.88	1.12	0.275	24.55	0.739	0.849
Ks, mm/h	1.46	3.37	2.28	0.52	22.81	0.267	-0.988
FC, %	30.4	39.66	34.44	2.40	6.97	0.239	-0.682
PWP, %	19.22	24.42	21.76	1.53	7.03	0.332	-1.197
AWC, %	8.67	15.65	12.68	1.86	14.67	-0.201	-0.796
W, %	15.19	32.56	24.32	3.24	13.32	-0.069	0.681

OM: organic matter, BD: bulk density, PNT: penetration resistance, Ks: saturated hydraulic conductivity, FC: field capacity, PWP: permanent wilting point, AWC: available water content, W: gravimetric water content

To evaluate the spatial variability of the soil physical properties, the exponential model for clay content, BD, PWP, and the spherical model for PNT, Ks and FC were selected with their biggest r^2 values and the smallest reduced sums of squares (RSS) values using the GS+ 9 package program (Table 2). The semivariograms of the soil properties indicated that the range in spatial correlation varied among soil properties (Figure 2). The shortest range (10.24 m) was observed for FC and the longest range (80.19 m) was observed for clay content. According to the results, the ranges of spatial influence for the soil physical properties were generally ≤ 80 m for clay, ≤ 38 m for PWP, ≤ 20 m for BD and Ks, and ≤ 12 m for PNT and FC.

Block-kriged maps of the soil properties were created by GS+ 9 program (Gamma Design, 2010), using 0.32 x 0.32 m^2 grid system with 8836 points (Figure 3). Clay content in soil generally increased in the east to west direction of the plot. On the contrary, high BD is found in the eastern part of the plot. While clay content and PNT values decreased at the center of plot, Ks values increased in the same positions of the study area. Water content at FC and PWP values increased in the south east and the western part of plot where the clay content in soil was high. The correlation matrix among the soil properties are given in Table 3.

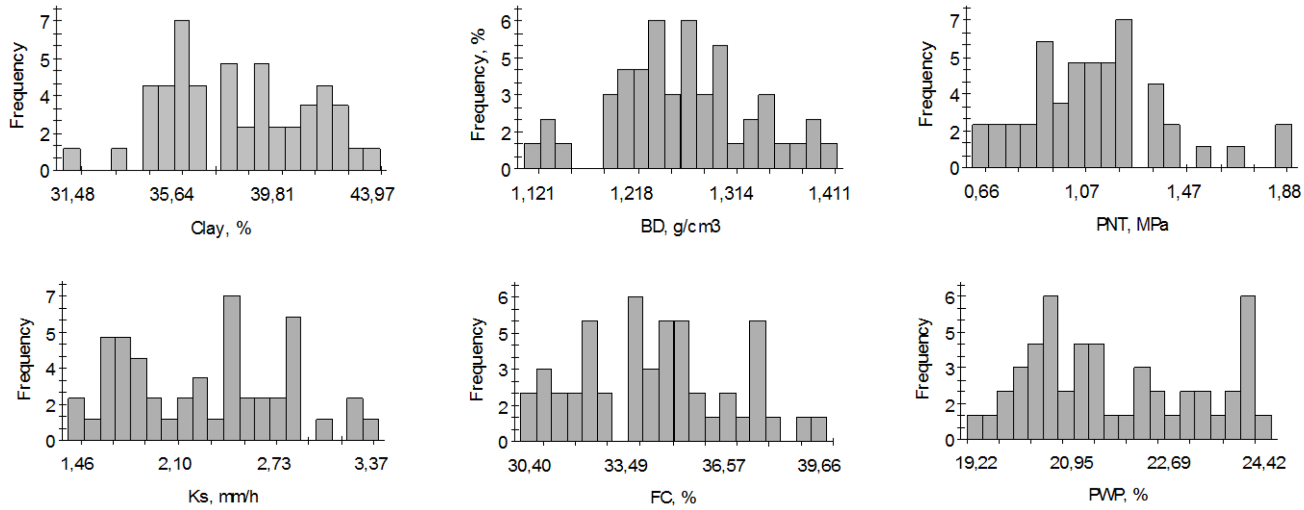

Figure 1. Frequency distribution of the soil physical properties.

Table 2. Semivariogram models and parameters for the soil properties.

	Model	Nugget, (C_0)	Sill, (C_0+C)	C_0/(C_0+C)	a	r^2	RSS	Cross Val. r^2
Clay	Exponential	3.750	28.490	13.16	80.19	0.723	16.20	0.541
BD	Exponential	0.00269	0.00599	44.91	19.67	0.786	7.68E-7	0.122
PNT	Spherical	0.0054	0.00807	6.70	12.17	0.533	3.73E-4	0.151
Ks	Spherical	0.050	0.313	15.97	18.40	0.635	9.39E-3	0.523
FC	Spherical	0.030	5.752	0.52	10.24	0.365	2.50	0.275
PWP	Exponential	0.950	4.910	19.35	37.41	0.814	0.787	0.443

Table 3. Correlation matrix among the soil properties

	Si	S	OM	BD	PNT	Ks	FC	PWP	AWC	W
C	-0.313*	-0.495**	0.365**	-0.365**	0.367**	-0.905**	0.497**	0.915**	-0.114	-0.347*
Si		-0.671**	0.157	0.149	0.032	0.596**	-0.165	-0.260	0.001	0.066
S			-0.429**	0.148	-0.316*	0.161	-0.236	-0.477**	0.089	0.211
OM				-0.286*	0.079	-0.105	0.303*	0.340*	0.111	-0.289*
BD					0.366**	0.340*	-0.761**	-0.577**	-0.505**	0.154
PNT						-0.288*	-0.139	0.209	-0.351*	-0.408**
Ks							-0.479**	-0.825**	0.063	0.259
FC								0.631**	0.768**	-0.156
PWP									-0.012	-0.414**
AWC										0.141

**correlation is significant at 0.01 level, *correlation is significant at 0.05 level. (C: clay, Si: silt, S: sand, OM: organic matter, BD: bulk density, PNT: penetration resistance, Ks: saturated hydraulic conductivity, FC: field capacity, PWP: permanent wilting point, AWC: available water content, W: gravimetric water content)

Discussion

Soil properties having a coefficient of variation (CV) between 0 and 15 % are considered least variable, 15 and 35 %, moderately variable, and bigger than 35 % highly variable (Ogunkunle, 1993). The CV values of the soil properties indicated that PNT (24.55%) and Ks (22.81%) were more variable in the field than BD (5.28%), FC (6.97%), PWP (7.03%) and clay (7.62%). Skewness and kurtosis values and frequency distributions for clay, BD, PNTR, Ks, FC and PWP indicated that the soil properties usually showed normal distribution (Table 1, Figure 1). Therefore, the original values of soil properties were not transformed. Warrick and Nielsen (1980) reported that the spatial variability of the static soil physical properties is commonly fitted to normal probability distributions; whereas the dynamic properties, related to water or solute movement, are usually lognormally distributed. Veronese-Junior et al. (2006) reported that coefficient of variation for PNT values for Brazilian Ferralsol decreased from the surface layers (52.31%) to the deepest layers (15.18%), and PNTR and moisture content values showed normal distribution. Utset and Cid (2001) found that PNT and BD in 30 x 30 m² plot of Rhodic Ferralsol are normally disturbed.

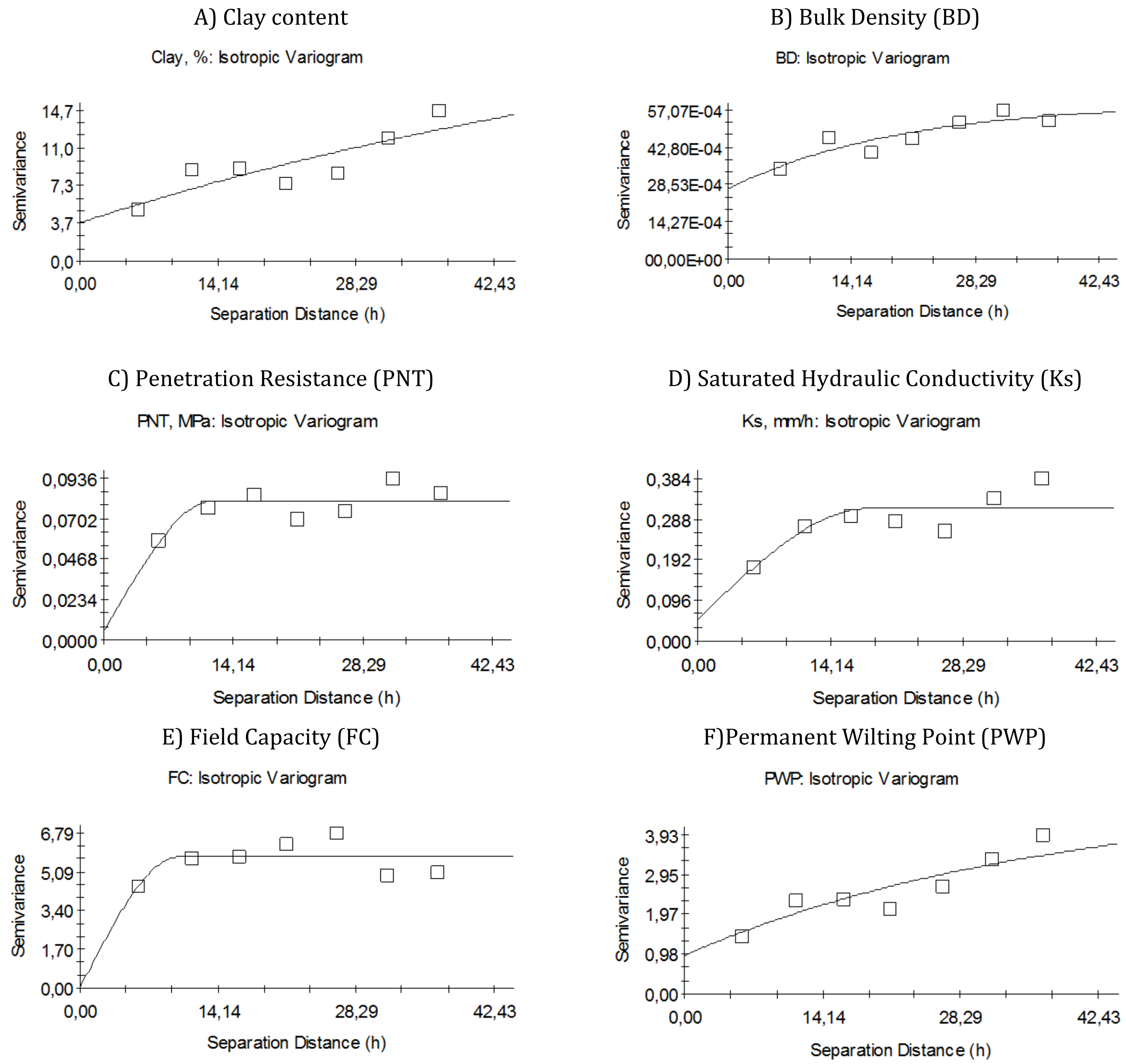

Figure 2. Isotropic variograms and models for the soil properties.

The range indicates the distance in a field where measured properties are no longer spatially correlated. Measured properties of the samples at a distance less than the range become more alike with decreasing distances between them (Tabi and Ogunkunle, 2007). The similar range for BD and Ks may be related to the interaction between soil structure and water flow. The nugget effect, which represents random variation caused mainly by the undetectable experimental error and field variation within the minimum sampling space (Cerri et al., 2004; Aşkın and Kızılkaya, 2006), was higher in clay content than in the other soil properties. Generally, the nugget values close to zero for the physical properties revealed that all variances of the soil properties were reasonably well explained at the sampling distance used in this study by the lag. A variable has strong spatial dependency if the ratio of nugget/sill is equal or less than 25%, moderate spatial dependency if the ratio is between 25 and 75%, and weak spatial dependency if the ratio is greater than 75% (Cambardella et al., 1994; Bo et al., 2003). Generally, strong spatial dependency of soil properties is related to structural intrinsic factors such as texture, parent material and mineralogy, and weak spatial dependency is related to random extrinsic factors such as plowing, fertilization and other soil management practices (Zheng et al., 2009). The ratios of nugget/sill in the soil physical properties, except BD, were less than 25% in

Table 2. Therefore, spatial dependence for these soil properties was strong. Spatial dependence of BD was moderate due to having 44.91% nugget/sill ratio. This indicates that soil plowing as an extrinsic factor weakened spatial dependency of BD in the field.

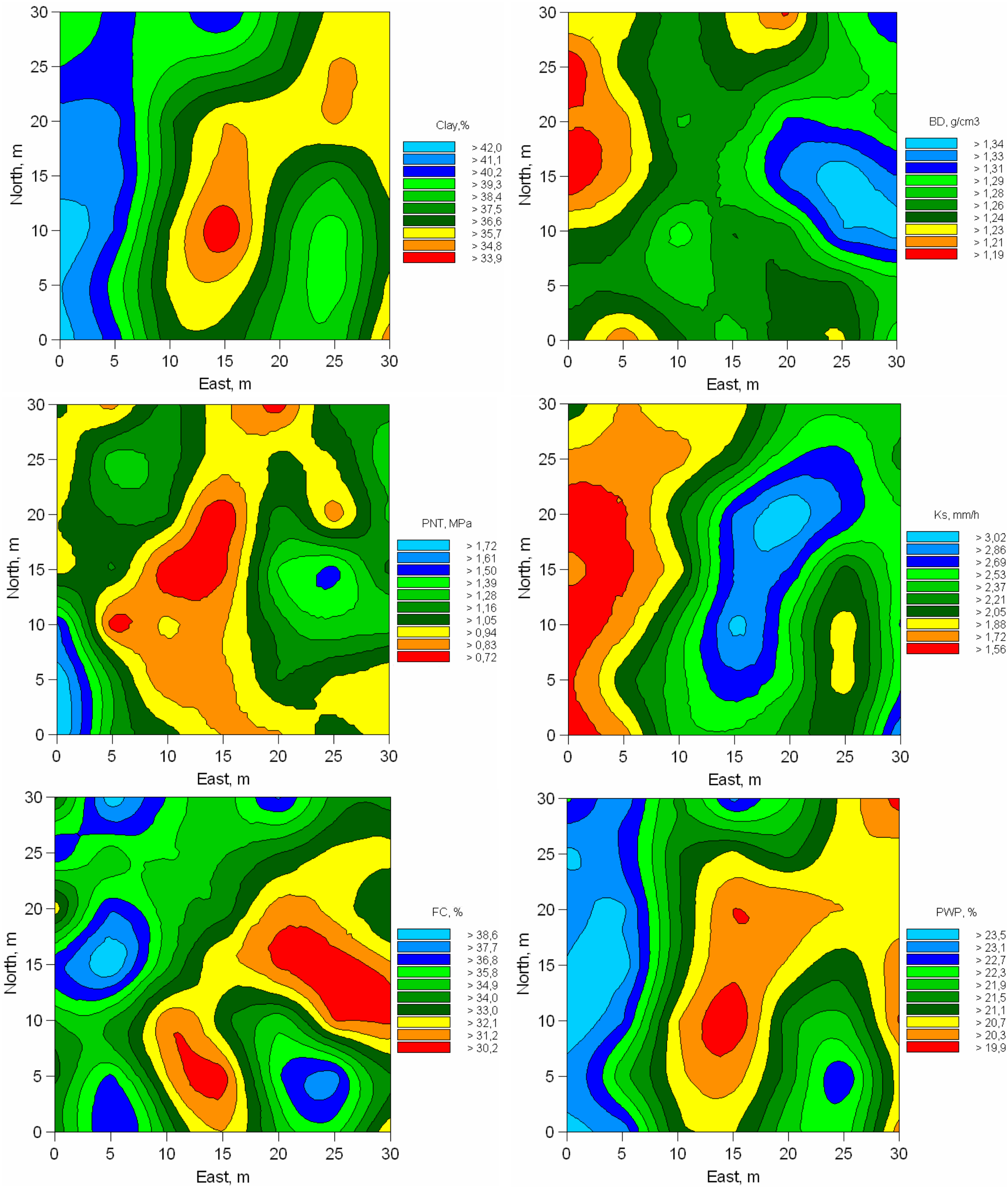

Figure 3. Block kriged maps for clay content, bulk density (BD), penetration resistance (PNT), saturated hydraulic conductivity (Ks), field capacity (FC) and permanent wilting point (PWP).

Cambardella et al. (1994) studied field-scale variability of physical and chemical soil characteristics at two sites undergoing different tillage practices in central Iowa. Soil bulk density on the conventional tillage site was moderately spatially dependent (nugget effect = 37%, range = 129 m), while bulk density at the no till site was also moderately spatially dependent for both the 0–7.5 cm and 7.5–15 cm sampled depths (nugget effects= 30% and 25%, ranges = 223 m and 115 m, respectively). In another study, Utset and Cid (2001) measured penetrometer resistance (PNT) in a deep clay soil immediately after tillage and before sugar cane seeding. They found that PNT semivariance was higher for dry soils and shows almost a pure nugget effect with an 80 m range, while irrigation yields a spatial structure with a range of about 8–10 m. PNT was spatially correlated with bulk density after irrigation. Cressie and Horton (1987) found that there was a strong spatial dependence (12 m lag distance) in infiltration rates for a silty clay loam undergoing moldboard plowing and chisel plow and no till had no spatial dependence over the same lag distance. van Es (1993) reported that the tillage effects on infiltration varied temporally within a season, and spatially within fields and between rows, under plowed and ridge-tilled corn. In another study, van Es et al. (1999) found that tillage effects were greatest for medium and fine textured soils, and spatial variability in water retention parameters was significant.

Bulk density had significant negative correlations with clay (-0.365**) and organic matter content (-0.286*) (Table 3). It is known that the variation in bulk densities is the result of differences in soil texture, organic matter contents and management practices (Wolf and Snyder, 2003). Penetration resistance is an empirical, easy and cheap measurement technique of soil strength, and widely used to assess soil compaction and the effects of soil management (O'Sullivan et al., 1987; Castrignanò et al., 2002). Critical PNT for successful root development in soil is about 1.7 MPa or 2.0 MPa (Canarache, 1990; Arshad et al., 1996). PNT values in this study reached to these critical levels in the south western and the eastern part of the plot. PNT had the significant positive correlations with clay content (0.367**) and BD (0.366**), and significant negative correlations with AWC (-0.351*) and gravimetric water content (-0.408**) (Table 3). Veronese-Junior et al. (2006) similarly reported that PNT values increased with decreasing soil moisture content. Utset and Cid (2001) determined that the PNT on a Rhodic Ferralsol over a 30 m x 30 m area after irrigation practices was considerably affected by the soil moisture condition, bulk density and micro topography.

Ks had significant negative correlations with clay content (-0.905**), PNT (-0.288*), FC (-0.479**) and PWP (-0.825**), and significant positive correlations with Si content (0.596**) and BD (0.340*) (Table 3). Candemir and Gülser (2012) determined that saturated hydraulic conductivity significantly increased with increasing sand and silt content and decreasing clay content. Iqbal et al. (2005) found that increased Ks values in surface horizons could be due to lower bulk density owing to the presence of root channels and macroporosities.

Both FC and PWP gave significant positive correlations with clay and organic matter content, and significant negative correlations with BD and Ks (Table 3). Iqbal et al. (2005) determined that the area in kriged maps with higher sand content had higher Ks values and lower clay content and lower water content at FC and PWP. In this study, spatial variations of soil hydraulic properties are generally controlled by the particle size distribution, especially clay content, as a fundamental factor.

Conclusion

According to the CV values, PNTR and Ks showed more variation in the field when comparing with the other soil physical properties. Generally, the range or the distance of spatial dependence for the soil physical parameters, except clay and PWP, varied between 10 m and 20 m. These are the distance between two sample-collecting points for soil hydraulic properties in the field. While the BD had moderate spatial dependence, the other soil physical properties had strong spatial dependence. Strong spatial dependency of the soil hydraulic properties (Ks, FC, PWP) may be attributed to clay content, and moderate spatial dependency of BD can be attributed to effect of soil tillage. There were strong relationships between the soil physical and hydraulic properties. Kriged maps illustrated positional similarity between the soil physical properties along the small scale plot of cultivated field.

As a result, soil hydraulic properties showed high spatial variability even if in 0.1 ha of small-scale plot in the field cultivated for preparing suitable seed bed and plant growth soil conditions. Therefore, expected yield from a field not only depends on soil fertility parameters, but also depends on variation of soil physical and hydraulic properties. In precision agricultural practices, heterogeneity and variation of soil physical parameters in a field due to soil plowing should be taken into consideration with other affecting factors for a successful site specific management.

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Prediction the soil erodibility and sediments load using soil attributes

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Abstract

Soil erodibility (K factor) is the most important tool for estimation the erosion. The aim of this study was to estimate the soil erodibility in Sanganeh area located in Naderi Kalat, Khorasan Razavi Province of northeastern Iran. The sediments load collected during the 17 rainfall events were measured at the end of 12 plots during 2009-2012. The K factor was calculated according to the USLE for each plot and rainfall event. The relationships between K factor and measured sediments load with soil attributes were studied. The results showed that calcium carbonate, SAR (sodium absorption ratio), silt, clay contents, and SI (structural stability index) were the most effective soil attributes for estimating the sediments load and OM (organic matter), sand, SI and calcium carbonate, silt, clay contents, and SI for K factor. The results of stepwise regression equations showed that the precision of regression equation derived from PCA for estimating the K factor and sediments load were more than ones derived from correlation test. According to the results of this research, it's recommended that PCA be applied for determination the effective soil attributes for estimating the K factor in USLE and sediments load in studied area.

Keywords: Soil erodibility, soil erosion, sediment

Introduction

Soil erosion is an important problem in agricultural lands worldwide (Kirkby and Morgan, 1980; Jianping, 1999). In many cases, soil erosion causes an almost irreversible decline in soil productivity and other soil functions (Biot and Lu, 1995; Bruce et al., 1995) and leads to environmental damage. Vegetation growth in semi-arid area is relatively slow, while rainfall events can be intense (Govers et al., 2006). In such area a sudden rainfall event may have a particularly large effect on erosion rates and erosion patterns. Rainfall characteristics, management practices, and ground cover are the key factors contributing to soil erosion (Molnár and Julien, 1998; Arnaez et al., 2007). However, very little quantitative information is available regarding the effects of rainfall intensity on soil erosion.

The Universal Soil Loss Equation (USLE) is an empirical erosion model for predicting long-term average annual soil loss resulting from rainfall events from field slopes in specified cropping and management systems and rangelands (Renard et al., 1997).

Many authors have used soil erodibility (K factor) in USLE as indicator of soil erosion (Barthès et al., 1999; Parysow et al., 2001) because soil erodibility is a measure of soil susceptibility to detachment and transport

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by the agents of erosion. The K factor is the integrated effect of rainfall and the resistance of the soil to particle detachment and subsequent transport. These processes are influenced by soil properties, such as particle size distribution, structural stability, organic matter content, soil chemistry and clay mineralogy and water transmission characteristics (Lal, 1994). It was originally derived from five variables, namely the silt plus the very fine sand content, the clay content, the organic matter content, an aggregation index, and a permeability index that have to be combined in a K factor nomograph (Wischmeier et al., 1971). A nomograph to estimate the K factor was derived by Wischmeier et al. (1971) from rainfall simulation experiments.

It was found that the K factor for a particular soil varies considerably on storm, season and year bases. The reason is mainly due to the variation in rainfall and antecedent soil conditions (Kirby and Mehuys, 1987; McConkey et al., 1997). Long term measurements from natural runoff plots are necessary to obtain a representative value for the K factor.

Soil erosion by water is a major problem in Iran. The purpose of this study was to use available data from natural runoff plots in north eastern Iran to have an approximation of soil erodibility and sediments load in the region.

Material and Methods

Description of studied area

Studied area is located in eastern of Kpoe Dagh catchment near the kalat town, is known as Shekar Kalat rangelands. The average annual precipitation and temperature is 257 mm and 15^{oC}, respectively. De Martonne's index for the area is 1.02 reflecting the semi-arid climate (Zangiabadi et al., 2010).

Determination of rainfall erosivity

The sediments load collected during the 17 rainfall events were measured in plots which had been prepared for this work from 2009 to 2012. For each rainfall, the duration of rainfall was divided into small uniform intervals and kinetic energy of each time was calculated following equation 1 (Wischmieir and Smith, 1978):

$$E = 11.87 + 783 \log I \quad (1)$$

Where E is the kinetic energy (J m^{-2} mm^{-1}) and I is the rainfall's intensity (mm h^{-1}) for each time intervals of rainfall. The total E of each rainfall event was determined based on the summation of all time intervals. Then, the maximum intensities of rainfall events for 30 (I_{30}) minute (mm h^{-1}) were calculated using time-height curves of rainfall events. Rainfall's erosivity was then calculated based on Eq. 2 below:

$$R = EI_{30} \quad (2)$$

Where R is rainfall's errosivity (MJ mm ha^{-1} yr^{-1}), E is total kinetic energy of rainfall, and I_{30} is the maximum intensity of 30 minute of rainfall events.

Determination of soil erodibility and soil attributes

USLE computes the average annual erosion on field slopes from the product of six factors representing rainfall erosivity (R), soil erodibility (K), slope length (L), slope steepness (S), cover and management practices (C), and supporting conservation practices (P). Hence the equation:

$$A = R \cdot K \cdot L \cdot S \cdot C \cdot P \quad (3)$$

where A is the computed spatial and temporal average soil loss per unit area (ton ha^{-1} yr^{-1}). Only two of the six components in the equation have units; the rainfall erosivity factor (R, MJ mm ha^{-1} h^{-1} yr^{-1}) and the soil erodibility factor (K, ton ha MJ^{-1} mm^{-1}). The K factor is defined as the rate of soil loss per erosion index unit for a specified soil as measured on a standard plot. For determining the K factor, 12 plots were selected in studied area and it was calculated according to the USLE (equation 3) for each plot and rainfall event. For this aim, R was calculated by Eq. 2 for rainfall events. A (sediments load) as a result of rainfall events was measured at the end of studied plots; L, S and C were calculated in relation to standard plot. Because of no management practices P value was equal to 1. After then, the average of K factor due to the different rainfall events was determined for each plot.

Soil samples were also collected from the 0–10 cm in each plot. Samples were air-dried and passed through a 2 mm sieve before measuring the chemical attributes. Soil physical and chemical attributes included particle size distribution by pipette method (Gee and Bouder, 1986) , organic matter (OM) by Dichromate oxidation (Walkley and Black, 1934), total $CaCO_3$ (TNV) by titration method with 6 M HCl, pH and electrical conductivity (EC) of saturated paste extract (Page et al., 1982), mean weight diameter (MWD) of wet aggregate using 4, 2, 1, 0.6, 0.25 mm sieves (Kemper and Rosenau, 1986), structural stability index (SI; Pieri, 1992) , and sodium absorption ratio (SAR) by Eqs. 4 and 5, respectively.

$$SAR = \frac{Na^{+}}{\sqrt{\frac{Ca^{2+} + Mg^{2+}}{2}}} \quad (4)$$

$$SI = \frac{(OC\%) \times 1,724}{\%\,(silt + clay)} \quad (5)$$

Where Na^{+}, Ca^{+2}, and Mg^{+2} are ionic concentrations in mMole Lit^{-1}.

In order to study the effects of soil attributes on soil erodibility and sediments load, the Pearson correlation coefficient was applied and stepwise regression was used to estimate the K factor and sediments load. For this, the soil attributes had the significant correlation with K factor and A contents in Eq. 3 were used as independent variables. In addition, principle component analysis (PCA) method was applied to select the independent variables for estimating K factor and A values, too. In this method, only the PCs with eigenvalues ≥1 were selected as independent variables. Within each PC, highly weighted attributes were defined as those with absolute values within 10% of the highest weighted loading. When more than one variable was retained in a PC, each was considered important and was retained as independent variables provided they were not correlated ($r < 0.60$) to each other (Andrews et al., 2002). Among well-correlated variables within a PC, the variable having the highest correlation sum was selected for the independent variables (Andrews and Carroll, 2001). Finally, by comparing the precision of regression and PCA method, the best method was introduced for estimating the K factor and A contents. JMP8 software was used for statistical analysis.

Results and Discussion

Statistics of studied soil properties

Some statistics of studied soil properties have been shown in Table 1. According to these data, the sand and silt contents are almost equal and the sand is mostly in the very fine fraction. The silt and very fine sand contents is slightly high, which reflects a higher sensitivity for erosion. Wischmeier and Mannering (1969) found that small increase in silt content has considerable impact on soil erodibility and sediments load. The MWD of soils has a sever limitation. Karimi et al. (2007) reported that soils with MWD value less than 0.5 mm have severe limitations for the formation of stable aggregates. Reasons of instability and sever limitation of aggregates in our soils seems to be the lack of organic carbon and presence of Na^{+} cations. Despite the fact that studied area is conserved against animal grazing and no agricultural practices are applied, but the area is located in an arid region and the vegetation is poor. The positive effects of organic carbon on aggregates stability and soil structure have been reported by Emami et al. (2012), Emami and Astaraei (2012) and Virto et al. (2011). In addition, electrical conductivity is high which may limit crop growth and organic carbon builds up and hence increases soil erosion. The mean value of SAR is also high, so it can degrade the soil structure.

Table 1. The ranges and mean values of studied soil attributes

Soil attributes	minimum	maximum	mean	Standard Deviation	CV (%)
OM (%)	0.52	2.23	1.33	0.56	36.93
Bd (g cm^{-3})	1.3	1.6	1.39	0.12	8.73
TNV (%)	1.5	10	4.3.6	2.47	55.37
EC (dS m^{-1})	2.3	10.4	5.28	2.00	37.62
SAR (-)	0.35	76.28	30.44	21.74	299.55
Sand (%)	25.42	52.24	43.33	5.95	13.81
Clay (%)	20	37	24.95	3.38	13.41
Silt (%)	23.76	51.58	31.72	1.54	17.13
MWD (mm)	0.13	0.74	0.30	0.15	60.04
pH (-)	6.99	7.96	7.36	0.08	4.14

Soil erodibility

The results of soil erodibility (K factor in USLE) calculated in 12 plots of studied area are shown in table 2. There were no conservation practices in the studied plots, therefore in equation 3 P factor was equal 1. In general, the values of soil erodibility in the area are low. This may be due to presence of vegetation, and high content of sand and clay. Vegetation tends to reduce the kinetic energy of rainfall drop impacts and as a result, soil detachment and soil erodibility decreases. With increasing sand content, the infiltration rate increases and runoff decreases (Santos et al., 2003). Furthermore, clay fractions could help decrease particle detachment; hence the soil erodibilty is decreased.

Table 2. The range and average of erodibility factor in studied plots

Plot No.	Average (ton ha MJ^{-1} mm^{-1})	Range (ton ha MJ^{-1} mm^{-1})	Standard Deviation (ton ha MJ^{-1} mm^{-1})	CV (%)
1	0. 37	0.18-0.59	0.002	68.55
2	1.76	0.99-2.3	0.016	111.78
3	2.13	1.47-4.66	0.004	84.47
4	0.41	0.14-0.84	0.003	107.6
5	0.26	0.11-0.39	0.001	87.04
6	0.20	0.12-0.30	0.002	97.76
7	3.03	1.13-5.40	0.022	88.09
8	2.49	0.97-4.69	0.023	93.19
9	1.81	0.94-3.47	0.023	128.53
10	2.75	1.09-3.51	0.022	127.85
11	0.73	0.41-0.96	0.006	104.73
12	0.29	0.12-0.77	0.002	99.76

Relationships between soil erodibility and soil attributes

The correlations between soil erodibility and soil properties are shown in Table 3. Since SAR and K factors had no normal distributions, the data for SAR and K factor were transferred to logarithmic scale and exponential function to get a normal distribution. As seen from the results, exp (K) had a negative and significant ($P < 0.05$) correlation with organic matter content, clay and soil structural stability index. Organic matter by coating the soil particles and creates a water repellent layer that prevents soil detachment and keep soil particles flocculated, therefore it decreases the soil erodibility and erosion. In our study the highest correlation coefficient was observed between K factor and clay content. Wang et al. (1994) indicated that the soil organic matter and clay contents are the principal factors that influenced soil anti-erodibility in the Loess Plateau and that the percentage of water stable aggregates was the best indicator. However, the correlations between OM and SI were also high. Kodesova et al., (2009) reported that presence of organic and clay coatings usually increase soil aggregate stability, hence soil degradation and erodibility is decreased. In our soils, sediments load had a negative and significant correlation with OM ($r = -0.71$), sand contents ($r = -0.72$), SI ($r = -0.71$) value, and calcium carbonates ($r = -0.65$) content. Sediments load and Log SAR ($r = 0.75$), and silt ($r = 0.70$) were also positively correlated.

Bonilla and Johnson (2012) by analyzing 535 soil datasets observed that soil erodibility decreases as the sand content increases ($r= −0.375$). They found no trend between clay content and the erodibility factor ($r=−0.033$) and the correlation coefficient between erodibility and silt content was higher than for the other soil particles ($r=0.607$). Similar to our results, Zhang et al. (2004) found the negative and significant correlation between soil erodibility and clay content ($r = -0.62$, $P<0.05$). According to the literatures the highest vulnerability to erosion by water occurred where soils were predominantly silty (Bonilla and Johnson, 2012; Duiker et al., 2001; Pérez-Rodríguez et al., 2007; Romero et al., 2007; Zhang et al., 2004). Similarly, Di Stefano and Ferro (2002) reported that detachment decreases as particle size either decreases or increases beyond the range of 20–200μm. Above this range, it is more difficult to detach and transport particles because of the particle mass, and below this range, cohesive forces counter particle detachment. Consequently, soil particles with diameters in the size fractions of silt, fine and very fine sand are more easily eroded. The same results were reported by Ampontuah et al. (2006), in two contrasting cultivated hill slopes of England. Their research was performed on fields containing about 67–80% silt, and were highly susceptible to water erosion. Wischmeier and Mannering (1969) found that soil content of the runoff was inversely related to organic matter content. Their analysis on silts, silt loams, loams and sandy loams textural classes showed that the inverse relation of erodibility to organic matter level was strong, but it significantly declined as the clay fraction became larger.

Table 3. The correlation coefficient between soil attributes with sediment content and soil erodibility factor in USLE

Soil attributes	OM	TNV	EC	$\log^{SAR}$	pH	BD	Silt	Clay	Sand	SI	MWD	A	e^K
OM	1												
TNV	-	1											
EC	-	-	1										
$\log^{SAR}$	-	-	-	1									
pH	-	-	-	-	1								
BD	-	-	-	-	-	1							
Silt	-	-	-	**0.71	-	-	1						
Clay	-	-	-	-	-	-	-	1					
Sand	0.60*	0.64*	-	-	-	-	-0.75**	-	1				
SI	0.99**	0.60*	-	-	-	-	-0.72**	-	0.62*	1			
MWD	-	-	-	-	-	-	-	-	-	-	1		
A	-0.71**	-0.65*	-	0.75**	-	-	0.70**	-	-0.72**	-0.71**	-	1	
e^K	-0.60*	-	-	-	-	-	-	-0.62*	-	-0.58*	-	-	1

+ OM: Organic matter, TNV: Calcium carbonate, EC: Electrical conductivity, SAR: Sodium adsorption ratio, BD: Bulk density, SI: Structural stability index, MWD: Mean weight diameter of aggregates, A: sediment content, K: Erodibility factor
++ **: significant at $P < 0.01$, *: significant at $P < 0.05$.

Our results showed that the highest correlation coefficient of sediments load is related to Log SAR. Dexter and Chant (1991) have shown that the amount of clay dispersion increased as SAR increased. In fact, due to sodium impact on clay dispersion and degradation of the soil structure, infiltration rates decreases and leads to more soil erosion. Therefore, the higher SAR in soil solution the more sediments load. Silt particles have low cohesive force and are easily transported by runoff; therefore they are more vulnerable to water erosion. On the contrary, sand content had a negative relationship with sediments load. Due to higher mass of sand particles, runoff cannot transport them. On the other hand, the presence of sand particles in soil cause an increase in macro pores, water infiltration, and consequently decrease in susceptibility to erosion and sediments load (Santos et al., 2003).

The sediments load had a negative but significant ($P < 0.05$) correlation with calcium carbonate ($r = -0.65$). Calcium cations originating from carbonate dissociation linked between organic and inorganic soil components, create cationic bridging effect (Baldock and Skjemstad, 2000), promotes aggregation, increases the soil resistance against rainfall drop impacts, splash erosion, and rainfall erosivity, and consequently, decreases the soil detachment and sediments load. Virto et al. (2011) also, reported that aggregation in many soils in semi-arid land is affected by their high carbonate contents. The role of carbonates, as a source of Ca, in promoting mineral bonds and mineral-SOM interactions through cation bridges has been described responsible for micro-aggregates formation and stability in several studies (Baldock and Skjemstad, 2000).

Estimating soil erodibilty and sediments load

Soil erodibilty and sediments load were estimated by stepwise regression. As mentioned before, soil attributes including structural stability index, sodium adsorption ratio, calcium carbonate equivalent, sand, silt, and organic matter, clay, and SI had significant correlation with sediments load, and erodibilty variables, respectively (Table 3). Therefore, they were regarded as independent variables for the above dependent variables. The results of stepwise regression analysis showed that among the independent variables TNV, log SAR, and silt attributes were effective variables for estimating the sediments load (Table 4). In addition, TNV in this equation is negative, so it has a reductive effect on sediments load, but the sign of SAR and silt contents is positive and by increasing these parameter values, the sediments load increases. Similarly, the clay and SI attributes were selected for estimating the soil erodibilty (exp K) variable (Table 4) and the negative sign shows any increase in both variables decreases that by increasing them, soil erodibility. The determination coefficient (R^2) of regression equations between measured and estimated soil erodibilty and sediments load were 0.6 and 0.74, respectively. Also, the RMSE of soil erodibility and sediment equations were 0.0074 and 0.0022, respectively. In addition the estimated soil erodibility and sediments load were significant at $P < 0.05$ and $P < 0.01$, respectively. As seen in Figure 1, the regression equation of sediments load is under-estimated and that of soil erodibility is over-estimated. The slope of linear equations for sediments load and soil erodibility variables are 0.62, and 0.41, respectively, which demonstrated that the slope of sediment equation is close to 1. In general, based on the R^2, RMSE, significance level and slope of regression line, the precision of sediment regression equation is large enough that can be reliablely predicted by TNV, SAR, and silt contents.

Table 4. Results of regression analsis for estimating the soil erodibility and sediment contents according to correlation test.

Dependent variable	Regression equation	RMSE	Prob. > F
A	A = -0.0008 – 0.0004TNV + 0.0034log(SAR) + 0.0002Si	0.0022	0.0091
Exp K	Exp (K) = 1.068 – 0.0001Cl – 0.002SI	0.0074	0.0148

A: Sediment content (tonha^{-1}yr^{-1}), K: soil erodibility factor (tonhaMj^{-1}mm^{-1}, TNV: equilibrium calcium carbonate, SAR: Sodium adsorption ratio, Si: silt percent, Cl: Clay percent, SI: Structural stability index.

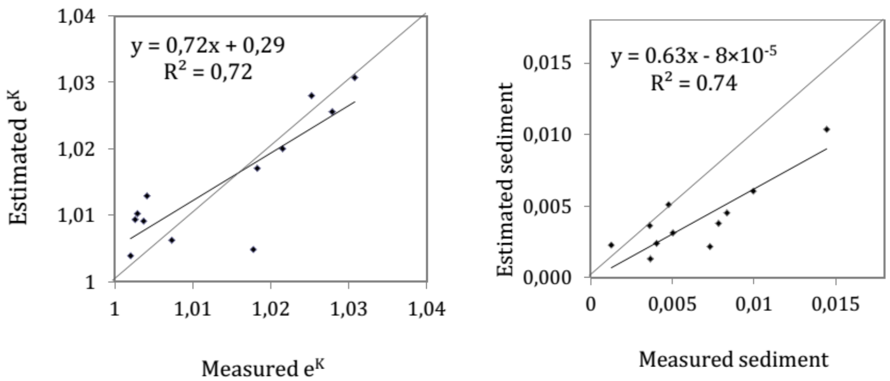

Figure 1. Results of estimated and measured soil erodibilty (left) and sediment content (right).

In addition, A and K factor were estimated based on the principle component analysis (PCA). The results of PCA are shown in Table 5. In this method, 4 principle components (PCs) which had the Eigenvalues more than 1 could explain 83.33 % of variations. The first PC with the highest loading effect and difference of less than10% could explain 44.88% of variations. The attributes of PC_1 consisted of TNV, silt, and SI which had high correlation with each other. Since these attributes had large loading effects, all of them were selected as principle variable at PC1. The second PC had 2 variables i.e. clay percent and bulk density (Bd) with the highest loading effect, which bulk density was chosen as an effective variable. In PC_3 only pH and for PC_4 EC and bulk density had the highest loading effects. Finally, 7 soil attributes i.e. silt, SI, TNV, bulk density, clay, EC and pH were selected as effective variables on sediment and soil erodibilty contents. The regression equations obtained selected based upon PCA is shown in Table 6. The results of stepwise regression equations derived from PCA showed that among the 7 soil attributes, 3 of them were selected for estimating the sediment and K factor. Hence TNV, silt, and clay percents were used for estimation the sediments load but clay and TNV attributes had the negative and silt had the positive effect on sediments load. Also silt, clay, and SI attributes were selected for estimating the K factor and all of them had the negative effect on K factor. Compared to equations of correlation test, The R^2 value of regression equation derived from PCA for estimating the K factor increased to 0.72. R^2 Value for sediment also slightly increased. In addition, RMSE values of these equations for both A and K factor decreased to 0.0068, and 0.0021, respectively. Similarly, the equations derived from PCA method were significant at $P < 0.01$ and 0.05 for estimating A and K factor, respectively. The slope of linear regression equations for both A and K factor were 0.74 and 0.71 and closer to 1. According to R^2, RMSE, and slope of linear regression equations, it can be concluded that PCA method is more precise than correlation test for estimating the A and K factor.

More detailed of regression equations clarified that silt and TNV were used for estimating the sediments load in both PCA and correlation test. TNV and silt had the reductive and incremental effect on A, respectively. Calcium carbonate may improve soil structure and decrease the soil erosion (Duiker, et al. 2001). Silt particles are sensitive to soil erosion (Wischmier and Mannering, 1969). Also clay and SI attributes were applied for estimating K factor in both PCA and correlation test methods and the reductive effect on K factor. Zhang et al. (2004) found that clay content had negative effect on K factor in USLE.

Table 5. The results of principle component analysis

PC4	PC3	PC2	PC1	Component number
1.09	1.48	1.99	4.94	Eigenvalue
9.90	13.42	18.14	44.88	Percent of Variance
83.33	64.43	63.01	44.88	Cumulative percent of Variance
	Communality			
-0.006	0.284	-0.087	0.407	TNV
-0.087	-0.175	0.361	0.333	OM
0.481	-0.290	-0.129	-0.160	EC
0.129	0.462	0.213	-0.304	Log^{SAR}
0.421	0.610	0.255	0.107	pH
-0.433	-0.094	0.571	-0.036	Bd
-0.142	0.116	0.248	-0.397	Silt
-0.385	0.241	-0.562	0.018	Clay
0.389	-0.275	0.121	0.358	Sand
-0.041	0.246	-0.046	0.419	SI
-0.235	0.016	0.106	0.358	MWD

Table 6. Results of regression equations for estimating the soil erodibility and sediment contents obtained according to principle component analysis.

Dependent variable	Regression equation	RMSE	Prob. > F
A	A = -0.0084 – 0.0008TNV + 0.0003Si - 0.0003Cl	0.0021	0.0073
Exp K	Exp (K) = 1.117 – 0.0011Si – 0.0018Cl - 0.0048SI	0.0068	0.0140

A: Sediment content ($tonha^{-1}yr^{-1}$), K: soil erodibility factor ($tonhaMj^{-1}mm^{-1}$, TNV: equilibrium calcium carbonate, Si: silt percent, Cl: Clay percent, SI: Structural stability index.

Conclusion

The results showed that calcium carbonate, SAR, silt, clay contents, and SI were the most effective soil attributes for estimating the sediments load and OM, sand, SI and calcium carbonate, silt, clay contents, and SI for K factor. The results of stepwise regression equations showed that R^2 value of regression equation derived from PCA for estimating the K factor and sediments load increased. In addition, RMSE values of these equations for both A and K factor decreased. According to the results of this research, it's recommended that PCA be applied for determination the effective soil attributes for estimating the K factor in USLE and sediments load in studied area.

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Surface soil factors and soil characteristics in geo-physical milieu of Kebbi State Nigeria

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Abstract

Soil factors and surface soil characteristics are important components of agricultural environment. They support surface and subsurface soils to perform many functions to agriculture and economic human developments. Understanding these factors would aid to the recognition of the values that our soil and land offered to humanity. It is therefore, aim of this study to visualise and examine the soil factors and surface soil characteristics in Kebbi State Nigeria. An Integrated Surface Soil Approach (ISSA) was used in the classification and description of soil environment in the study region. The factors constituted in the ISSA are important components of soil science that theories and practice(s) noted to provide ideas on how soil environment functioned. The results indicate that the surface soil environments around Arewa, Argungu, Augie, Birnin Kebbi and Dandi are physically familiar with the following surface soil characteristics: bad-lands, blown-out-lands, cirque-lands, fertile-lands, gullied-lands, miscellaneous and rock-outcrops.The major soil factors observed hat played an important role in surface soil manipulations and soil formation are alluvial, colluvial, fluvial and lacustrine; ant, earthworms and termite; and various forms of surface relief supported by temperature, rainfall, relative humidity and wind. Overall, the surface soil environment of the region was describe according to their physical appearance into fadama clay soils, fadama clay-loam soils, dryland sandy soils, dryland sandy-loam soils, dryland stony soils and organic-mineral soils.

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Introduction

Soil science has been considered as an applied science that focussed on improving the understanding of soils and how they function (Thompson and Rimmer, 2008). It is one of the objectives of this science to generate soil information that is useful in the understanding of the true nature and properties of soil (FAO, 2006). This information has been considered as part of sustainable agriculture and human development in the 21st Century (DESA, 2013).Therefore, the future of soil science emphasise the need to integrate ideas so as to obtain necessary soil information which could be use in many aspects of agriculture and sustainable economic development (Hartemink, 2006). Integrated idea through field assessment has been reported to provide adequate knowledge, detail information and overall insight tothe understanding of many important components of soil environment (Olson, 1981). The question here is 'what factors will be considered to achieve the need of soil science?'. Sometimes, answer to this type of question could be difficult primarily due to the fact that many issues and research interests are involved, although weights are always given to the

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objectives outline in a particular study. And for this, this study lays its emphasis on soil forming factors and surface soil characteristics. Information on these factors is very limited and scarce in the study region (KARDA, 1997; Usman, 2007).

The potential benefits of gathering information on surface soil characteristics and soil factors in the region is largely related to the need for informed priority to improve soil quality and environmental surroundings for diverse crop productions (Hartemink, 2006). Unfortunately, the ways of collection and management of the soil information in the study regionare not able to gather the information required for the proper management of surface soil quality. Accurate soil information about the current status of surface soil characteristic and soil factors is critical in achieving the goals of sustainable agriculture in the 21st Century (DESA, 2013). Without proper assessments of the important soil components such as surface characteristics, soil factors and land use activities, knowledge of managing soils is almost unfeasible. Without clear and sound approach, adequate and reliable soil information is unpractical. And, without an international field guides such as FAO guidelines for soil description (FAO, 2006), understanding the classes of each soil characteristics and soil factor is impossible. Therefore, it can be justified that the need for soil assessment in the study region is vital to efficient soil management and environmental conservation (Usman, 2007). Thus, the objective of the study was to assess and discuss the major classes and characteristics of surface soil features and soil factors around Arewa, Argungu, Augie, Birnin Kebbi and Dandi areas of Kebbi State, Nigeria.

Material and Methods

Study region

Kebbi State is located geographically in the north-western part of Nigeria in sub-Saharan West African region (Figure 1). The State has a total land area of 36,229 km^2 of which 12,600 km^2 is under cultivated within the latitude 10^o 30^o 5'N and longitude 3.5^o and 6^o E (KARDA, 1997). Socially and culturally, the State has been dominated by Hausa-Fulani who depend fully on crop production, cattle rearing, fishing and mining. Their population is believed to bearound 3, 630, 9313 people according to National Population Commission of Nigeria Kebbi State chapter (NPCN-KB, 2007). The land is quite important resource to million people in the State and is been utilized for many purposes – cropping, irrigation, grazing among others.

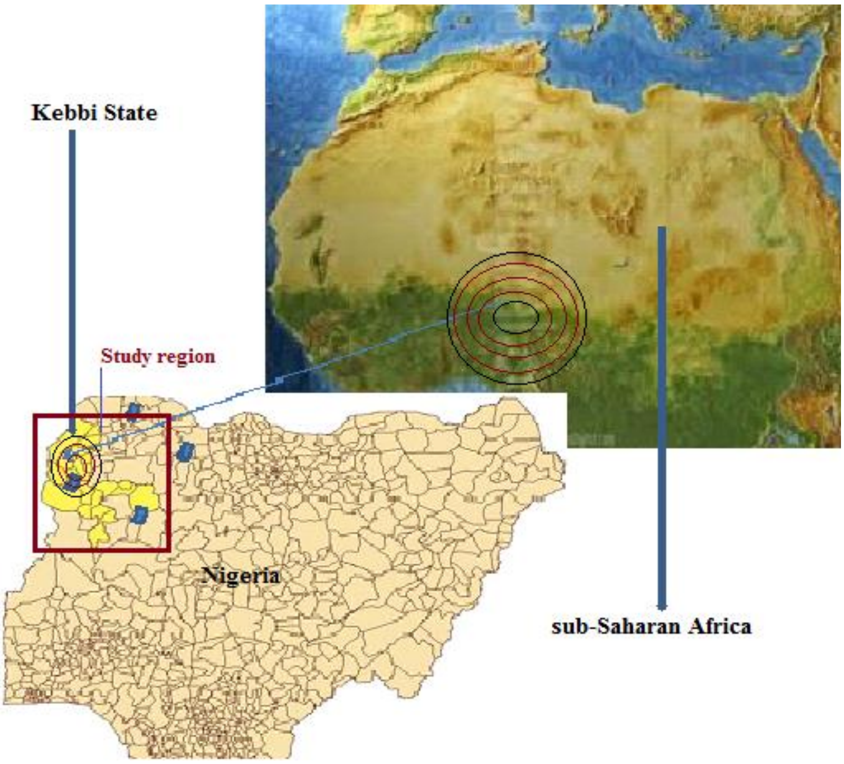

Figure 1. Location map of the study region from sub-Saharan Africa. The concentric ovals at the two parts of the map depicted the position of Nigeria and Kebbi State on the sub-Saharan African's map and Nigeria's map, accordingly. The yellow area at the map of Nigeria shows the position of the study area and the blue spots show the sites of the bordering states of Sokoto, Zamfara and Niger, consequently.

Field visit exercise

Field visit exercise was aimlessly carried out on 74 different sites between 2009 and 2011 around Arewa, Argungu, Augie, Birnin Kebbi and Dandi local government areas of Kebbi State (Figure 2). These sites were considered because of their relevant to crop production in the State. During the visit, soil factors (parent materials, organisms, topography) and surface soil characteristics were assessed and classified, accordingly. It is important to mention that priority was only given to those soil classes, which occur more often withinthe 74 areas visited. Guidelines of soil assessment by the FAO (2006), USDA-NRCS (2002) and FAO-SWALIM (2007) were employed as complete guides in the field.

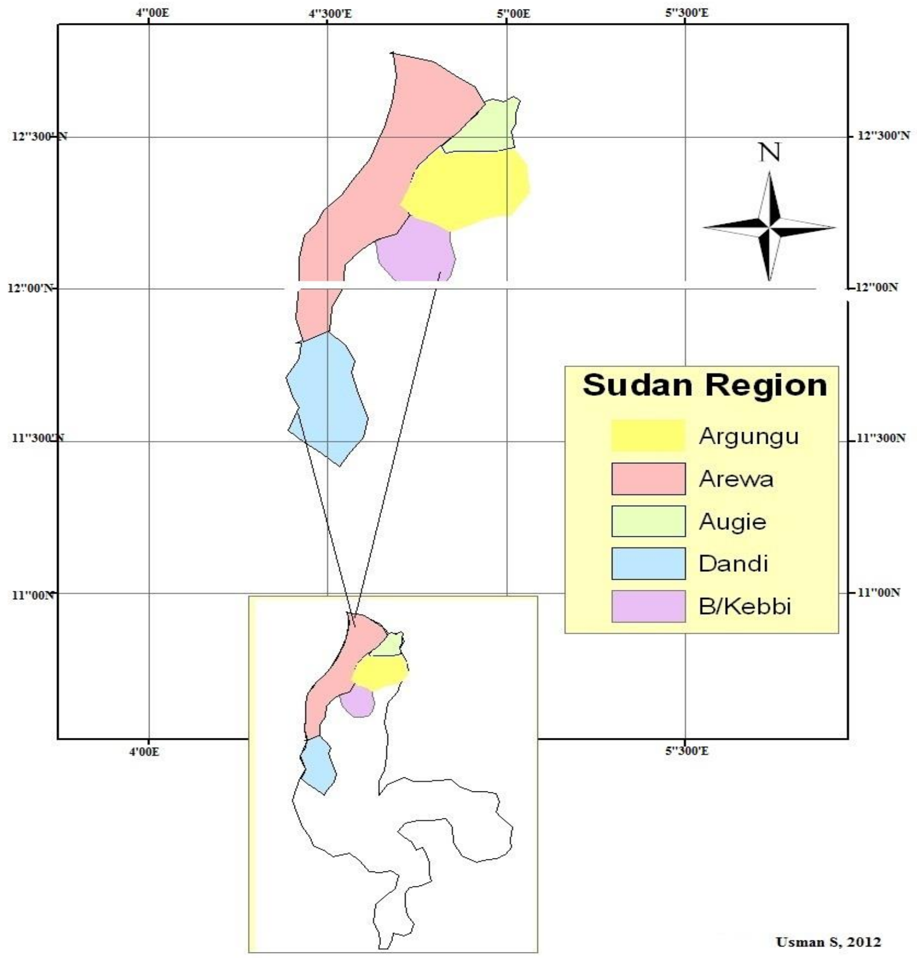

Figure 2. Map of the study sites

Soils and their geographical distributions around the 74 sites visited

The major surface soil types around the 74 sites visited in the study region are characterised as Aridisols (USDA soil order), Calcisols (FAO soil order) and Vertisols (FAO-USDA soil order) (Usman, 2013a).Aridisols and Calcisols are dryland soils described as well-drained, low organic matter, low water holding capacity and up-and-down undulating landform (Yalwa, 2008). These soils are believed to have been formed many years ago under aridity and sand dunes of sub-Saharan desert (Ahn, 1970). Geographically, they are distributed uniformly as sands, mineral stones and limestone particles covering large areas of land (Usman, 2007). The vertisols on the other hand, is a fadama soil that has been attributed with cracks during the dry season, shrink and swelling due to high clay content (Usman, 2013a).

Approach used

In order to facilitate acquisition of relevant primary information needed for this study, an Integrated Surface Soil Approach (ISSA) was designed and used from 2009 to 2011. The factors constituted in this approach were in line with the information presented in FAO Guideline for soil descriptions (FAO, 2006), Somalia Field Survey Manual (FAO-SWALIM, 2007) as well as Soils and the environment: A guide to soil surveys and their applications (Olson, 1981).

Definition of the approach

Integrated Surface Soil Approach (ISSA) is considered as direct method of visual and digital assessments of geo-physical surface soil conditions within a scope of various ideas, skills and knowledge on factors that could potentially explain the natural surface conditions of soil environment. The factors constituted in the ISSA are important components of soil science that theories and practice(s) noted to develop trust in the understanding of the overall image of soil environment (Olson, 1981; Jenney, 1994; Usman, 2013b). The concept of the approach is depicted in Figure 3.

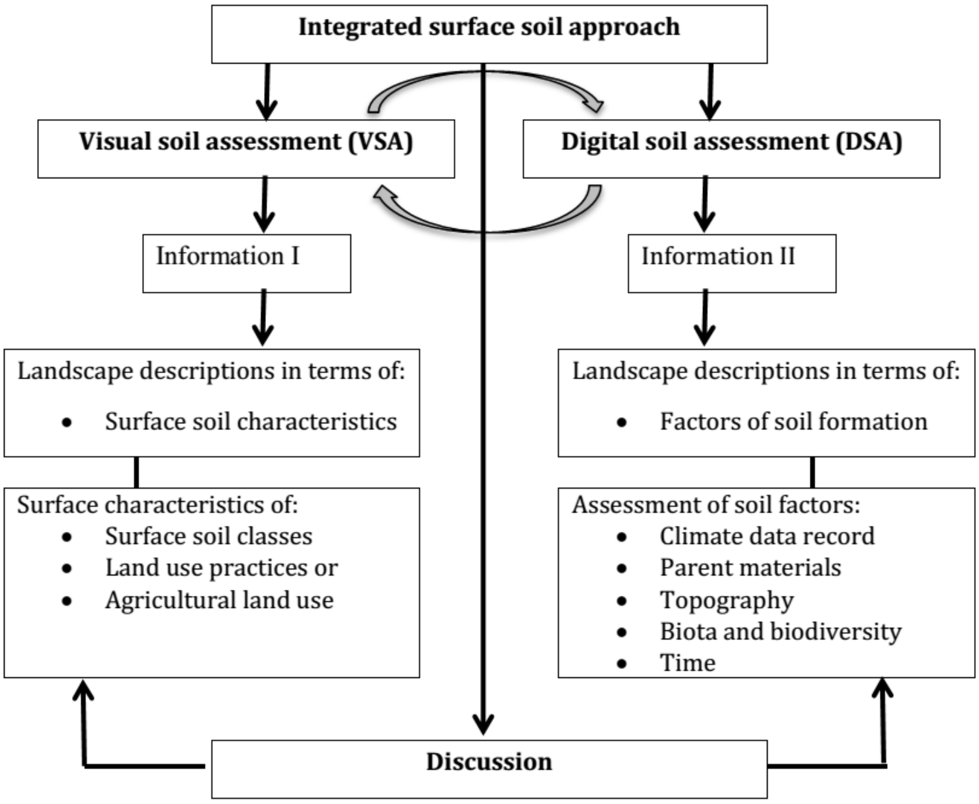

Figure 3. Integrated Surface Soil Approach (ISSA)

Figure 3 describes the conceptual framework of the ISSA. The approach has two independent components: VSA and DSA. However, the two arrows between VSA and DSA provide linkages that help to understand the current status of the surface soil components intended to assess and visualise in the study area. These linkages served as meansto record the results of the assessment together with some examples of the images of surface soil characteristics (Information I) and soil formation factors (Information II). Finally, the overall results were discussed according to the information obtained under VSA and DSA tallied with the surface soil characteristics and factors of soil formation of the study region.

Digital Soil Assessment (DSA)

The DSA was adapted to provide an overview of the true picture of surface soil characteristics and soil factors in the study region, and the concept is depicted in Figure 4. Simple procedure of photograph using Cyber-shot DSC-W510 (12.1 mega pixels) SONY camera was used in the field to take records of cloud as related to rainfall and temperature, parent materials, presence of soil organisms, characteristics of topography and land use as related to landform 's formation of the study region.

Visual Soil Assessment (VSA)

The VSA was used to evaluate parent materials, organisms, topography and those surface soil characteristics, which are visible and can be assessed directly by the naked eye primarily to explain the current status of surface soil in the study region. This type of assessment was considered important in assessing the surface soil properties, which are capable of changing as a result ofdiverse land use practices (FAO, 2008; Soil Survey Staff, 2010). In addition, previous climate data for rainfall, temperature, wind speed and relative humidityas well as the dynamic formation of the atmosphere were considered as preliminary information to further understand the physical milieu of the region.

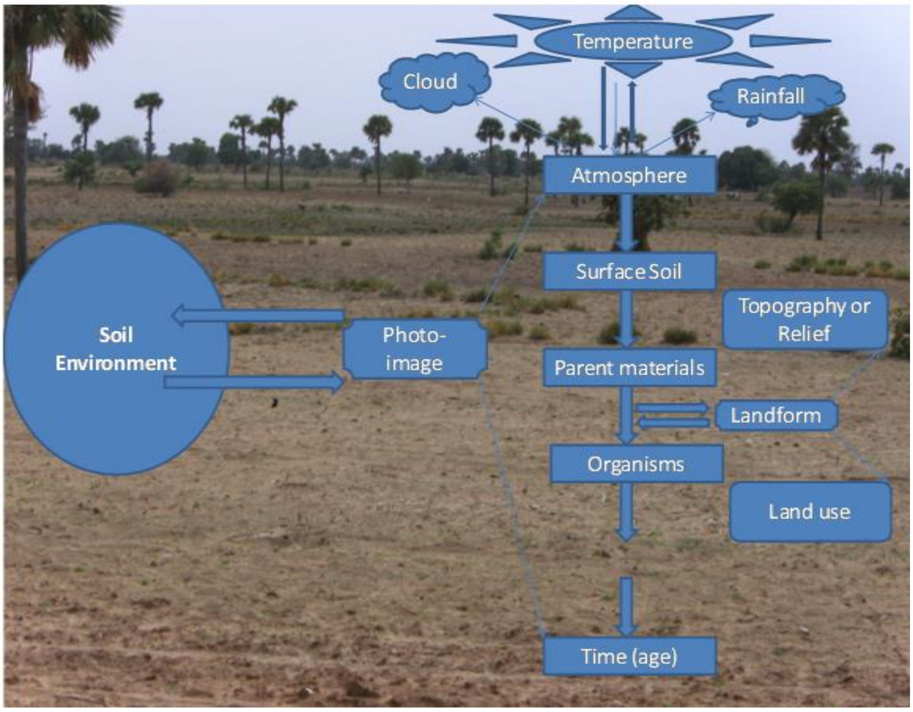

Figure 4. Digital Soil Assessment (DSA). Arrows at the top indicate the relationship between the three components of atmosphere (i.e. temperature, rainfall and cloud) considered in this assessment. Similarly, arrows at the middle indicate the relationship between soil factors, land and land use at the surface soil environment.

Results and Discussion

Soil factors

Soil factors are: climate + parent materials + topography + biota + time (Jenney, 1994). The survey on these factors in the study region is discussed below.

Climate

In order to understand the climate condition of the study region, four factors were considered namely – rainfall, temperature, relative humidity and wind speed. Physically, these factors are influence by aridity, poor vegetation cover and temperatures of annual and daily fluctuations as a result of periodic energy input of the sun (Yalwa, 2008). According to local meteorological data record in the region, the annual rainfall is variable and declining, it is 600 mm to 875 mm, and on average 650 mm during the period 1995 to 2010. Monthly temperature throughout the year falls between 25^{0}C and 45^{0}C.The relative humidity also varies slightly, being around 50% to 80% during the rainy season and decreased to low as 14% in dry period. The major winds occur around Decembers and January with an average wind speed of up to 18 m/s. This clearly indicates that the region has a tropical weather conditions and can be characterised into three seasons, namely: rainy, dry and hot. Rainy (wet) season starts normally from May/June to September/October, and dry season starts from December to early April whereas hot season begins in April and lasted in May as noted between 2009 and 2011. Thus, the atmospheric sky condition in the study region can be explained according to the physical nature of the sky in rainy season (Figure 5).

Generally, this physical nature of the atmospheric sky condition in the region can be grouped into four dynamic formations: the clear sky, the bright sky, the dust sky, and the cloudy sky.The clear sky normally occurs from mid-October and March to early April usually brings stable atmospheric conditions with no cloud, no dust. During the months of December and early January, the sky experiences dust condition making atmosphere unstable due to accumulation of million dust particles overlooking the atmospheric surface environment. The sky become bright mostly in the mid of April through May and June causing rapid temperature increases as a result of direct solar heat radiation to the surface soils (temperature is from 30-45^{0}C).Cloudsky happens during the rainy season when the atmosphere saturated by air causing different forms of clouds with different shapes, sizes and colours (Figure 5).

Figure 5. Physical condition of atmospheric sky in the study region

Parent materials

Table 1 gives the summary of the major classes of parent materials in the study region. These parent materials are grouped according to origin of their formations, noted from igneous to metamorphic and finally to consolidated and unconsolidated classes.

Table 1. Major classes of parent materials in the study region

Major class[1]	**Group[1]**	**Type[1]**	**Remarks[2]**
Igneous rock	Basic	Dolerite Ironstone	Not good for agriculture but can be used in the context of soil protection
Metamorphic rock	Basic	Phyllite (Peltic-R) limestone (marble)	Good for building and water ways (drainage)
Sedimentary rock (Consolidated)	Clastic sediments	Sandstone silt, clay and clay-stone Limestone Sand Silt	Require well-sustainable management practices for agricultural improvement
Sedimentary rock (Unconsolidated)	Carbonate, Fluvial Colluvial Alluvial Lacstrine Estuarine	Clay Loam Clay Clay-loam Sand Silt Slope deposits	Good for wide range agricultural activities: cereal farming, irrigation and horticultural cropping system; still required regular soil management Practices.

[1]According to FAO (2006) guidelines for soil description; [2]Based on VSA in the field

Lithologically, the surface soil parent materials in the study region vary significantly probably due to landform variations and differences in land use activities. In dryland sites, large portion of the land has been covered by sedimentary sand and silt particles of which are clastic and carbonated anthropogenic sediments by their physical appearances. And, in fadama sites, the land is covered by clay and clay-silt/loam particles, which is believed to have been originated from alluvial, colluvial, fluvial and lacustrine deposited particles (Table 1). Typical examples of these parent particles are depicted in Figure 6 and 7.

Physically, it is believe that the result of diverse agricultural activities has caused many surface soil changes, and probably led to the continuous formation of other soil particles (Brady and Weil, 2007). These particles are classified into: fluvial such as pure sand, gravel and clay; alluvial such as clay, clay loam; lacustrine such as sand-loam, silt and clay; estuarine such as fine sand and clay particles and many others (Table 1; Figure

7). However, the dynamic changes on these parent particles could be related to factors such as the surface climate, deforestation, bush burning, soil erosion, drought and addition of organic materialsthat contribute significantly to soil formation (Jenney, 1994).

Figure 6. Examples of some major classes of parent materials in the study region: (a) basic igneous rock, (b) peltic metamorphic rock, (c) fluvial Sedimentary rock [unconsolidated sand and gravel] and (d) clastic sedimentary rock [consolidated sandstone]

Characteristically, the surface agricultural soils of the study region can be categorised according to their current parent particles into – fadama clay soils, fadama alluvial soils, dryland sandy to sandy-loam soils, dryland stony soils, and organic-mineral upland soils.

Topography

Table 2 outlines the major classes of surface topography in the study region. The shapes and positions of each class have been characterised, accordingly. Physically, some classes are flat and level while others are sloppy, up-and-down and gently-slope depending on the nature and condition of their formation in the field.

Table 2. Major classes of topography in the study region

Class[1]	Shape[1]	Position[1]	Remarks[2]
Flat	Straight	Summit	Visible: 1m – 100m or <
Level	Straight	Shoulder	Visible: 1m – 30m only
Very gentle slope	Concave	Middle	Visible but: 0.5m or >
Slopping area	Concave	Lower	Not visible: 0.0m – 0.0m
Sloppy-site	Concave	All-over	Open milieu: visible
Sloppy	Linear	Back-slope	Only visible at very close
Flat	Shallow	All-over	Only visible at very close
Up-and-down	Back-slope	Partly	Visible: 1m – 300m
Gently-slope	Contour	Middle	Visible at short distance
Gently-slope	Convex	Partly	Visible: 1m – 260m

[1]According to USDA-NRCS (2002) guideline; [2]Based on VSA in the field

The topography varied from flat to level and gentle to sloppy (Table 2). Most of the flat reliefs are straight and are visible from 1m – 100m. This flat sites show little or no impact of surface erosion as compare with the very gently and sloppysites as physically noted between 2009 and 2011. It is believed that there are considerable surface relief's changes particularly in the dryland sites - mainly anthropogenic human activities such as deforestation. These changes have created some major surface imbalances between soil structure, soil texture and basic parent particles. Typical examples of some major classes of topography are depicted in Figure 8.

Figure 7. Examples of some major groups and types of consolidated and unconsolidated particles in the study region: (a) clastic sediments sandstone, (b) lacstrine clay soil, (c) organic limestone (carbonate), (d) lacstrine sand, (e)clastic sandstone, and (f) estuarine marine clay

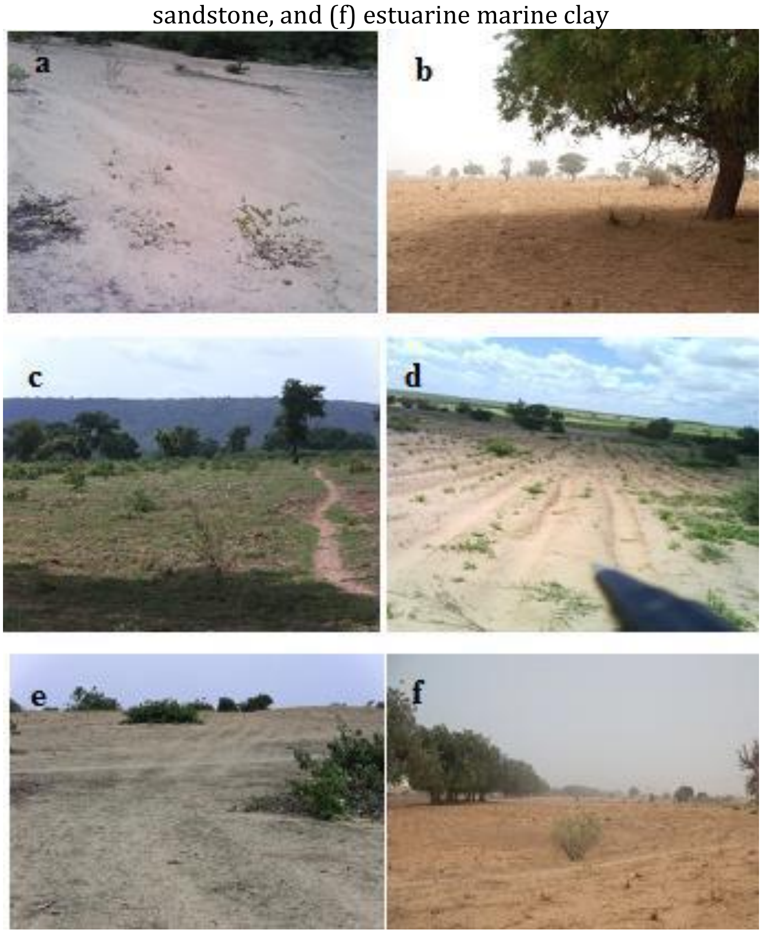

Figure 8. Examples of some major surface soil reliefs in the Sudan Savannah.(a) Very gentle slopping area, (b) Almost flat relief area, (c) Straight relief area (d) Strongly slopping area, (e) Up-and-down area, and (f) Valley area

Soil biota and biodiversity

The major types of soil organisms observed are termites, earthworms, ants and nematodes. These organisms were physically assessed according to the nature of their presence, biodiversity or activities and appearance on/within surface soil voids. Remarkably, these organisms play important role in the breakdown and decomposition of many organic materials in soil (Ritz et al., 2010). They contribute widely in the improvement and transformation of soil quality, soil organic matter and soil nutrients availability (Brussaardet al., 2007; Li et al., 2014; Castro-Huerta et al., 2015).Typical examples of biota and their biodiversity are depicted in Figure 9.

Figure 9. Some major activities of biota and their biodiversity in study region: (a), (b) and (d) indications of biological activities and (c) mobility of termite population

Surface soil characteristics

Table 3 gives the major surface characteristics in the study region.

Table 3. Classification of major surface characteristics in the study region

Class[1]	Remarks 1[2]	Remarks 2[2]
Miscellaneous	Eroded soil environment	No vegetation, few grass, and no original surface soil features
Blown-out land	Desertification is common	Winnowing and mass movement of soil particles
Cirque land	Area dominated by rock with cirque shapes	No trees with only few grasses
Gullied land	Areas with deep cut of V and U shapes gullies	Original surface soil parent particles have been disappeared
Rock-outcrop	Bare bedrocks areas	No space for grasses and trees
Slickness	Fine-textured soil areas	Plant and shrubs areas
Water	Rivers areas	Fishing and irrigation farming
Bad land	Bad soils	No agricultural function

[1]According to FAO (2006) guideline for soil description; [2]Based on VSA in the field

Physically, the most risk and poor surface characteristic in this classification are gullied land areas, which most of them are bad-lands. These areas require an engineering and well planned sustainable land management. By contrast, the rock-outcrop areas could be considered as support to surface soil, they bind soil particles and thus, minimise the risk of erosion (FAO, 2006). Water areas are important sites for irrigation and other agricultural activities in the region. Typical examples of these surface characteristics are depicted in Figure 10.

Figure 10. Examples of some major surface characteristics in the study region: (a) miscellaneous area, (b) rock-outcrop area, (c) cirque land, (d) bad land, (e) water body area, and (f) Slickness area

Time

The period of time in the formation of soil, is a factor that can be related to the historical background of human development and agricultural activities in the region. It is believe that the surface soil of the region received changes long time ago related to the human settlements in the region (e.g. Ahn, 1970).

Land use activities

Table 4 presents the major land use activities in the study region. The results conformed to other previous studies in the region (Mortimore, 1989; Baker, 2000; Usman, 2007). The typical examples are depicted in Figure 11.

Table 4. Major land use practices in study region

Land use activities[1]	**Remarks[1]**
Agricultural land use	Agriculture is the economic development of the State.
Dry farming system	Cereals (millet, sorghum), legumes (cowpea, ground nut) are the major crops growing in dry farming system of the State.
Mono-cropping	
Inter-cropping	
Crop rotation	
Mixed farming	
Fadama land use	Important land areas for rice and wide range of horticultural crops (tomato, chilli, leaves, okra, etc).
Irrigation system	
Sowing method	
Horticultural	
Orchard farming system	Plantation of mango, guava, cashew, are common.
Fruit trees plantation	
Pastoralistfarming system	Cattle, sheep's, goats, are the common animals under trans-human system.
Extensive nomadic	
Extensive non-nomadic	
Intensive rearing	Local hens, layers and broilers.
Poultry farming system	
Extensive system (local)	
Intensive (Business)	Mainly grasses and shrubs of various types.
Grazing land use	
Grass land area	

[1]According to the current status of agricultural sites via VSA in the field

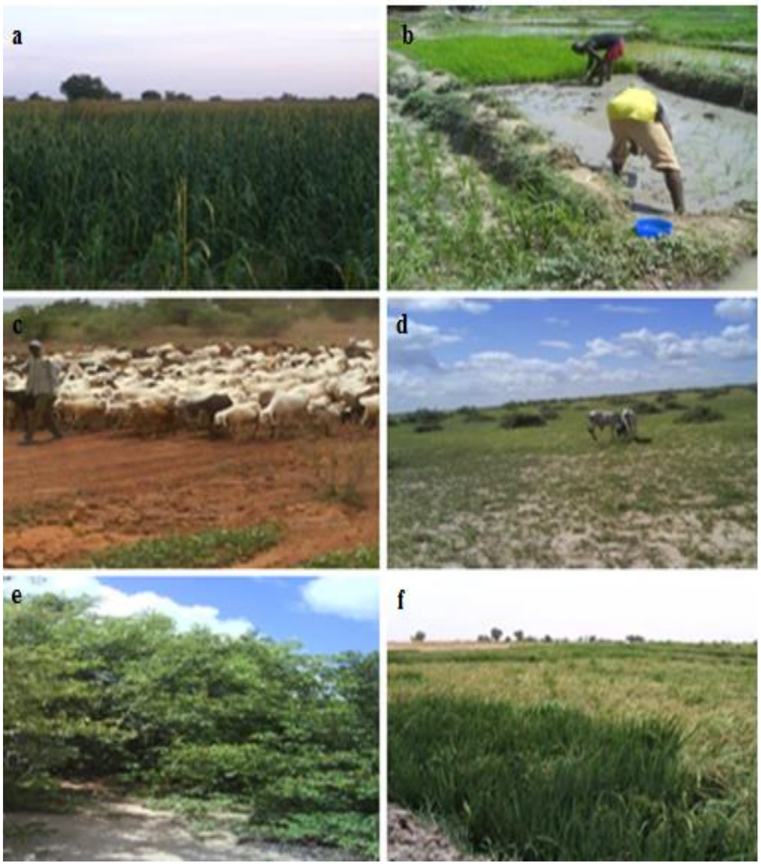

Figure 11. Examples of land use activities in the study region. (a) dry farming system (millet production), (b) irrigation system, (c) nomadic pastoralist system, (d) grazing land, (e) grazing land, and (f) fadama rice production (sawing)

Conclusion

The study captured some values of surface soil environment in the study region. Soil factors, surface soil characteristics and land use activities are covered. It has been understood that a very close and interdependent relationship had always existed between all phases of soil components in the study region. Soil parent materials and biota were found interacted with one another, adding values to soil quality and soil fertility. Also, climate factors such as rainfall, temperature, relative humidity and wind were also noted to have independently interconnected with soil particles and added values tosoil for different agricultural land use activities.

An alternative approach, as adapted in this study, would give necessary information to the local people and government in general usefully, understanding both the values of their land resources and current status of its condition related to future management and transformation. The difficulties of this approach however, are the fact that it requires time, careful observation and field visit all over the region as well as additional guidelines for environmental soil assessment. Thus, further assessment is required to determine if improvement can be made to the approach for wide use to other local environments.

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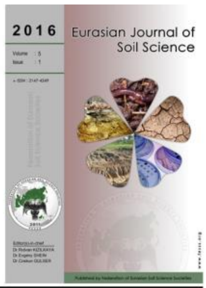

The assessment of groundwater geochemistry of some wells in Rafsanjan plain, Iran

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Abstract

Water quality is the critical factor that influence on human health and quantity and quality of grain production in semi-humid and semi-arid area. Groundwater and irrigation water quality play important roles in main production this crop. For this purpose, 94 well water samples were taken from 25 wells and samples analyzed. The results showed that four main types of water were found: Na-Cl, K-Cl, Na-SO_4, and K-SO_4. It seems that most wells in terms of water quality (salinity and alkalinity) and based on Wilcox diagram have critical status. The analysis suggested that more than 87% of the well water samples have high values of EC that these values are higher than into critical limit EC value for irrigation water, which may be due to the sandy soils in this area. Most groundwater were relatively unsuitable for irrigation but it could be used by application of correct management such as removing and reducing the ion concentrations of Cl^-, SO_4^{2-}, Na^+ and total hardness in groundwater and also the concentrated deep groundwater was required treatment to reduce the salinity and sodium hazard. Given that irrigation water quality in this area was relatively unsuitable for most agriculture production but pistachio tree was adapted to this area conditions. The integrated management of groundwater for irrigation is the way to solve water quality issues not only in Rafsanjan area, but also in other arid and semi-arid areas.

Keywords: Irrigation, saline, water quality, groundwater, Rafsanjan

Introduction

Water quality is the critical factor that influences on human health and quantity and quality of grain production in arid and semi-arid areas. Pistachio *(Pistacia Vera L.)* nuts are an important product of Iran and the USA (the world's first and second producers, respectively), but interest in this species as an alternative to traditional fruit crops is growing in other countries. Rafsanjan has been recognized as one of the largest pistachio production sites, not only in Iran but also worldwide. Rafsanjan area is water scarcity due to general low precipitation, high evaporation and the temporal and spatial distribution of rainfall. The mean of relative moisture is 7.33 %. The mean annual precipitation in this area ranged between 97 to more than 100

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mm/yr. The ranges of temperature in this area is between -8°c in winter to 43°c in summer. Pistachio is a major crop in Rafsanjan area. Pistachio nuts can be grown in an arid and semi-arid climatic conditions and its tolerance to salinity but drought and saline stress are a major cause of yield loss (Behboudian et al., 1986; Goldhamer et al., 1987). The growth season was divided into three phenological stages (Goldhamer, 1995): stage *I* starts at the beginning of the nut growth and ends when its maximum size is reached; during stage *II* the shell hardening takes place and finally, the stage *III* is the period of kernel growth (Goldhamer, 1995). Two immediate responses to this challenge are the efficient use of irrigation technology and the use of alternative sources of water. In the arid and semi-arid zones, the production of fruits and nuts are fully dependent upon irrigation (Naor, 2006).

The substantial investments associated with orchard establishment can only be justified if production is high and stable throughout the life of the orchard. Therefore, orchards have been developed mostly in lands where irrigation water supply was abundant, even in drought years. Increasing water use efficiency in irrigated agriculture and promoting dry land farming will both play a significant role in maintaining food security (Deng et al., 2006; Ribolzi et al., 2011). Conditions of water quality and quantity are two key factors impacting groundwater utilization for irrigation. Therefore Groundwater and Irrigation water quality play essential role in agricultural practices and particularly in pistachio harvesting. Consider of this area is located in arid and semi-arid climate and there has high evaporation therefore observed saline and alkaline soil and water in this area. Saline groundwater is often found at shallow depth in irrigated areas of arid and semi-arid regions and is associated with problems of soil salinization and land degradation. In saline soil and alkaline soil observed Low hydraulic potential and bad effects of ions such as: Cl^-, HCO_3^-, Boron and particularly Na^+ due to increase Na/K, Na/Ca, Cl/NO_3, Mg/Ca in plant and nonequivalent between nutrient concentration and therefore decrease plant growth (Hosseinifard et al., 2005a; 2005b).

Increase salt concentration in soil and water causing potassium sorption decrease by plant and K^+ deficiency in plant (Hosseinifard et al., 2010). Groundwater, which contains numerous natural ions and may be polluted by human activities, seriously influences agricultural utilization. Moreover, irrigation water with excessive ions also impacts the environment. For example, the most common ions in groundwater are chloride (Cl^-) and sodium (Na^+), particularly in coastal aquifers. When water with high Cl^- and Na^+ concentrations is used for irrigation, many plants suffer from toxicity and retardation growth, resulting in yield reduction (Karaivazoglou et al., 2005; Grieve et al., 2006).

Groundwater quality typically contains a variety of hydrochemical parameters Therefore, the assessment of groundwater used for irrigation subject to hydrochemical parameters should be performed using a multivariate approach (Khan et al., 2006; Jang and Chen, 2009). Improved water management can also help minimize offsite water quality impacts of irrigated production. Irrigated agriculture affects water quality in several ways, including higher chemical-use rates associated with irrigated crop production, increased field salinity and erosion due to applied water, accelerated pollutant transport with drainage flows, degradation due to increased deep percolation to saline formations, and greater in stream pollutant concentrations due to reduced flows. Strategies to improve the Nation's water quality must address the effect of irrigation on surface and ground water bodies (Rogers, 1996; Liu et al., 2009). However groundwater is one of important resource of water supply for irrigation water in arid and semi-arid areas. The objective of this study was to determine irrigation water quality in Rafsanjan area and assay management practices on applied ground water in this area.

Material and Methods

Description of the study area

This study was conducted in the commercial orchards in some pistachio growing areas of Rafsanjan. Rafsanjan is an area in southeast Iran (Kerman province), this area located between longitude: 55˚, 59', 30"E and latitude 31˚, 13"N. The mean annual precipitation of the region is less than 100 mm. The mean annual potential evapotranspiration is more than 3000 mm. Soil moisture and temperature regimes are aridic and thermic, respectively. Groundwater has been used for various purposes, such as drinking, agricultural, domestic, and industrial needs. The most important economic activity of this area is agriculture. In the selected orchards, the common irrigation intervals are about 48 days.

Sample collection and analysis

From Twenty five areas in Rafsanjan region, groundwater samples were selected based on the preliminary field survey from Rafsanjan in the Kerman Province, July 2011. The area studied occupies about 135 km^2, with a mean altitude of 1,469 m.a.s.l. (Figure 1). Most of these wells supply water for gardening and irrigation. Samples were collected after a pumping time of about 30 min. Samples were analyzed in the laboratory for the major ions employing standard methods. The analyses were carried out within 48 h of collection. Care was taken that the pH, electrical conductivity (EC), HCO_3^-, and Ca^{2+} ions were analyzed within 4 h of sampling. The pH and EC were measured. Calcium (Ca^{2+}) and magnesium (Mg^{2+}) were determined titrimetric ally using standard EDTA. Chloride (Cl^-) was determined by standard $AgNO_3$ titration method. Carbonate (CO_3^{2-}) and bicarbonate (HCO_3^-) were determined by titration with HCl. Sodium (Na^+) and potassium (K^+) were measured by flame photometry, sulfate (SO_4^{2-}) by spectrophotometric turbidimetry. Total dissolved solids (TDS) were computed by multiplying the EC ($dS.m^{-1}$) by a factor of 640. The alkalinity (sodium) hazard of water is described by the sodium adsorption ratio (SAR) (Hosseinifard and Aminiyan, 2015):

$$SAR = \frac{Na}{\sqrt{\frac{(Ca + Mg)}{2}}} \quad \text{Eq. (1)}$$

The ionic symbols indicate concentrations of the ions in the water in mill equivalent per liter. Total hardness (TH) of groundwater was calculated using following equation (Sawyer et al., 2003):

$$TH\ (mg\ CaCO_3/\ lit) = (Ca^{2+} + Mg^{2+})\ meq.l^{-1} \times 50 \quad \text{Eq. (2)}$$

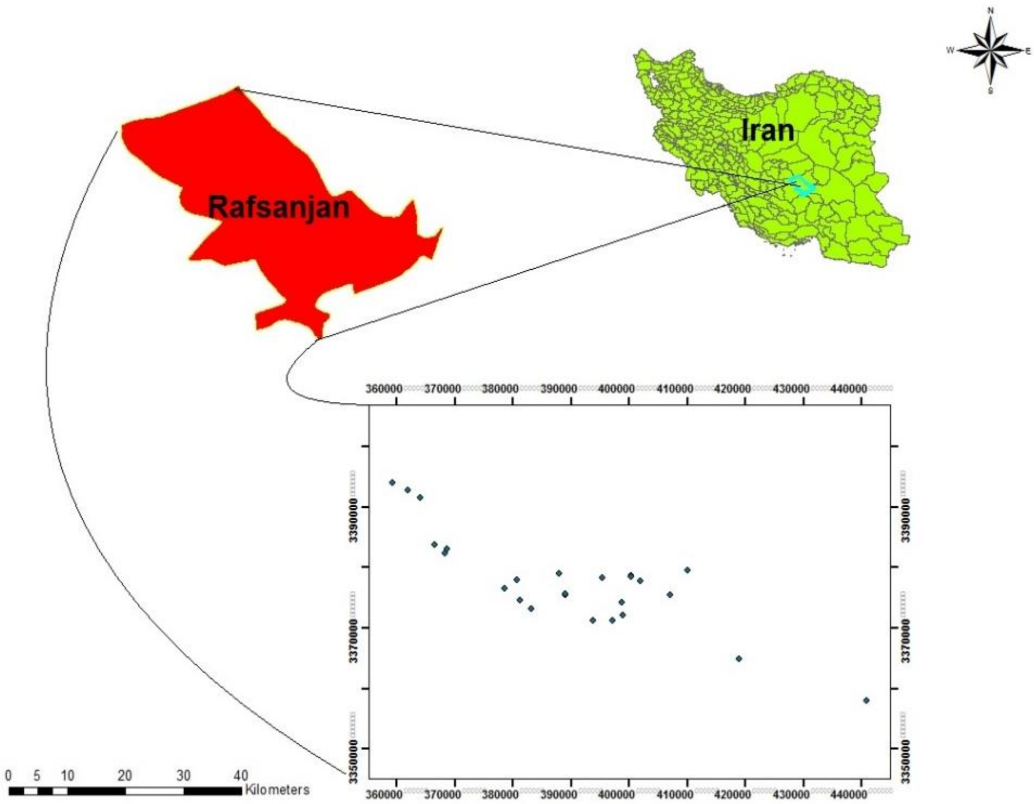

Figure 1. The location of the groundwater sampling wells

Data of statistical analysis

Statistical analysis and water samples chemical analysis were carried out using SAS (version 9.2), MINITAB (version 14) and Rockworks (version 15) water quality software respectively. Also the GIS map depicted by GS plus (Version 7) software.

Results and Discussion

The chemical compositions of the well samples were statistically analyzed and the results are given in. The results showed that concentration of K^+ and Na^+ were dominant cations and Cl^- and SO_4^{2-} were main anions in water samples (Table 1). Thus, the order of cation abundance is $K^+ > Na^+ > Ca^{2+} > Mg^{2+}$ and the order of anion abundance is $Cl^- > SO_4^{2-} > HCO_3^-$.

Table 1. Summary statistics of chemical compositions of major ions (mg. L^{-1}) in water samples.

Variable	Na	Mg	Ca	K	Cl	HCO_3	SO_4	pH	EC	TDS	TH
Mean	732.67	177.69	351.94	967.90	1876.41	55.00	757.69	7.51	6.64	4249.19	1620.21
Min	151.80	16.80	72.00	343.20	280.00	6.10	134.40	6.80	1.90	1216.00	330.00
Max	2417.30	864.00	1760.00	26220.80	4987.50	146.40	4701.60	8.40	23.90	1529.00	7375.00
Median	541.65	132.00	258.00	854.10	1400.00	48.80	516.96	7.50	4.94	3168.00	1230.00
N*	94	94	94	94	94	94	94	94	94	94	94

* N: Total sample

Correlation assessment between dissolved ions in well samples suggested that may found their origin. The ions were correlated together, they have similar origin usually. The results showed that between Cl^- and Ca^{2+}, Mg^{2+} and Na^+ there was significant correlation (r= 0.74), (r= 0.57), (r= 0.46) respectively (Figure 2). Therefore the possible origin of these cations may be considered salts such as: $CaCl_2$, $MgCl_2$, KCl and NaCl. Due to have high significant correlation between Ca^{2+} and Mg^{2+} cations (*$p \leq 0.001$*), perhaps may there was origin such as Dolomite mineral (Ca, $MgCO_3$) for this cations and also there was negative significant correlation between Cl^- and HCO_3^- anions (Table 2).

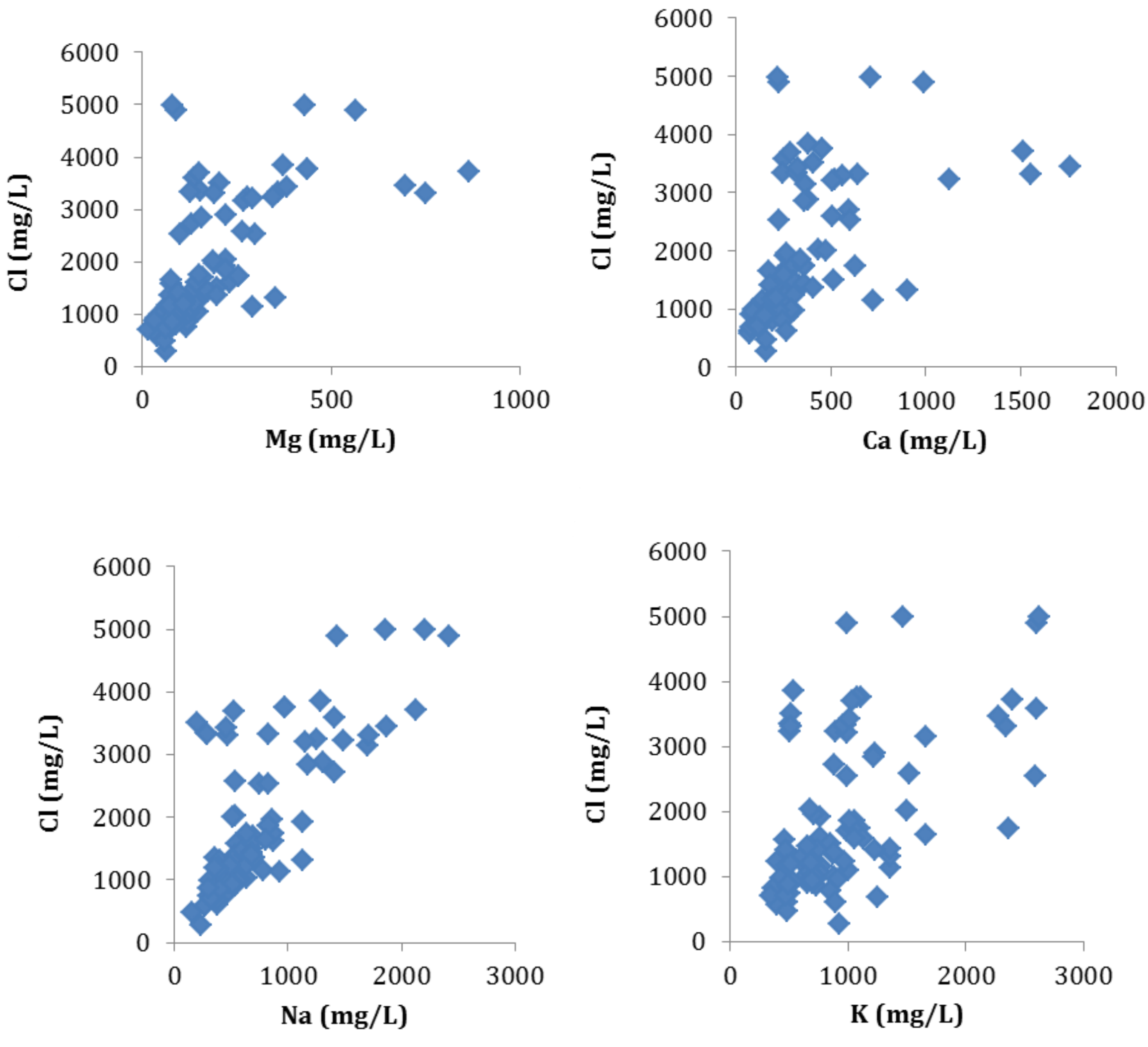

Figure 2. Relationships between the major cations and Cl anion.

Table 2. Pearson correlation between hydrochemical characteristics of groundwater samples.

	Na	Mg	Ca	Cl	HCO_3	pH	EC	SO_4
Mg	0.57	1						
Ca	0.53	0.89	1					
Cl	0.74	0.57	0.46	1				
HCO_3	-0.18	-0.13	-0.12	-0.22	1			
pH	0.42	0.66	0.71	0.22	-0.08	1		
EC	0.90	0.82	0.79	0.77	-0.19	0.61	1	
SO_4	0.40	0.63	0.74	0.16	-0.08	0.70	0.59	1
K	0.70	0.35	0.38	0.54	-0.31	0.41	0.70	0.55

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

Piper Diagram

Based on dominant cations and anions in well water samples, four main types of water were found (Figure 3): Na-Cl, K-Cl, Na-SO_4, and K-SO_4. This result showed this water types very similar to ocean water and very saline area's water and also result suggested that, non-carbonatic alkaline ions were more than 50% and alkaline and very acidic ions were dominant in well water samples.

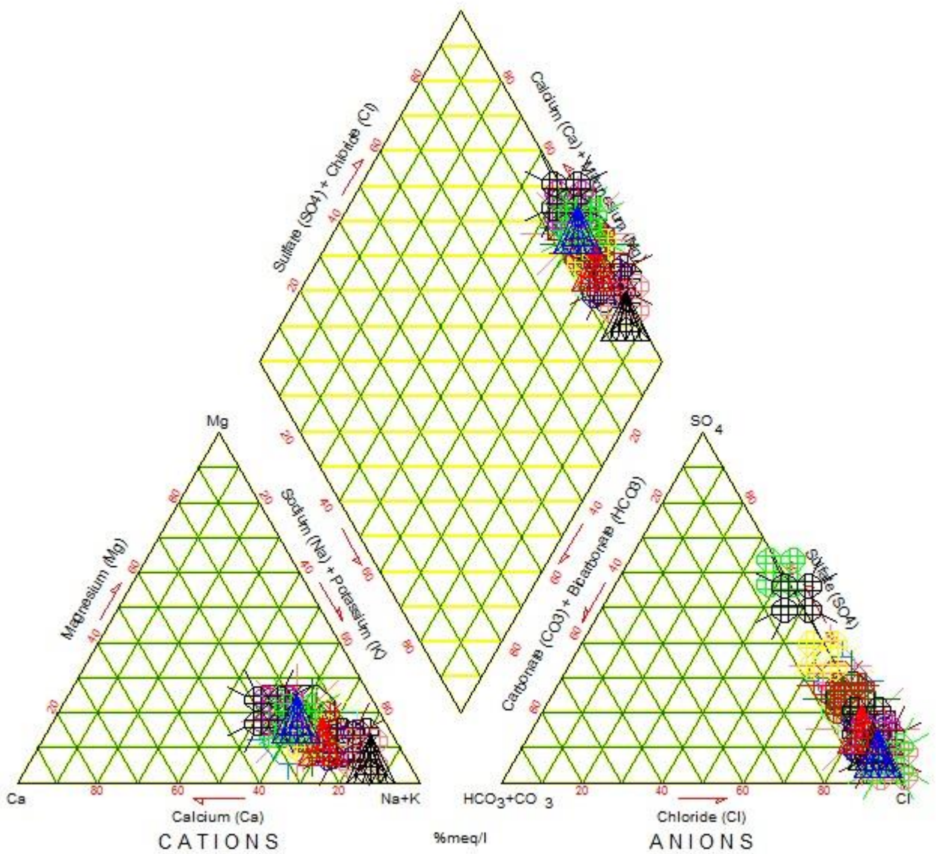

Figure 3. Piper diagram for the well water samples of the studied area

Irrigation water quality

Salinity is the total amount of inorganic solid material dissolved in any natural water, and water salinization refers to an increase in TDS and overall chemical content of water (Ritcher and Kreitler, 1993). Water that enters to the soil is subject to chemical, physical, and biological changes. Classification of well water based on TDS (Freeze and Cherry, 1979) indicates that primary of the samples are brackish water with TDS ranges from 1216 to 1529 with an average of 4249.19 mg L^{-1}. The analysis suggested that more than eighty-seven percentage of the well water samples have high values of EC that these values are higher than into critical limit EC value for irrigation water (Figure 4), which may be due to the sandy soils in this area (Figure 5).

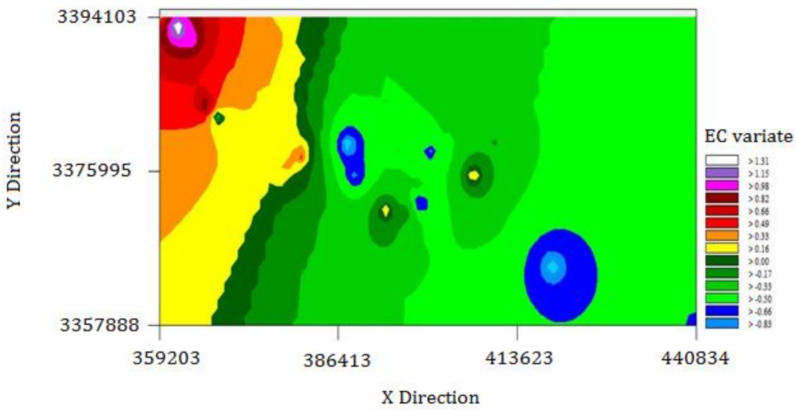

Figure 4. Spatial varibility of EC (dS/m) in Rafsanjan well water samples

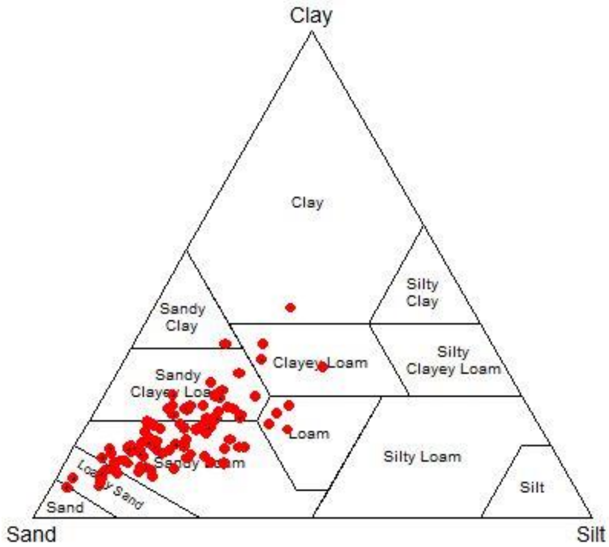

Figure 5. Soil sample texture of studied area.

Hardness of water depends dominantly upon the amounts of divalent metallic cations, of which Ca^{2+} and Mg^{2+} are the more abundant in groundwater. The hardness values in well water samples range from 330 to 7375 (mg L^{-1} $CaCO_3$), the average being 1620.21 (mg L^{-1} $CaCO_3$). The most recommended limit of total hardness is 80–100 mg l^{-1} $CaCO_3$ (Freeze and Cherry, 1979). Well water samples exceeding the limit of 300 (mg L^{-1} $CaCO_3$) is considered to be very hard (Sawyer et al., 2003). Therefore most water samples fall in the very hard waters category. Sodium concentration is important when evaluating the suitability of quality water for irrigation. High concentrations of Na^+ are unfavorable in water because Na^+ is adsorbed onto the soil cation exchange sites, causing soil aggregates to disperse, reducing its permeability.

The SAR, which indicates the effect of relative cation concentration on Na^+ accumulation in the soil, is used for evaluating the alkalinity of irrigation water. The calculated SAR ranged from 4.95 to 16.6. In order to identify the availability of waters for irrigation use, The parameters such as electrical conductivity, sodium adsorption ratio (SAR), and percent sodium (Na%) were estimated to assess the suitability of surface water and groundwater for irrigation purpose. The irrigation waters classification diagrams were used to assess the water quality (Richards, 1954; Wilcox, 1955), (Figure 6). According to this graph, water classes of water samples are mainly (60%) fall in the category C4-S4, 20% of water samples fall in C4-S3 and 20% of water sample fall in C4-S2.

Water samples are located in these categories special management for salinity and alkalinity control may be required, and plants with good salt tolerance should be selected and can be grown. Therefore, based on (Figure 6) the salinity hazard in well water samples is regarded as very high, but the Na^+ hazard is regarded as high to very high. Given that physiologic and phonologic characteristics of pistachio tree and there is sandy loam dominant soil texture (Figure 5) in this area, pistachio tree is tolerance to salinity and alkalinity condition and it adapted to this area conditions.

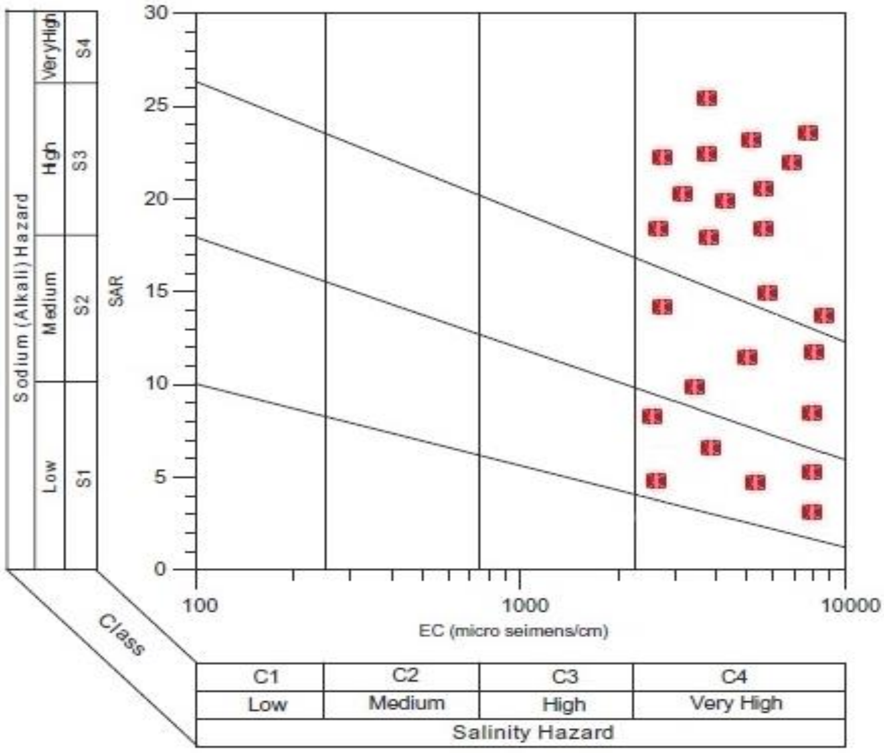

Figure 6. Diagram for irrigation waters classification Wilcox, (1955).

Hierarchical cluster analysis

Hierarchical cluster analysis (HCA) is used to test water quality data and determine if samples can be grouped into hydrochemical groups. The hierarchical cluster analysis method was used to group water samples into significant different clusters. Two groups were generated from hierarchical cluster analysis (Figure 7). Most of water samples were classified as group (II). In the each group, subareas well water samples had similar hydrochemical characteristics but between group I and group (II) there was different hydrochemical characteristics. Both groups consist of two subgroups.

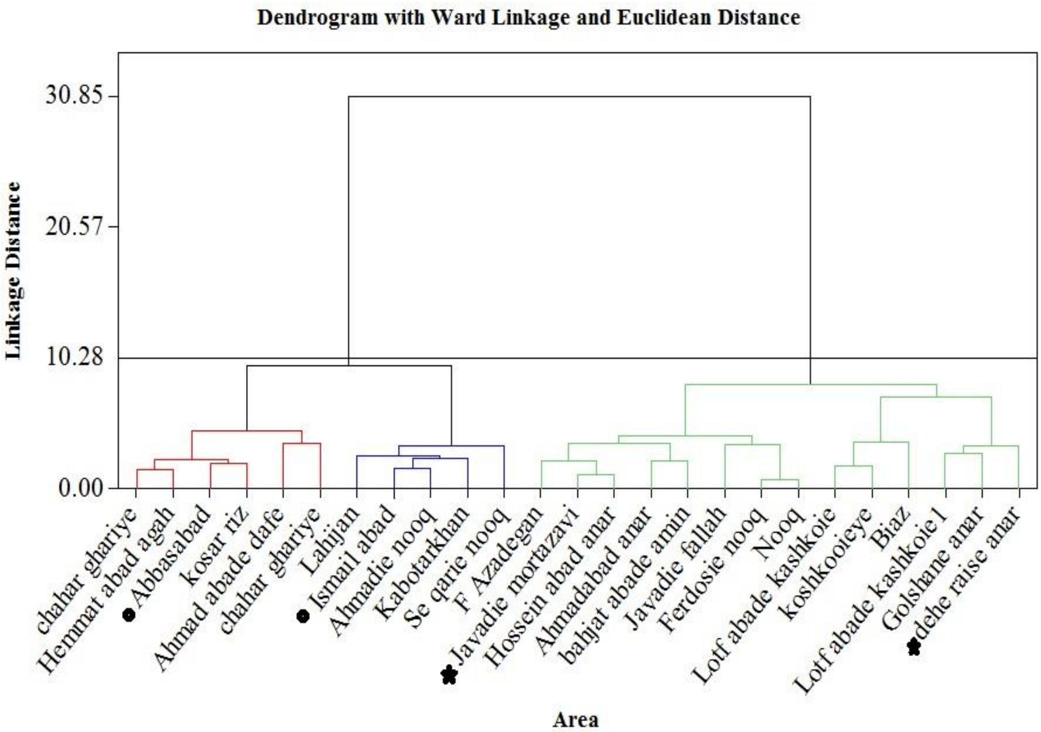

Figure 7. Dendrogram generated from HCA of well water samples chemistry data.

From each group two subarea selected and stiff diagram was mapped (Figure 8). Stiff diagrams plot milli-equivalent concentrations of cations on the left side of the diagram and of anions on the right. Each ion is plotted as a point, and the points are connected to form a polygonal shape. The ions are plotted in a consistent order (Na+K across from Cl; Ca across from HCO_3 + CO_3; Mg across from SO_4) so that each polygon becomes that sample's "signature". The greatest polygon was dehe raise anar and smallest polygon was abbasabad. Therefore observed that concentration of cations and anions in dehe raise anar were more than to the other subareas. From subareas selected Abbasabad, Ismail Abad and Javadie Mortazavi are expected that have similar water quality.

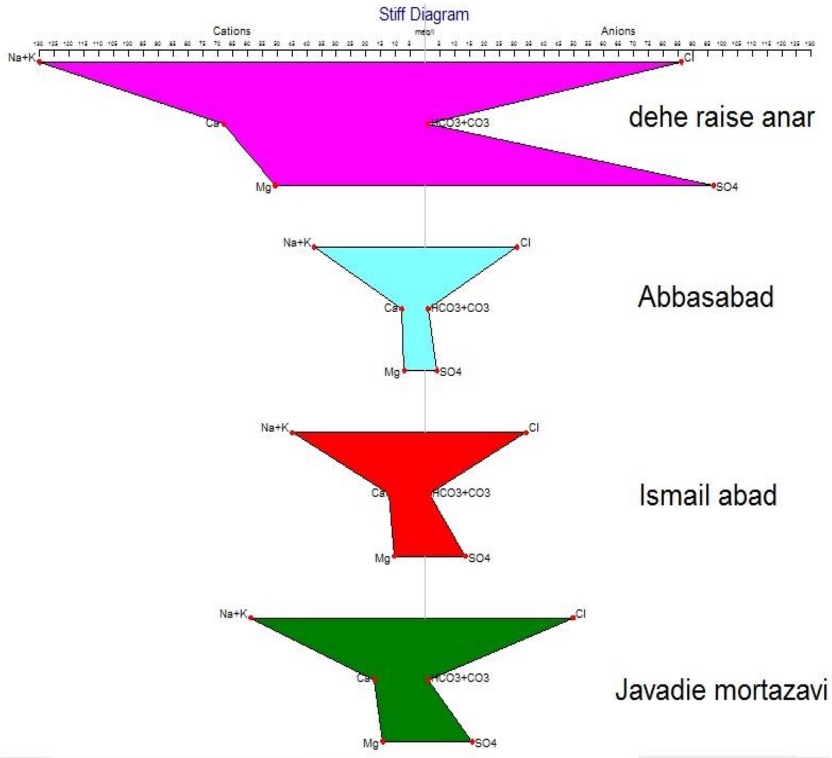

Figure 8. Stiff diagram of subarea from Rafsanjan area

Principal Component Analysis (PCA)

The multivariate statistical techniques such as principal component analysis (PCA) have widely been used as unbiased method in analysis of water quality data for drawing meaningful information. PCA is a very powerful technique applied to reduce the dimensionality of a data set consisting of a large number of inter-related variables, while retaining as much as possible the variability present in data set. This reduction is achieved by transforming the data set into a new set of variables, the principal components (PCs), which are orthogonal (non-correlated) and are arranged in decreasing order of importance. Mathematically, the PCs are computed from covariance or other cross-product matrix, which describes the dispersion of the multiple measured parameters to obtain eigenvalues and eigenvectors. Principal components are the linear combinations of the original variables and the eigenvectors. The results showed that approximately 82% of the variation is explained by two main components; thus, the summary data successfully and with high variance is very good (Figure 9).

More assessments can be observed between PC1 with the greatest amount of variance explained is 68% versus 14% PC2. As seen in PC1 variables pH and bicarbonate is placed in front of the other variables measured. So it seems the difference in water quality of wells due to different amounts of pH and bicarbonate. PC2 has positive relationship with Na (Eigenvalue: 0.241), K, Cl, pH, TDS and SAR whereas PC2 has negative relationship with the other parameters (Table 3).

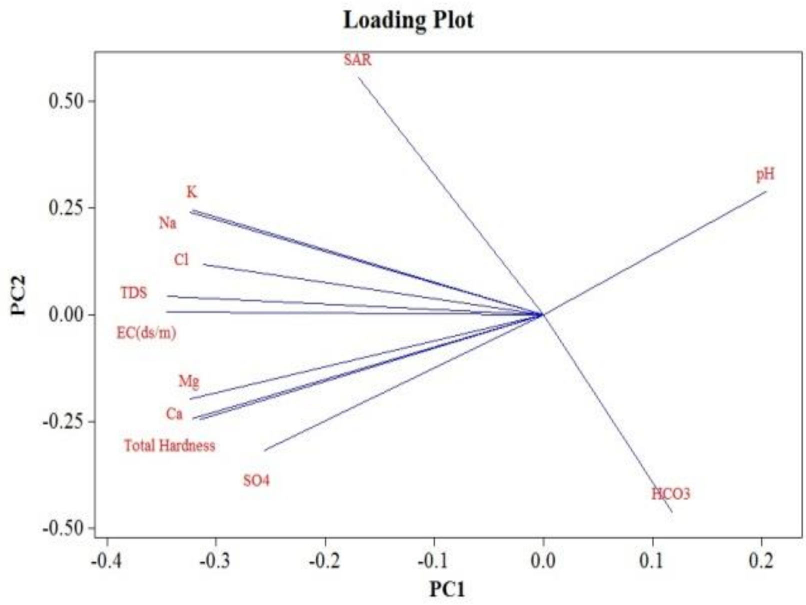

Figure 9. Principal Component Analysis (PCA) loading plot.

Table 3. Relationship PC1 and PC2 with ions and chemical parameters

Variable	PC1	PC2
Na	-0.324	0.241
Mg	-0.324	-0.197
Ca	-0.315	-0.246
K	-0.321	0.247
Cl	-0.311	0.119
HCO_3	0.118	-0.461
SO_4	-0.256	-0.317
pH	0.204	0.290
EC(ds/m)	-0.345	0.006
Total Hardness	-0.321	-0.244
TDS	-0.344	0.044
SAR	-0.169	0.555

Conclusion

The groundwater were sampled and analyzed and assessed the water quality for irrigation. Hierarchical cluster analysis is a useful method to group water samples. The indicators to water quality assessment were TDS, Na, HCO_3, SO_4, pH, Cl, total hardness, SAR and EC from principal component analysis. The high concentration of Na, K, Ca, Mg, HCO_3, Cl, and SO4 were correlated with natural environment conditions. The exceed concentration of salts indicated anthropogenic and geologic impact on groundwater. The fuzzy membership and multivariate statistical techniques (HCA, PCA) are the useful and objective methods for water quality evaluations. The reservoir water and deep groundwater can be used for irrigation water but it was required correct management of ground water. Most groundwater were relatively unsuitable for irrigation but it could be used by application of correct management such as removing and reducing the concentrations of Cl, SO_4, Na and total hardness in groundwater and also the concentrated deep groundwater was required treatment to reduce the salinity and sodium hazard. Given that irrigation water quality in this area was relatively unsuitable for most agriculture production but pistachio tree was adapted to this area conditions. The integrated management of groundwater for irrigation is the way to solve water quality issues not only in Rafsanjan area, but also in other arid and semi-arid areas.

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An application of Embedded Markov chain for soil sequences: Case study in North Western part of Algeria

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Abstract

Embedded Markov chain (EMC) has long history in geological domains, particularly to define the most representative sequences from statigraphic logs. In other words, what is viewed as a meaningless and disordered stratigraphic layer stack can be reorganized in a meaningful sequence by using EMC. This method was transposed in this paper to obtain soil sequences from data retrieved from soil map made by authors, covering a part of the region of Traras (N.W. of Algeria) and containing 13 major soil types. Each major soil type occupies at least one polygon in the map and allow to establish soil adjacencies, which have been tabulated in a matrix regardless to the direction. Three EMC methods have been tested, Walker, Harper and Türk using Strati-signal software and to erect soil relationship diagrams (SRD) representing the most significant links between soils. Significant test is the main difference between the above mentioned three EMC methods. It has been shown that Harper method is quite insensitive to small number of transitions. Besides, all three methods agreed for one soil sequence made by four soils: lithics leptosols- cambisols chormics- cambisols calcarics- fluvisols representing theoretical catena the most representative to the study area. This soil sequence is relevant to the study region and even to the whole Mediterranean region, and is commanded by the topography and the Mediterranean bioclimate. Walker SRD is the most realistic but the most difficult to interpret because of the high number of soil links, Harper SRD gives interesting results. Although the results didn't bring something new to the soil interpretation and soil pedogensis but EMC applied to a finer scale may highlights other hidden relationships between soils.

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Introduction

It is well known from readings that Markov chain is based upon the assumption that knowledge of the past can sometimes be useful to explain the present and vice versa. This assumption was originally related to time series events, and has been applied to space phenomena as well. This "spatial" markovian assumption is closely akin to the first principle of Tobler (1970): "All things are related but nearby things are more related than distant things". Geologists are the most prolific for applying Markov chain models to their studies, and have developed several methods mainly in

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embedded Markov chain (Gingerich, 1969; Selley, 1969; Walker, 1979; Harper, 1984a; Türk, 1982; Ndiaye, 2007); unlike pedologists are less inclined to apply this stochastic model. Nevertheless, concerning Markov chain in soil studies several works have been done such as Burgess and Webster (1984a,b), Li et al. (1999), Li et al. (2004), Li and Zhang (2006), Sun et al. (2013), but none of them have worked with embedded Markov chain.

The key question is to know whether the occurrence of a soil at one point is completely dependent of the contiguous soil which means that the hypothesis of randomness must be disproved statistically.

In this article, embedded Markov chain (EMC) methods was applied to soil map covering the eastern part of Traras region located on the north western part of Algeria in Tlemcen vicinity. The purpose is to explore spatial links between soils in order to extract soil sequences the most representative. It is worth mentioning that soil sequence in this study has the same meaning as catena, and it can be defined as: “A chain, string, or connected series of soils, related by their sequence in a landscape. Synonymous in part with toposequence chronosequence. The variability of soils in a topographic sequence is a function of gradient and position on the slope” (Birkeland, 1999). The concept of catena is fundamental to explain the sequence of soils following both hill-slopes and landscapes. Water and gravity are the main forces involved to sequential variation of soils. This led us to propose the use of Markov chain as an analytical tool in the study of horizontal relationship of soil types.

Material and Methods

Brief presentation of soil map

The study area is located in the eastern part of the Mountains of Traras and covers an area of about 963km^2, under semi-arid Mediterranean bioclimate. Vegetation is structured principally by arar tree (*Tetraclinis articulate* (Vahl) link.) and from an altitude of about 450m it is associated with holm oak (*Quercus rotundifolia* Lam.). On higher altitudes cork oak (*Quecus suber* L.) is present as relict species on acidic substratum. Aleppo pine (*Pinus halepensis* Mill.) occupies naturally the shallowest soils. *Juniperus phoenicea* L. is observed mainly on littoral dunes.

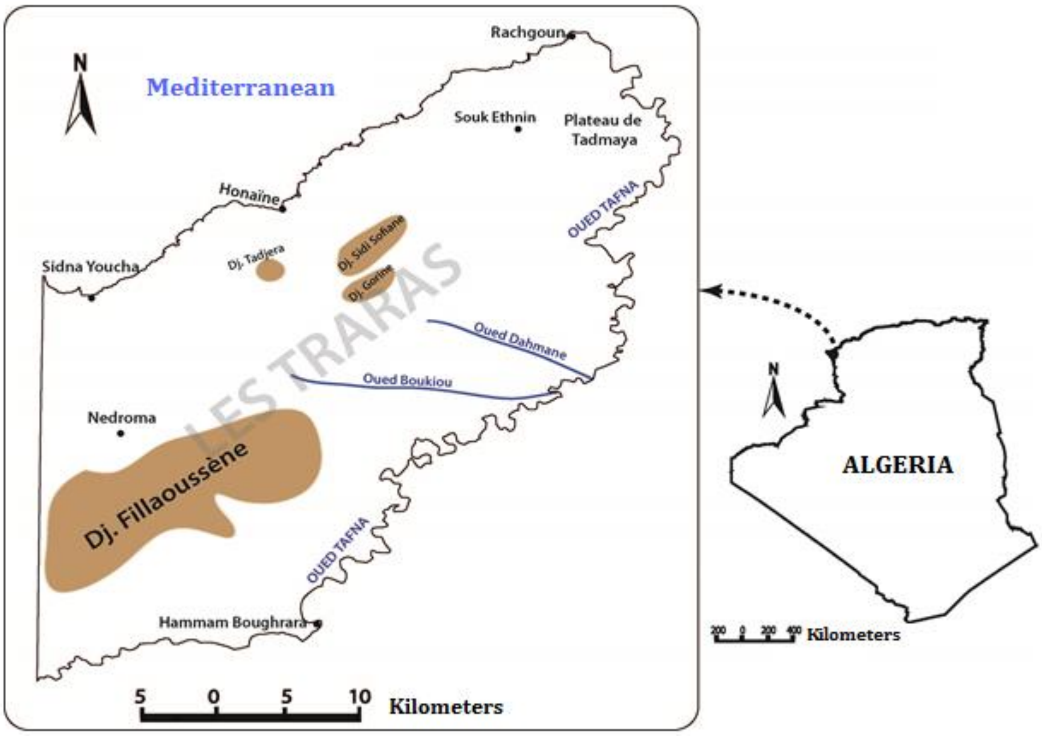

Figure 1. Geographic location of the study area

Soil map was constructed by authors following the most classical method based on JENNY's model (Jenny, 1949). Three main covariables, namely lithology, landform and vegetation cover have been used in order to build chorological laws allowing inference by GIS queries. Originally, the French soil referential (Baize and Girard, 2008) was utilized to define the 13 major soil types of the map. The corresponding soil reference group following FAO-WRB (2006) was roughly defined (Figure 2, Table 1).

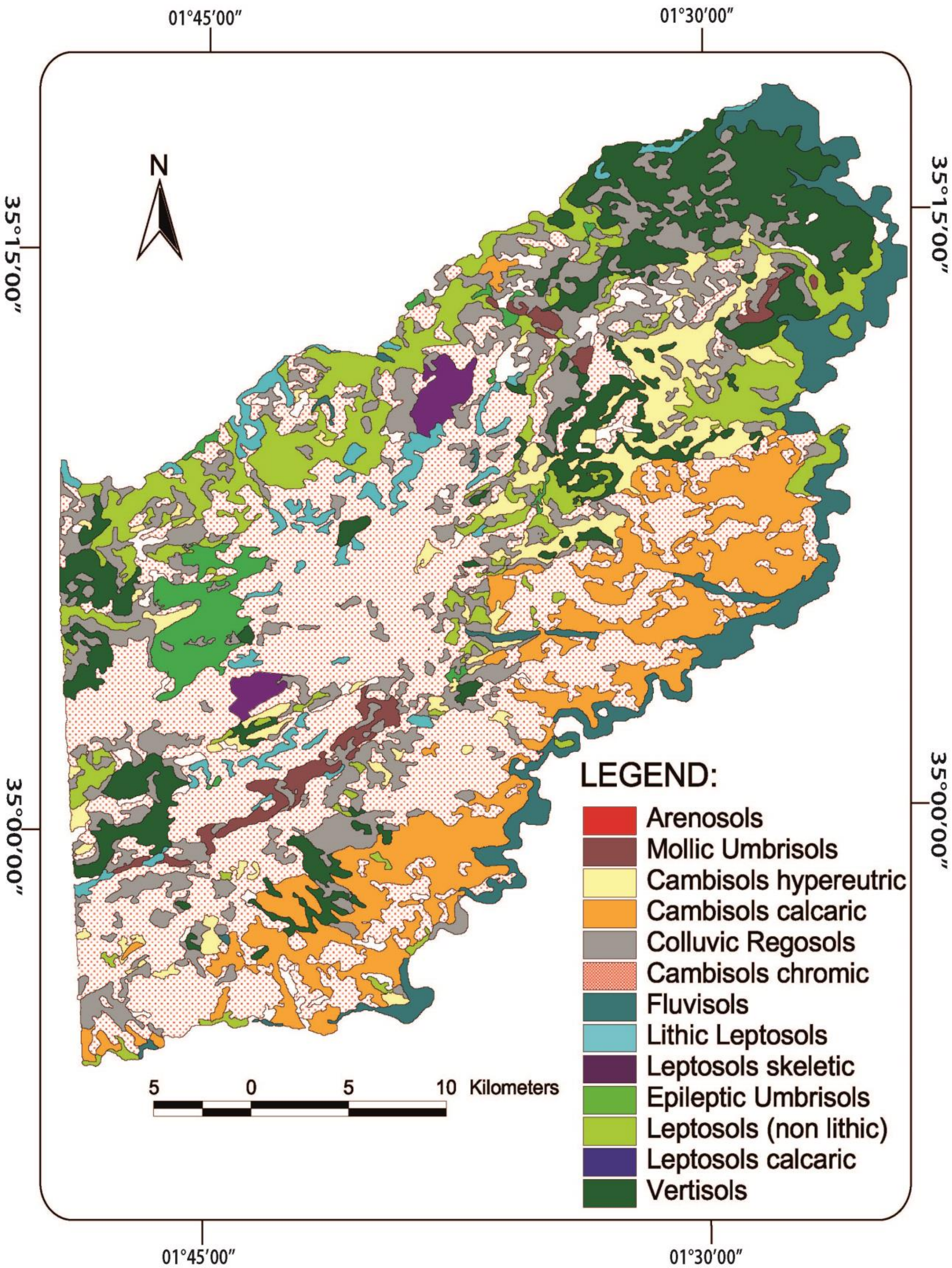

Figure 2. Soil map

Table 1. Major soil units' abbreviation and their correspondence between RP (2008) and FAO-WRB (2006)

Major soil unit following R.P. (2008)	Major soil unit following FAO-WRB (2006)	Abbreviation
Arénosols	Arenosols	ARN
Lithosols	Lithics leptosols	LLP
Régosols	Leptosols non lithics	LNL
Peyrosols	Letosols skeletics	LSK
Rendisols	Leptosols calcarics	LCA
Calcosols	Cambisols calcarics	CCA
Calcisols	Cambisols hypereutrics	CHY
Rankosols	Epileptics umbrisols	EUM
Fersialsols	Cambisols chromics	CCH
Fluviosols	Fluvisols	FLV
Colluviosols	Colluvics regosol	CLV
Brunisols saturés	Mollics umbrisols	MUM
Vertisols	Vertisols	VRS

Method

The EMC has long history and is rooted in the work of Gingerich (1969), Selley (1969), Read (1969), Türk (1979, 1982), Walker (1979), Harper (1984), Powers and Easterling (1982) and many others. Numerous techniques exist to apply EMC; all are based on the same principle, and can be divided into five steps (Xu and Maccarthy, 1998; Labat, 2004):

1. Tabulate the number of instances of the transition (transition frequency matrix) from which transition probability matrix(P_{ij}) is computed by dividing each value by the appropriate row total;
2. Compute independent trials (probability) matrix $e_{ij} = C_j/(T - C_i)$ where $e_{ij}$is the probability of passing from facies i to facies *j*, C_i and $C_j$are the number of occurrences facies *i* and facies *j*, *T* is the total number of transitions of the whole matrix (P_{ij}).
3. Deducing difference matrix $(D_{ij} = P_{ij} - e_{ij})$;
4. Test for randomness and significance;
5. Construct a relationship diagram from the positive values of the difference matrix or the resulting values of any significance test used.

Gingerich (1969) and Selley (1969) methods are considered as the earliest in the EMC field. However they did not solve correctly the problem of randomness tests as well as the determination of a given difference (for more details readers can refer to the review article of Xu and Maccarthy (1998) and the comment of this article made by Türk (2002). Some modifications have been advocated by Türk (1979, 1982), Powers and Easterling (1982), Harper (1984a,b); improvements were made in order to detect outliers in the original transition matrix and to test randomness of the sequence. All these methods were developed by geologists for lithostratigraphic column data where direction is taken into account (top to bottom or bottom to top).

The main differences that exist between geological and current data are: the first one are in one dimension (stratigraphic column) and the second in two dimensions (soil map). In addition, data have been introduced regardless to the direction, hence transition from soil A to soil B is the same from B to A and the consequence is a symmetric matrix. Furthermore, transition of one soil with itself is not allowed, so the diagonal of the transition matrix is equal to zero, which is a prerequisite of the EMC definition.

The main impediment for applying EMC methods is the tediousness of the calculation, increasing the risk of error. Thus, in this case study, Strati-signal software developed by Ndiaye (Ndiaye, 2007; Ndiaye et al. 2014) has been used to relieve calculation tasks. Strati-signal allows three main EMC methods: Walker, Harper and Türk. The simplest method is Walker which follows all steps above-mentioned without any test of significance of the D_{ij} matrix positive values. Harper method is similar to the previous one but uses binomial probability to rule out the null hypothesis of randomness of each transition link between soils. Türk method uses quite different approach, by introducing the idea of iterative proportional fitting (IPF) to calculate a valid randomized matrix to compare data, and to test it using quasi-independence concept. Hence, the transition matrix is split into noise and signal matrix (expectancy matrix) and to check the residuals with chi-square significance method (Türk, 1982).

These three methods have been used in the current case study in order to perform some comparisons, and to check out the fitness of EMC soil map spatial analysis. The results are presented on tables and soil relationship diagram (SRD) by analogy to facies relationship diagram (FRD) of geologists.

From the soil map (Figure 2) adjacency matrix was tabulated (Table 2) each value represent the number of times where soil A is neighboring (adjacent) with soil B. For practical purposes, each major soil type was designated with an abbreviation as presented in the Table 1.

Table 2. Adjacency matrix stemmed from the above soil mapR_i and C_i total rows and columns respectively.

	ARN	LLP	LNL	LSK	LCA	CCA	CHY	EUM	CCH	FLV	CLV	MUM	VRS	***R***
ARN	0	1	0	0	0	0	0	0	0	1	0	0	0	2
LLP	1	0	13	3	8	0	1	1	43	1	14	5	10	100
LNL	0	13	0	12	18	12	41	2	79	10	125	6	27	345
LSK	0	3	12	0	6	1	5	0	15	0	21	4	1	68
LCA	0	8	18	6	0	14	16	0	46	2	44	4	17	175
CCA	0	0	12	1	14	0	11	0	69	22	32	0	10	171
CHY	0	1	41	5	16	11	0	1	55	4	85	0	33	252
EUM	0	1	2	0	0	0	1	0	3	0	5	0	0	12
CCH	0	43	79	15	46	69	55	3	0	25	184	14	42	575
FLV	1	1	10	0	2	22	4	0	25	0	8	0	2	75
CLV	0	14	125	21	44	32	85	5	184	8	0	25	78	621
MUM	0	5	6	4	4	0	0	0	14	0	25	0	4	62
VRS	0	10	27	1	17	10	33	0	42	2	78	4	0	224
C_j	2	100	345	68	175	171	252	12	575	75	621	62	224	2682

The adjacency matrix is a large database (13 soil types and 2682 soil transitions counted) is also called frequency matrix, was introduced into the Strati-signal software to perform the three above mentioned EMC methods by keeping software computation defaults.

Results and Discussion

A χ^2 test has been used in order to check for the Markov property, in other words to rule-out the null hypothesis of randomness. The results are:

Computed χ^2=512.3

Number of degree of freedom=131

Critical χ^2 at 95% confidence level=124.3

The null hypothesis is rejected and data should not be random, exhibiting a strong first-order Markovian property.

From Table 3 representing Walker matrix, the positive values are ranged between 0.472 and 0.001 and represent about 58 links between soils, which is quite high to represent on SRD and difficult to unravel and interpret. That could be the weakness of this method especially if dealing with relatively big matrix. Hence, the highest values were selected, totalizing about 80% of the cumulative values, representing 16 links between soils (differences≥0.044, from Walker matrix). By assuming that the remaining 20% shared by 42 links (differences<0.044) are more likely to bring noise into the SRD impeding a clear interpretation.

Table 3. Walker matrix. Positive differences are in bold and are used to build the SRD (Soil Relationship Diagram).

	ARN	LLP	LNL	LSK	LCA	CCA	CHY	EUM	CCH	FLV	CLV	MUM	VRS
ARN	0.000	0.463	-0.129	-0.025	-0.065	-0.064	-0.094	-0.004	-0.215	0.472	-0.232	-0.023	-0.084
LLP	0.009	0.000	-0.004	0.004	0.012	-0.066	-0.088	0.005	0.207	-0.019	-0.101	0.026	0.013
LNL	-0.001	-0.005	0.000	0.006	-0.023	-0.038	0.011	0.001	-0.017	-0.003	0.097	-0.009	-0.018
LSK	-0.001	0.006	0.044	0.000	0.021	-0.051	-0.023	-0.005	0.001	-0.029	0.071	0.035	-0.071
LCA	-0.001	0.006	-0.035	0.007	0.000	0.012	-0.009	-0.005	0.033	-0.018	0.004	-0.002	0.008
CCA	-0.001	-0.040	-0.067	-0.021	0.012	0.000	-0.036	-0.005	0.175	0.099	-0.060	-0.025	-0.031
CHY	-0.001	-0.037	0.021	-0.008	-0.009	-0.027	0.000	-0.001	-0.018	-0.015	0.082	-0.026	0.039
EUM	-0.001	0.046	0.037	-0.025	-0.066	-0.064	-0.011	0.000	0.035	-0.028	0.184	-0.023	-0.084
CCH	-0.001	0.027	-0.026	-0.006	-0.003	0.039	-0.024	0.000	0.000	0.008	0.025	-0.005	-0.033
FLV	0.013	-0.025	0.001	-0.026	-0.040	0.228	-0.043	-0.005	0.113	0.000	-0.132	-0.024	-0.059

The Harper method results are presented in the Table 4 where binomial probability values are all <0.005 which means that they are highly significant with >99.5% confidence level (Xu and Maccarthy, 1998; Sarmah, 2013). Number of links is dropped from 58 links in the previous method to only 10 links.

Table 4. Binomial probability values following HARPER method.

Transitions	Binomial probability
FLV→CCA	1,21E-09
CCA→FLV	9,10E-09
CCA→CCH	2,84E-07
LLP→CCH	2,33E-06
LNL→CLV	5,61E-05
CCH→CCA	8,43E-04
VRS→CLV	9,61E-04
CCH→LLP	0,00224241
CHY→CLV	0,00245536
MUM→CLV	0,00275467

Table 5. Significance difference values according to TÜRK method.

Transitions	Significant difference values
FLV→ARN	0,952
ARN→FLV	0,952
LLP→ARN	0,935
ARN→LLP	0,935
FLV→CCA	0,802
CCA→FLV	0,802
CCA→CCH	0,573
CCH→CCA	0,573
CCA→LCA	0,470
LCA→CCA	0,470
LLP→CCH	0,436
CCH→LLP	0,436
FLV→CCH	0,402
CCH→FLV	0,402
VRS→CHY	0,367
CHY→CCH	0,367

Türk method shows more links between soil units 16 (Table 5) instead of 10 by Harper method. The resulted SRD of the three methods is represented Figure 3.

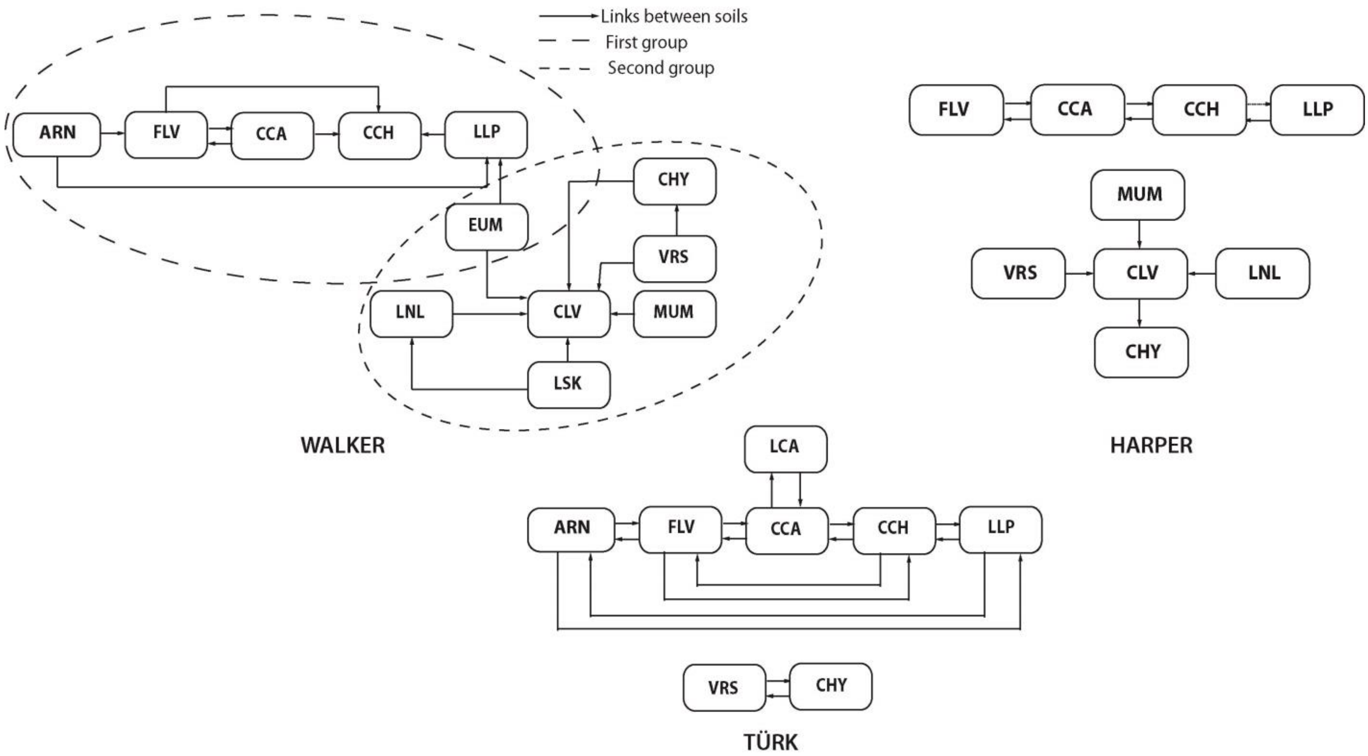

Figure 3. Soil relationship diagram (SRD) following Walker, Harper and Türk methods

The SRD drawn from Walker matrix shows two distinctive groups (Figure 3). The first one is structured around the cambisols chromics (CCH) which are considered as the climax soils in the Mediterranean region. FLV is also linked to CCH because ancient terraces are able to evolve to CCH by fersiallitization, which is a long-term pedogenic phenomenon. So FLV-CCH is more likely to be defined as a topo-chronosequence and not to complete the cyclic sequence FLV-CCA-CCH-LLP-FLV. A pedological cyclic sequence must obligatory pass through LLP or LNL which are both considered as rejuvenating step.

The second group is structured around colluvic regosols (CLV) where it plays the role of a receptacle of eroded material coming from the other soils. However, Walker SRD raised one problem concerning ARN which is represented by only one polygon on the map, but it presents the largest differences with FLV and LLP because it is linked only with these two soil types. Hence, it is considered as an outlier since it affects negatively the other links. Similar problem has been pointed out by Mastej (2002) presuming that they must have at least 5 transitions for credible results although single case with lower number of transitions is acceptable. The same observation can be made for the Türk SRD concerning ARN. Conversely, in Harper SRD the ARN is not represented at all, and the first soil sequence is reduced to only four types of soil (FLV, CCA, CCH, LLP), which can be found in the other methods. CLV is the main soil structuring the second sequence by both Walker and Harper methods but it doesn't exist in Türk method. According to this method, links are not enough significant to be represented and are considered as noise and hence, eliminated. However, it further stresses the link between VRS and CHY which is substantiated by Walker SRD. This link is confirmed in the field; cambisols hypereutrics (CHY) occur essentially on marls and can be evolved, by transition with cambisols vertics, towards vertisols (VRS) wherever topography permits in bowl-like depressions.

So if the ARN have brought confusion, it should be overlooked and rebuild a new matrix with 12 soil types instead of 13, and all the procedure is then restarted.

Computed χ^2=446.5

Number of degree of freedom=109

Critical χ^2 at 95% confidence level=124.3

The null hypothesis is rejected and data should not be random, exhibiting a strong first-order Markovian property.

In the Walker method the 80% of the cumulative values correspond to 19 out of 54 links instead of 16 out of 58. The results are shown in Figure 4.

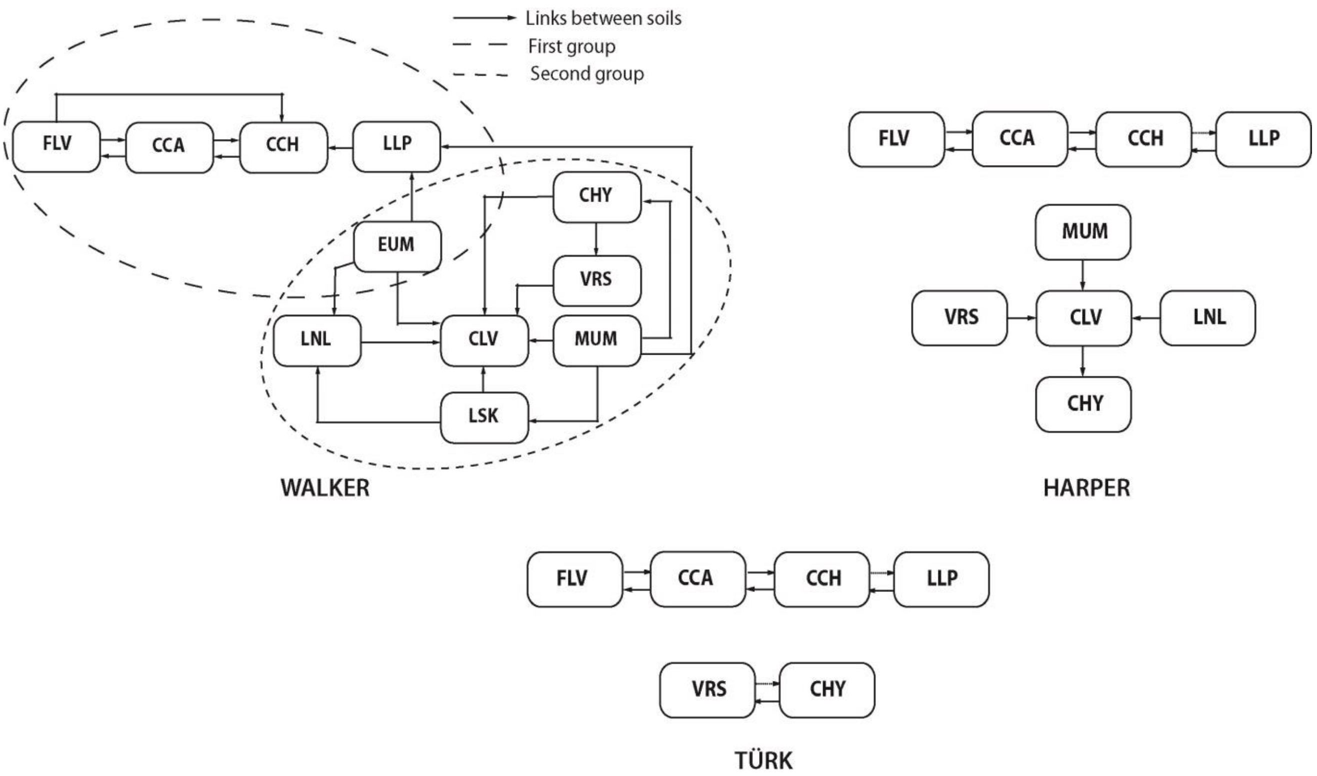

Figure 4. Soil relationship diagram (SRD) following WALKER, HARPER and TÜRK methods without ARN

With or without ARN, Harper SRD doesn't change at all. So this method is not affected by low number of transitions. However, in Türk SRD by losing 4 links and one soil type (LCA) it supports Harper results concerning the first sequence. Walker method seems to be the most realistic but the weakness of the method lies on the significance of the relationships between soil types. The ceiling 80% was defined arbitrary; conversely, by introducing significance test information might be lost that can be reliable. However, Harper SRD is the most relevant to the current data, because it shows two separate sequence of soils as it is mentioned before: the first FLV-CCA-CCH-LLP (Figure 5) begins with lithics leptosols occurring on the steepest slopes maintaining them by rejuvenation. On lower altitudes and less steep slopes, truncated cambisols chormics are widely spread, they exist on almost all kind of substratum bearing forested maquis; they are followed by cambisols calcarics on less vegetated areas because of the human impact (low altitudes and gentle slopes). In pedogenic point of view the CCA are developed from eroded material coming from the CCH. Finally, CCA are naturally connected with fluvisols bordering wadi Tafna the main stream of the region, with its tributaries wadi Boukiou and wadi Dahmane.

The second sequence is associated to the steep slopes relief with the colluvics regosols (CLV) occurring on the foothills. The relation of these two sequences can be seen in the Walker SRD, where LLP plays a pivotal role between the two sequences.

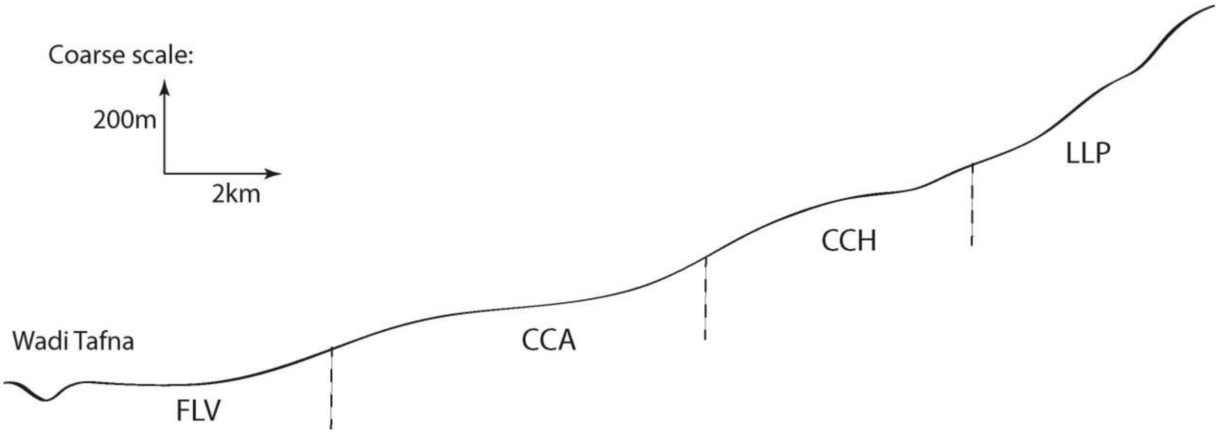

Figure 5. Theoretical catena of the Traras region with EMC method (the 1st sequence)

Conclusion

The present study reveals that it is worthwhile to apply embedded Markov chain analysis for soil map spatial analysis. However, the questioning arises from this study is, if it is mathematically fair to apply an EMC to data from a 2 dimension map with a symmetric matrix. By letting this question to statisticians, the results are conclusive and the catena is still remains a powerful means to understand soils. Theoretical catena highlighted by EMC method is the most representative of the study area and even for the whole Mediterranean region.

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Identification of strain types of some *Beet necrotic yellow vein virus* isolates determined in Northern and Central Parts of Turkey

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Abstract

Beet necrotic yellow vein virus (BNYVV), the agent of rhizomania disease, causes severe economic losses in sugar beet fields in all over the world. The virus is transmitted by a plasmodiophorid vector, *Polymyxa betae* Keskin. Twenty soil samples, collected from sugar beet fields in northern and central parts of Turkey during surveys in 2004 and 2005 and known to be infested with viruliferous cultures of *P. betae* carrying BNYVV, were selected and used in this study. Sample selection was made according to symptom expression of beet seedlings in preliminary bait plant tests and locations of the soil samples that accurately represent the region from which they were taken. Total RNAs were extracted from sugar beet plants grown in these soils and used to amplify RNA-2 (nt. 19-1088) and RNA-3 (nt. 50-1268) of BNYVV by reverse transcription polymerase chain reaction (RT-PCR) method. Restriction fragment length polymorphism (RFLP) analysis of PCR-amplified products showed that most of BNYVV isolates studied were A-type strain, however, two isolates did not exactly match the band profile of A-type strain. Additionally, the presence of BNYVV RNA-5 component was investigated by RT-PCR using the primers specific for P26 coding region. Four samples belonging to three provinces were found to be involving RNA-5 segment (20%).

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Introduction

Many soil-borne viruses are known to infect sugar beet (*Beta vulgaris* L.) worldwide. Among them, *Beet necrotic yellow vein virus* (BNYVV), which is the agent of rhizomania disease, is transmitted via the plasmodiophorid *Polymyxa betae* Keskin (Abe and Tamada, 1986) and causes severe economic losses in sugar beet fields in all over the world. BNYVV is type species of the genus *Benyvirus* and possesses a multipartite positive single stranded RNA genome, with four or five components (Tamada, 1999). RNA-1 and RNA-2 are required for replication, assembly and cell-to-cell movement, RNA silencing suppression and vector transmission of the virus. The RNA-3, which encodes for a 25kDa protein (p25), is responsible for pathogenicity and production of typical disease symptoms in sugar beet roots (Tamada et al. 1999; Chiba et al. 2008). RNA-4 coded p31 protein is more directly responsible for vector transmission and root-specific suppression of RNA silencing (Rahim et al. 2007; Peltier et al. 2008). The RNA-5 encodes 26kDa protein (p26) and is known to be an additional pathogenicity factor (Tamada et al. 1989). Also, BNYVV isolates

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containing RNA-5 have been reported to be more pathogenic than RNA-5 lacking isolates (Tamada et al. 1996; Miyanishi et al. 1999).

Three major strain types of BNYVV have been identified using molecular analysis (Kruse et al. 1994; Koenig et al. 1995). The A-type strain, which is the most widespread (Schirmer et al. 2005), has been previously detected in Turkey (Kruse et al. 1994; Kutluk Yilmaz et al. 2007a), however the B-type strain is mainly found in Germany, France (Kruse et al. 1994; Koenig et al. 2008), Japan (Miyanishi et al. 1999), Iran (Sohi and Maleki, 2004) Belgium, the United Kingdom (Ratti et al. 2005) and China (Li et al. 2008). The P-type strain is closely related to the A type, but contains a fifth RNA and to date it has been recorded in Kazakhstan (Koenig and Lennefors, 2000), France (Schirmer et al. 2005) and the United Kingdom (Ward et al. 2007). The other BNYVV isolates containing RNA-5 segment have been suggested to be named as the J-type strain which possess two deletions at amino acid positions 77 and 227-229 in p26, compared to the P-type strain (Schirmer et al. 2005). The J-type widely distributed in Asia, especially in Japan (Tamada et al. 1989) and China (Li et al. 2008) whereas it was recorded in a field of Germany (Koenig et al. 2008). More recently, the J-type RNA-5 containing BNYVV isolates were also detected in Turkey (Kutluk Yilmaz et al. 2016).

Although increases in sugar beet yields following some chemical treatments to control rhizomania disease were obtained, the use of these products is being phased out because of health hazards and adverse effects on the environment associated with their use. Alternatively, different methods have been tried for the control of rhizomania disease (Burketova et al. 1996; Wang et al. 2003; Akca et al. 2005; Akca et al. 2014). But, their efficiency not sufficient in eliminated BNYVV soil inoculum (Bragard et al. 2013). *P. betae* sporosori is able to persist in the soil for up to 25 years while remaining viruliferous (Abe and Tamada, 1986), therefore, the most reliable solution is use of resistant sugar beet cultivars.

In Turkey, rhizomania disease was reported in 1987 (Koch, 1987), and then it has been observed in most provinces where sugar beet is cultivated (Kaya, 2009; Kutluk Yilmaz and Arli-Sokmen, 2010; Yardimci and Culal-Kılıc, 2011; Kutluk Yilmaz et al. 2016).

In the current study, twenty BNYVV isolates were selected according to their symptom expression and geographic origin from the northern and central parts of Turkey. The BNYVV strain types were determined using reverse transcription-polymerase chain reaction (RT-PCR) and restriction fragment length polymorphism (RFLP) analyses, and the relationship between the phenotypic appearance of BNYVV on the sugar beet bait plants and strain types were investigated.

Material and Methods

Bait plant technique

In this study, twenty BNYVV isolates belonging to six provinces (Amasya, Cankiri, Corum, Kastamonu, Samsun and Tokat), out of 156 BNYVV-infected ones (Kutluk Yilmaz and Arli-Sokmen, 2010), were selected according to their symptom expression and geographic origin in order to be used in molecular studies (Figure 1, Table 1).

Figure 1. The dark colored inner area in the map of Turkey shows the region soil sampled.

Table 1. Geographic origin and symptom expression of BNYVV isolates.

Province	District	Isolate no	Infection phenotypes	
Corum	Iskilip	114	No visible symptom	
Samsun	Bafra	421		
Tokat	Erbaa	291		
Amasya	Centrum	178	Light chlorosis	
Samsun	Kavak	40		
Tokat	Erbaa	103		
	Pazar	210		
	Zile	262		
Amasya	Suluova	175	Chlorosis	
	Tasova	249		
Kastamonu	Sarayovasi	176		
Samsun	Vezirkopru	30		
Tokat	Artova	325		
	Niksar	202		
	Niksar	275		
Cankiri	Kizilirmak	164	Leaf rolling+curling	
Samsun	Vezirkopru	85		
Amasya	Tasova	186	Green vein banding + distortion	
Corum	Osmancik	106		
Tokat	Zile	324	Necrotic lesions	
Corum	Osmancik	150	Chlorotic lesions + chlorosis	

In the bait plant experiment, two 300 mL plastic pots were filled with each soil sample (20 samples) mixed with sterile sand (1 part soil: 1 part sand), and 10 sugar beet seeds of the rhizomania-susceptible (cv. Arosa) were sown in each pot for the bait plant test technique (Burcky and Buttner, 1985). The plants were grown under controlled conditions of 12-h photoperiod, at 20°C (night) and 25°C (day) temperatures. All plants were equally watered generously every week with Hoagland's solution. After growing sugar beet seedlings six weeks, leaf symptoms on bait plants were recorded. In the harvest, plant roots were carefully washed in running top water. After that the combined roots of each pot were divided into two parts. One was used to test for the presence of BNYVV by ELISA and the other part for RNA extraction. Plant material was stored at -

20°C until used. In this study, soil samples infested with BNYVV P- type and B- type supplied by Claude Bragard (Universite Catholique de Louvain, Louvainla-Neuve, Belgium) was used as a reference material.

Enzyme linked immunosorbent assay (ELISA)

The bait plants were tested by double-antibody sandwich (DAS)-ELISA according to Clark and Adams (1977) using a specific polyclonal antiserum (Bioreba AG, Switzerland) to identify BNYVV. The optical density was measured at 405 nm using the micropleyt reader (Tecan Spectra II, Austria) and a sample was considered as positive when absorbance value at 405 nm was more than two times the mean of the negative control (Meunier et al. 2003).

Reverse transcription polymerase chain reaction (RT-PCR)

Total nucleic acids were extracted from 100 mg of roots from cv. Arosa using an RNeasy Plant Mini Kit (Qiagen, Germany) as described in the manufacturer's instructions. One-step reverse transcription polymerase chain reaction (RT-PCR) was performed as described in the manufacturer's manual (Qiagen, USA) using thermocycler (Bio-Rad, USA) for BNYVV RNA-2 and RNA-3. For the detection of BNYVV RNA-2 and RNA-3, the primers proposed by Kruse et al. (1994) were used (BNYVV-2F: CCATTGAATAGAATTTCACC and BNYVV-2R: CCCCATAGTAATTTTAACTC for BNYVV RNA-2; BNYVV-3F: GTGATATATGTGAGGACGCT and BNYVV-3R: CCGTGAAATCAC-GTGTAGTT for BNYVV RNA-3). The RT-PCR reaction was done as follows: 10.5 µl RNase-free water, 5 µl 5X Qiagen OneStep RT-PCR buffer, 1 µl dNTPs mix (400 µM), 5 µl 5X Q solution, 0.25 µl each of forward and reverse primers (0.6 µM), 1 µl Qiagen OneStep RT-PCR Enzyme mix and 2 µl RNA sample were added to the mixture. Reverse transcription of 30 min at 50°C and 15 min at 95°C was performed. Then 35 cycles composed of denaturation for 30 s at 94°C, annealing for 30 s at 41°C for RNA-2 (30 s at 48°C for RNA-3), and elongation for 1 min at 72°C, were carried out. A final elongation of 7 min at 72°C was added.

One-step RT-PCR was performed using a Superscript® III One-Step RT-PCR System with Platinum *Taq* DNA polymerase kit (Invitrogen) using thermocycler (Bio-Rad, USA) for BNYVV RNA-5. The upstream (BN5/F1: GTTTTTCCGCTCGCACAAGCG) and the downstream (BN5/R1: CGAGCCCGT-AAACACCGCATA) primers (Schirmer et al. 2005) which are specific for RNA-5 were used. The RT-PCR reaction was done as follows: 9.5 µl RNase-free water, 12.5 µl 2X reaction buffer, 0.5 µl each of forward and reverse primers (10 µM), 1 µl Superscript III RT/Platinum Taq mix and 1 µl RNA sample were added to the mixture. The following reaction conditions were conducted: 55°C for 30 min and 94°C for 2 min followed by 40 cycles of 94°C for 15 s, 59°C for 30 s, 68°C for 1 min, and final elongation step for 5 min at 68°C.

The samples were analyzed on 1% ethidium bromide agarose gel in 1X Tris-borate-EDTA (TBE) buffer using the Gel Doc 2000 Systems (Bio-Rad, USA).

RFLP analysis for the characterization of BNYVV strain types

Twenty isolates were used for RLFP analysis (Table1). The primers BNYVV-2F and BNYVV-2R; BNYVV-3F and BNYVV-3R were used to amplify the RNA-2 (nt. 19-1088) and RNA-3 (nt. 50-1268), respectively and followed by restriction cut with enzymes *EcoRI* and *EcoRI*, *BamHI*, *StyI*. Digestions were done as previously described by Kruse et al. (1994) and the restrictions patterns were analyzed by electrophoresis in 1% agarose gel stained with ethidium bromide.

Results

Detection of BNYVV

In a previous study, BNYVV was detected in 156 of the 510 sugar beet fields in the northern and central parts of Turkey (Kutluk Yilmaz and Arli-Sokmen, 2010). In the bait plant test with BNYVV-positive soil samples, sugar beet seedlings of rhizomania susceptible cv. Arosa showed different kinds of virus symptoms such as chlorosis, leaf rolling+curling, green vein banding + distortion, necrotic lesions and chlorotic lesions+chlorosis in growth room conditions (Table 1). Besides this, these kind of symptoms were not detected all plants, some of BNYVV-infected plants gave no visible symptom. The presence of some symptomatic differences in bait plant tests raises the question whether different BNYVV strain types are present in this region. Therefore, a total of twenty BNYVV-infested soil samples were selected according to their symptom expression and geographic origin from northern and central parts of Turkey. The rhizomania susceptible (cv. Arosa) cultivar was grown in these soils. To confirm of the presence of BNYVV in bait plant samples, DAS-ELISA test was done. All of the samples tested were positive in ELISA for BNYVV (Figure 1; Table 2).

Table 2. The presence of BNYVV and characterization of BNYVV strain types in northern and central parts of Turkey.

Province	District	Isolate No	ELISA absorbance values	RFLP pattern	RNA-5 positive*
Amasya	Centrum	178	1.246 (+)	A	-
	Suluova	175	0.537 (+)	A	-
	Tasova	186	0.339 (+)	A	-
	Tasova	249	2.743 (+)	A	-
Cankiri	Kizilirmak	164	0.469 (+)	A	-
Corum	Iskilip	114	0.400 (+)	A	-
	Osmancik	106	0.747 (+)	A	-
	Osmancik	150	0.997 (+)	A	+
Kastamonu	Sarayovasi	176	0.364 (+)	A	-
Samsun	Bafra	421	0.317 (+)	A	+
	Kavak	40	0.243 (+)	A	+
	Vezirkopru	30	1.017 (+)	A	-
	Vezirkopru	85	0.217 (+)	A	-
Tokat	Artova	325	2.927 (+)	A	-
	Erbaa	103	0.343 (+)	A	+
	Erbaa	291	2.829 (+)	A	-
	Niksar	202	2.911 (+)	A	-
	Niksar	275	2.820 (+)	A	-
	Pazar	210	2.782 (+)	A	-
	Zile	262	2.837 (+)	A	-
	Zile	324	0.600 (+)	A	-
Total (%)		20			4 (% 20)

*+: determined; -: not determined.

RFLP analyses of BNYVV isolates

RT-PCR studies were done by using the primers specific to RNA-2 (nt. 19-1088) and RNA-3 (nt. 50-1268). RFLP analysis of the PCR products revealed that BNYVV isolates in the region were A-type strain (Figure 2, 3 and 4), despite two isolates (421 and 186 No) did not exactly fit the band profile of A type isolates (Figure 3). The isolate 421 gave no visible symptom on sugar beet seedlings (cv. Arosa) in bait plant test, whereas the isolate 186 showed green vein banding and leaf deformation (Table 1). Besides this, no BNYVV B-type infections were found in the region (Table 2).

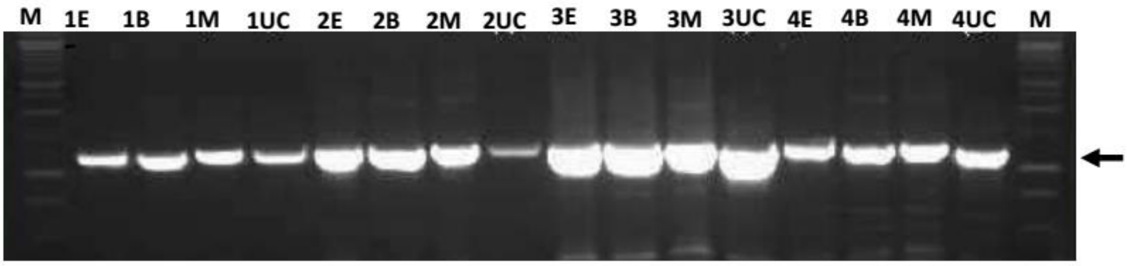

Figure 2. RLFP analysis for RNA-3 of Turkish BNYVV isolates. M: 1kb DNA Ladder (Promega, USA). The black arrow indicates BNYVV-specific 1218-bp DNA fragment. Lane 1-4, from a bait plant grown in the soil sample No. 178; lane 5-8 from a bait plant in the soil sample No. 262; lane 9-12, from a bait plant grown in the soil sample No. 325; lane 13-16, from a bait plant in the soil sample No. 175. E: *EcoR*I, B: *BamH*I, M: *Msp*I, UC: Uncut PCR product.

Detection of BNYVV RNA-5

To test whether the samples involves the fifth RNA segment, they were subjected to RT-PCR analysis with BNYVV RNA-5 specific primers (Schirmer et al. 2005). Four BNYVV-positive samples (20%) produced the expected amplicon of 885 bp in PCR amplification by using BN5/F1 and BN5/R1 primers (Data not shown). In this study, RNA-5 was determined in Corum, Samsun and Tokat provinces (Figure 1 and Table 1). This result indicated that BNYVV isolates containing RNA-5 segment seems to be not located in this region. The BNYVV isolates (40, 103 and 150) with RNA-5 showed light chlorosis and chlorotic lesions+chlorosis types of symptom on sugar beet seedlings in bait plant tests, whereas the isolate (412) gave no visible symptom (Table 1).

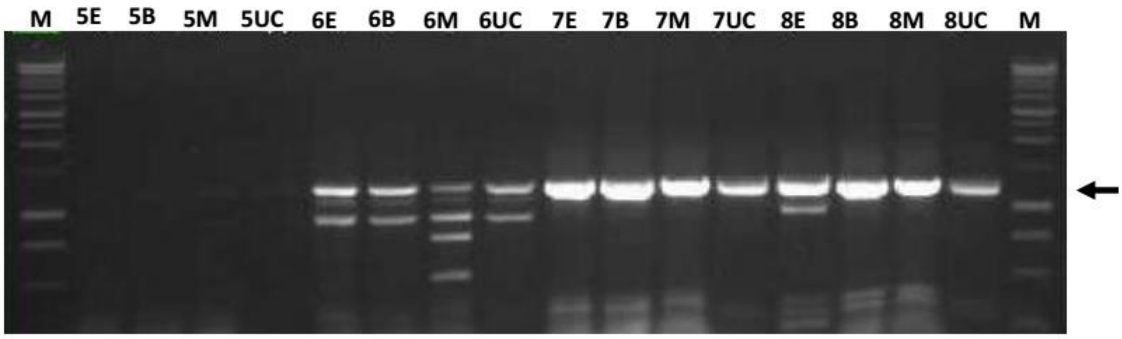

Figure 3. RLFP analysis for RNA-3 of Turkish BNYVV isolates. M: 1kb DNA Ladder (Promega, USA). The black arrow indicates BNYVV-specific 1218-bp DNA fragment. Lane 1-4, from a bait plant grown in the soil sample No. 275; lane 5-8 from a bait plant in the soil sample No. 421; lane 9-12, from a bait plant grown in the soil sample No. 106; lane 13-16, from a bait plant in the soil sample No. 186. E: *EcoR*I, B: *BamH*I, M: *Msp*I, UC: Uncut PCR product.

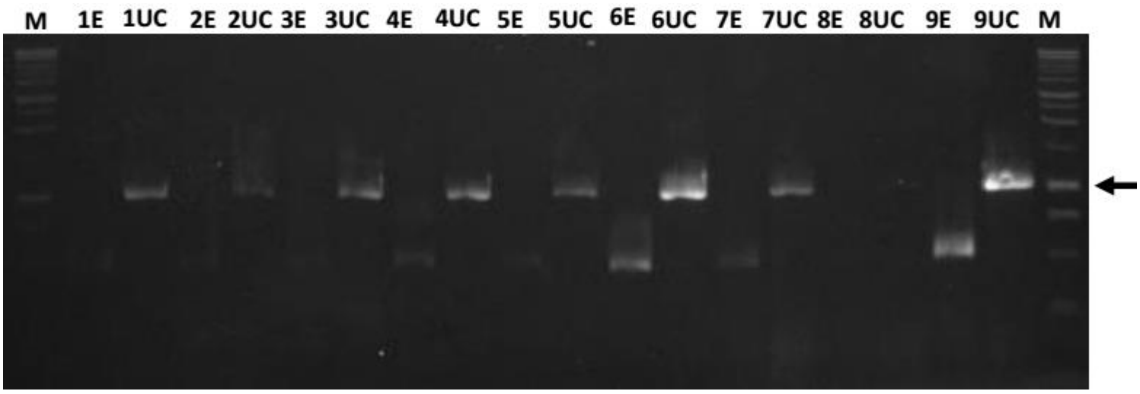

Figure 4. RLFP analysis for RNA-2 of Turkish BNYVV isolates. M: 1kb DNA Ladder (Promega, USA). The black arrow indicates BNYVV-specific 1070-bp DNA fragment. Lane 1-2, from a bait plant grown in the soil sample No. 186; lane 3-4 from a bait plant in the soil sample No. 178; lane 5-6 from a bait plant grown in the soil sample No. 106; lane 7-8, from a bait plant in the soil sample No. 85; lane 9-10 from a bait plant grown in the soil sample No. 325; lane 11-12 from a bait plant grown in the soil sample No. 291; lane 13-14 from a bait plant grown in the soil sample No. 175; E: *EcoR*I, UC: Uncut PCR product.

Discussion

In the current study, RFLP analysis was conducted for strain determination (A and B type) of BNYVV isolates selected according to their geographic origin and symptom expressions such as chlorosis, leaf rolling+curling, green vein banding+distortion, necrotic lesions and chlorotic lesions+chlorosis (Table 1) in bait plants grown in soils from northern and central parts of Turkey (Figure 1). Restriction patterns obtained for all BNYVV isolates were identical to A-type strain, despite two isolates (421 and 186) did not exactly fit the band profile of A-type isolates (Figure 3, Table 2). These isolates may differ in polymorphic sites and could be different variants of BNYVV. Polymorphism may further be investigated by sequencing. Interestingly, the isolate 421 gave no visible symptom on sugar beet seedlings (cv. Arosa) in bait plant tests, whereas the isolate 186 showed green vein banding and leaf deformation (Table 1). Similarly, A-type BNYVV have been previously reported in Turkey (Kruse et al. 1994; Kutluk Yilmaz et al. 2007; Chiba et al. 2011; Kutluk Yilmaz et al. 2016) and in its neighboring counties for example Iran (Sohi and Melaki, 2004) and Greece (Kruse et al. 1994; Pavli et al. 2011). No B-type BNYVV infections were found in tested samples in the region whereas the B-type BNYVV (Sohi and Maleki, 2004) was recorded in Iran.

Additionally, BNYVV RNA-5 segment was detected in four samples belonging to Corum, Samsun and Tokat provinces (Figure 1 and Table 2). In a previous study, Kutluk Yilmaz et al. (2016) indicated that BNYVV populations containing RNA-5 is highly widespread in sugar beet production areas in Turkey. Besides this, nucleotide sequencing and phylogenetic analyses showed that RNA-5 of Turkish BNYVV isolates is closer to the J-type BNYVV isolates than the P-type isolates (Kutluk Yilmaz et al. 2016). On the other hand, the J-type widely distributed in Asia (Tamada et al. 1989; Li et al. 2008) whereas it was recorded in a field of Germany (Koenig et al. 2008).

From RNAs of BNYVV, the RNA-3 encoded p25 controls rhizomania symptoms in sugar beet roots and severe symptom expression in Chenopodiaceae hosts (Tamada et al. 1999; Jupin et al. 1992). Besides this, the severity of symptoms in sugar beet was increased by the additional presence of RNA-5 (Tamada et al. 1996; Miyanishi et al. 1999). In this study, two of RNA-5 containing isolates (40 and 103) showed chlorosis type of symptom on the susceptible cv. Arosa, whereas the isolate 150 gave chlorotic lesions+chlorosis type of symptom. Also, the isolate 421 (with RNA-5 segment) gave no visible symptom (Table 1) and its obtained restriction patterns did not exactly fit the band profile of A-type isolates (Figure 3). In the previous study, chlorosis type of symptom has been typically associated with BNYVV (Rush et al. 2006). Also they indicated that some BNYVV-infected plants never exhibited foliar symptoms. Similarly, in the current study, some of BNYVV-infected plants gave no visible symptom (Table 1). On the other hand, it is known that high soil pH and lime ($CaCO_3$) contents cause chlorosis by affecting nutrient sufficiency in soils (Kutluk and Arli Sokmen, 2007). Kutluk Yilmaz et al. (2010) reported that increasing lime and exchangeable Mg contents of soils increased soil pH and induced BNYVV and *Beet soil-borne virus* (BSBV) infections transmitted by vector *P. betae*, respectively. There are very few published studies about different infection phenotypes of BNYVV populations on sugar beet. Some researchers indicated that the resistant plants after rub inoculation displayed a range of symptoms from no visible lesion or necrotic lesions at the inoculation site, whereas the susceptible plants showed bright yellow lesions (Chiba et al. 2008).

Consequently, the majority of the samples contained A-type BNYVV (80%), but in 20% of the samples, A-type BNYVV with RNA-5 was identified. There was no any interaction found between symptom expression and strain types of BNYVV populations. Moreover, RNA-5 segment did not seem to be directly associated with symptom severity on sugar beet seedlings (rhizomania susceptible cv. Arosa) in bait plants in this study.

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Evaluation of heavy metal complex phytotoxicity

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Abstract

The experimental data dealing with the effect of heavy metals contained in the technogenic contaminated soils on plant objects under controlled conditions was discussed. The aim of this work is to define the quantitative indicators of copper and zinc potential phytotoxicity, namely germination energy, simultaneous germination and duration of the test plants. It was found that the activity of the test plant growth is linked with copper and zinc complex action. Joint effect of copper and zinc is manifested both in inhibition of lettuce growth and determined, above all, by the nature contamination, soil properties and biological specificity of the test plants.

Keywords: Phytotoxicity, copper, zinc, biological test culture.

Introduction

Electroplating enterprise waste, called galvanic slime (GS), has a negative impact on atmosphere, hydrosphere and soil cover. As a result, such waste influences plants and animal organisms state (Kasimov, 2011; Baiseitova and Sartayeva, 2014; Black et.al., 1999). The migration of chemical elements, contained in stockpiled GS, is the result of physical and chemical processes under the influence of climatic and weather factors. The contamination, precipitation and infiltration through a layer of waste may spread into adjacent soil areas. While estimating ecotoxicological effects, combined effects of technological waste should be studied. It is very important to estimate the most effective phytotoxic waste using biological methods of analysis. These methods allow to find in addition to the general non-specific effects on biotest and to determine some specific reactions of individual chemicals or groups of substances (Olkhovich and Musienko, 2005).

Most of researchers paid attention to the problem of copper and zinc effect on different plants (Baiseitova and Sartayeva, 2014; Black et al., 1999; Olkhovich and Musienko, 2005; Grodzinsky et al., 2006; Hubachov, 2010; Mayachkina and Chugunov, 2009). But in spite of numerous studies, the problem of their translocation into plants remains open to discussion (Gruzdev, 2010; Gladkov, 2010). The research is rather difficult because of different soil conditions and various types of soil sensitivity. In this connection, it seems appropriate to conduct the study for specific areas which are most inclined to heavy metal contamination under homogeneous soil and climatic conditions (Grodzinsky et al., 2006; Hubachov, 2010).

The **e**xperiments studying the influence of different pollutants on plants in a controlled environment allows to solve many problems: a) to find out the causes of different plant resistance and the tendency to adapt to toxicants; b) to reveal the influence of the most important factors; c) to find the lethal dose of pollutant, etc. (Grodzinsky et al., 2006; Hubachov, 2010). Special test plants having the greatest sensitivity to various

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ecotoxicants were usually used for biological indication of the total soil pollution. These investigations have shown that the sensitivity of biological method may be compared with the instrumental one and sometimes even exceeds it (Olkhovich and Musienko, 2005; Mayachkina and Chugunov, 2009). In this regard, the aim of this study was to determine the potential phytotoxicity of galvanic production waste using the example of copper-zinc GS model. Cu and Zn metals were selected because they possess the greatest concentrations of GS in heavy industry and are the metals of high-class risk.

The aim of this study was to determine the effect of heavy metals, contained in technogenic contaminated soil on plants under controlled conditions. According to our aim the effect of Cu and Zn on the indexes of seed germination, initial growth and biomass of test plant were modeled and studied. In accordance with our aim the following tasks were put: to create an experimental model in vitro and to study lettuce (Odessa curly hair) its growth beginning and its sprout biomass.

Material and Methods

The determination of soil toxicity degree was performed by bioassay method (Olkhovich and Musienko, 2005). This method is the study of test object reaction to the pollutants action and allows getting an integral evaluation of phytotoxicity degree. Lettuce seeds (Odessa curly hair) were taken as a test object and the duration of seed germination and their energy in different GS contaminated soil layers were taken as a measure of toxicity.

The laboratory experimental model was devised to study copper-zinc GS phytotoxicity. Preliminarily contaminated by GS soil samples such as haplic arenosol, mollic gleyic fluvisol, gleyic chernozem, calcic voronic chernozem were used in the experiment. The top layers of soil were selected from the following depths: 0-5, 10-15, 20-25, 50-75, 100 cm. The selected soil was dried to constant weight in the open air. The air-dry soil 1500 g was put into plastic pots of 15 cm high and 9 cm in diameter. 15 seeds were placed in each vessel, pre-soaked in water for a day at a depth of 1 cm. A constant temperature 20 °C was maintained during the process of germination.

The duration and simultaneous germination energy were used to evaluate the effect of Cu and Zn in soil on seeds (Ubugunov and Dorzhonova, 2010; FR.1.39.2006.02264, 2009). Under germination is meant the number of seeds germinated for 7 days and expressed as a percentage to total number of seeds taken for germination, and under germination energy is meant the number of seeds germinated for the first 3 days of germination percentage to the total number of seeds taken for germination. Daily records of sprouted seeds were also used for a more precise characterization of germination speed. Therefore, the simultaneous germination energy was determined by the formula

$$D = \frac{P}{A}, \quad (1)$$

where D – simultaneous germination (average percentage of seeds germinated for the first day); P – percentage of complete germination; A – the number germination days.

The duration of germination is calculated:

$$C = \frac{(a \cdot 1) + (b \cdot 2) + (d \cdot 3) + \ldots}{(a + b + d + \ldots)}, \quad (2)$$

where C – is the average duration of one seed a day; a – a number of seeds germinated for one day; b – the number of seeds germinated for two days; d – the number of seeds germinated for three days, etc.

In addition to germination indicators, the growth rate was determined by seeds which characterize the viability of the plant best of all. The value of the control index (L_0) and experienced (L_{ex}) seeds were calculated as the mean arithmetic (L_{meam}) from the data totality about the length of vegetative part or the seedlings roots (FR 1.39.2006.02264, 2009).

$$L_{cp} = \frac{\sum L_i}{n}, \quad (3)$$

where L_i – the maximum length of the vegetative part or the root of each seedling; $\sum$ – the sum; n – the total number of experimental seedlings.

To evaluate the significance of differences between the experimental variants the whole experimental process was performed in triplicate.

Results and Discussion

Comparative data (Table 1) of Cu and Zn limited concentration* (LC) (LC (Cu) = 3 mg/kg; LC (Zn) = 23 mg/kg) in test soils were described in paper (Datsenko and Svashenko, 2015).

The result of Table 1 allow to formulate the following data:

- all Cu layers have the low concentration level;
- the concentration level for Zn was defined by soil character and it changes from very great in the upper layers to critical concentration in the lower ones (Damage determination from soil concentration by chemical substances).
-

Table 1. The index of the harmfulness of Cu and Zn in the contaminated soils in comparison with their limiting concentration in usual soils

Elements	The index exceeded hazard control samples	The index exceeded hazard in the respective layer (cm) of soil				
		0-5	10-15	20-25	50-75	100
Haplic arenosol						
Cu	0,75 LC	7.18 LC	1.36 LC	1.68 LC	1.68 LC	1.97 LC
Zn	0,28 LC	21.65 LC	12.10 LC	10.80 LC	3.93 LC	0.65 LC
Mollic gleyic fluvisol						
Cu	0,57 LC	7.70 LC	1.10 LC	0.50 LC	0.60 LC	0.60 LC
Zn	0,08 LC	33.50 LC	23.35 LC	7.90 LC	0.74 LC	0.15 LC
Gleyic chernozem						
Cu	0,89 LC	3.29 LC	1.84 LC	1.69 LC	1.09 LC	1.81 LC
Zn	0,17 LC	114.60 LC	32.65 LC	2.53 LC	0.30 LC	0.26 LC
Calcic voronic chernozem						
Cu	7,10 LC	36.57 LC	5.54 LC	5.75 LC	4.87 LC	7.08 LC
Zn	3,12 LC	229.30 LC	7.53 LC	1.59 LC	2.39 LC	1.73 LC

* LC – normative – is the quantity of unhealthy substances in surrounding components (water, air, soil). At constant contacts or at the influence of definite period they do not practically influence the human health and do not give rise to unhappy consequences for human posterity. Such normative is determined by law and is recommended to competent offices.

The analysis of morphometric parameters in lettuce shoots (Figure 1) grown in all soil tests showed that both inhibition and stimulating of the growth in the vegetative part take place. Similar effects were also observed for the growth of the root system.

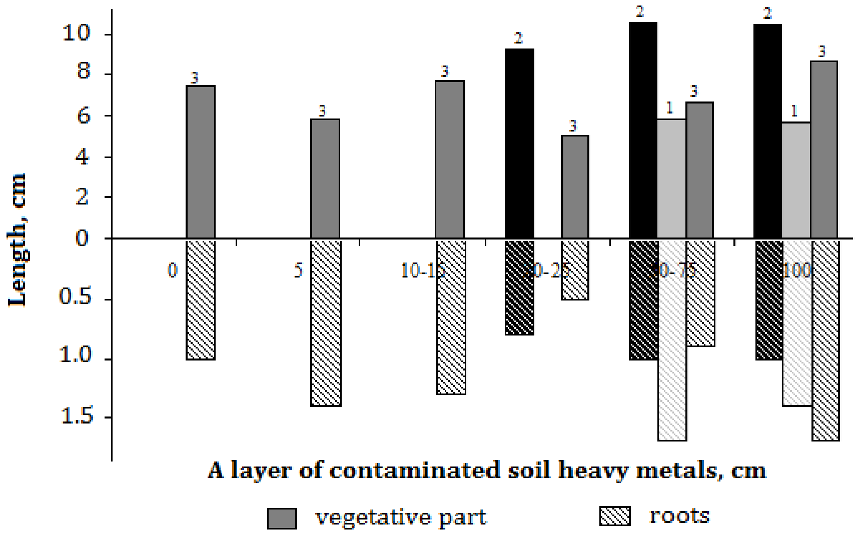

Figure 1. The length of lettuce sprouts (30 days cultivation) depending on the contaminated soil layer of heavy metals: 1 – mollic gleyic fluvisol; 2 – gleyic chernozem; 3 – typical heavy black earth

Haplic arenosol soil is characterized by a significant inhibition of test culture growth. The lettuce seeds spring up only in the lower layers of the soil under test (20-25, 50-75, 100 cm) and all germs were killed in 20 days. However, the biotesting studies of the rest test soils showed some stimulating development and test culture growth. Almost all layers showed a significant stimulation for calcic voronic chernozem soils. The shoot growth occurred throughout the whole growing period (30 days). The length change of both root and vegetative part of seedlings which depends on hazard index exceeding (LC) metal-toxicants shows the absence of unfavorable phytotoxic action. The average length of vegetative part and root system (L_{meam}) is the same and in some layers even greater than analogous indices in the control sample (L_0).

However, the analogy is not so much expressed and the stimulating effect is observed in the lower soil layers (20-25, 50-75, 100 cm) in mollic gleyic fluvisol and gleyic chernozem. The test soils impacted positively on the above-ground part and lettuce sprouts of root system showed good development. The aerial length in the gleyic chernozem is equal to 10 cm and 5.7 cm in the mollic gleyic fluvisol loam correspondingly. The root system development is equal to 1 cm in the gleyic chernozem and 1.5 cm in the mollic gleyic fluvisol (Figure 1). Significant seed sensitivity to copper and zinc was seen in all experimental samples. According to the results obtained, the reducing of metal concentration in the layers (50-75, 100 cm) decreases the metal toxic action. It is vividly expressed in the testimony of vigor (Figure 2). It is seen in the figure that it is 2.3-9 % in the haplic arenosol soils; 11-27 % in the mollic gleyic fluvisol; 42-47 % in the gleyic chernozem and 40-49 % in a typical heavy black earth.

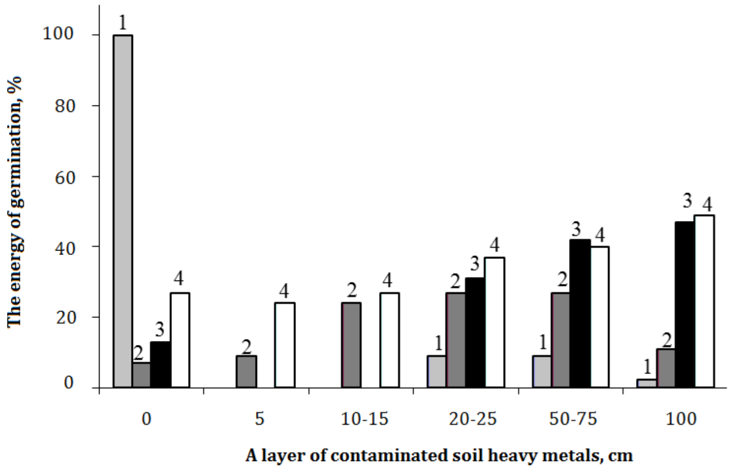

Figure 2. Lettuce seed dependence on the contaminated heavy metals soil layer: 1 – haplic arenosol; 2 – mollic gleyic fluvisol; 3 – gleyic chernozem; 4 – typical heavy black earth

Among the indicators of seed germination under GS soil contaminated conditions in a model proved to be the indicators of germination and their duration (Figure 3). The stimulating average effect of metals on the lettuce growth up to 7 days was stronger but its toxic action was weaker. Increasing the period of lettuce growth from 7 to 30 days, the growth character of the plants varies: the significant metal depressing effect is greater. The germination index in almost all cases of tested soils is fairly high: 9–73 % in the haplic arenosol sandy soil; 7–28 % in the mollic gleyic fluvisol loam; 13–42 % in the gleyic chernozem; 20–45 % in heavy loamy typical chernozem (Figure 3a). However, the duration index of germination is not high enough in all tested soil layers: 0,3–16 % in haplic arenosol; 1–4,4 % in the mollic gleyic fluvisol; 1,2–5.2 % in the gleyic chernozem; 0,9–6,0 % in the typical heavy black earth (Figure 3b).

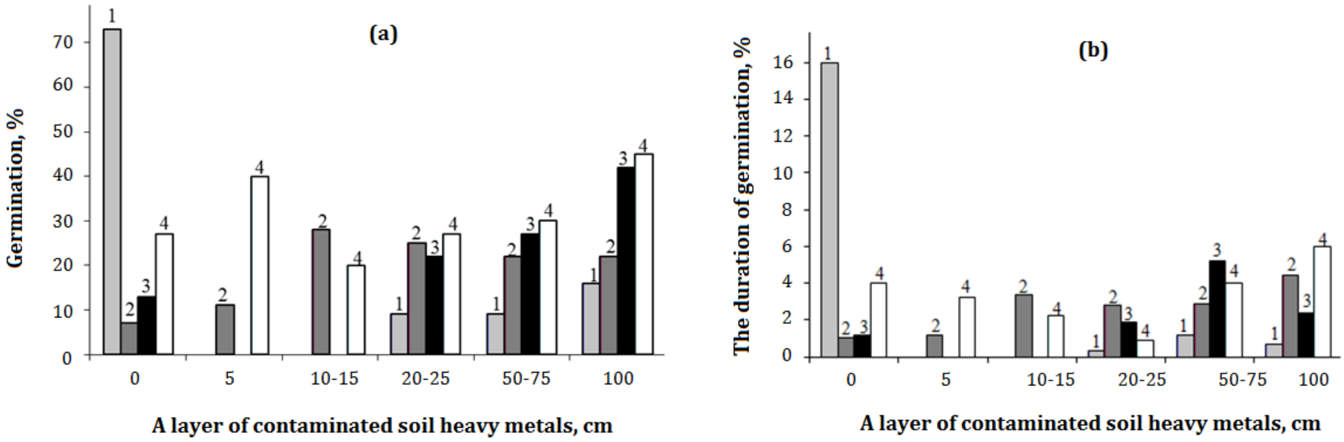

Figure 3. The dependence of lettuce seed germination (a) and there duration (b) on contaminated heavy metal soil: 1 – haplic arenosol; 2 – mollic gleyic fluvisol; 3 – gleyic chernozem; 4 – typical heavy black earth

The most informative among the growth and development of the tested culture in the model soil contamination is the germination activity indicator (Figure 4). The simultaneous of seed germination is relatively high in black chernozem soils: 1.3–4.7 % in gleyic chernozem; 3.4–7.0 % in heavy loamy chernozem. The same indicator is significantly lower in sandy soils and amounts to: 0.3–1.2 % in the haplic arenosol (except the control sample); 0.2–1.9 % in the mollic gleyic fluvisol.

Such indications of germination, growth and seed development of tested plants under heavy metal contamination can be explained by the fact that Zn and Cu belong to the metal group of average absorption by plants (Gruzdev, 2010). That is why at the initial terms the seeds possess sufficient nutrient potential to suppress the heavy metal effect. However, the inhibitory effect of metal-toxicants increased at the later development stages.

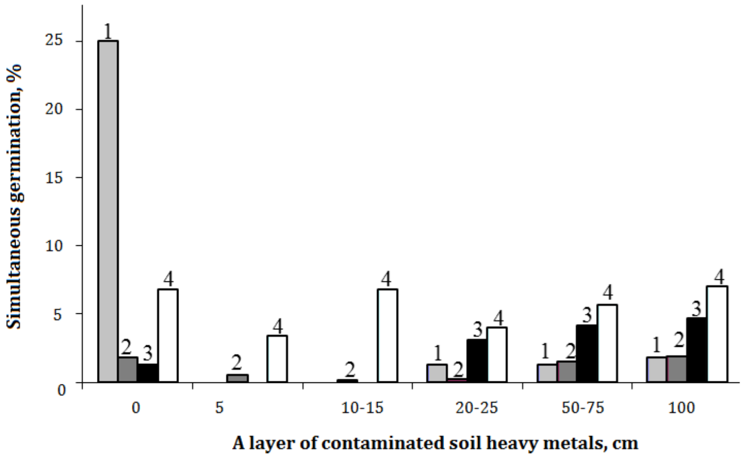

Figure 4. Seed germination activity dependence on the contaminated heavy metals soil of lettuce from the layer: 1 – gleyic fluvisol; 2 – mollic gleyic fluvisol; 3 – a typical heavy black earth, 4 – haplic arenosol

It should be also noted that for black earth typical loam (Figure 1) in the upper layers (5, 10–15 cm) the highest Cu and Zn content was obtained. The excess of their LC is equal to: 5.54–36.57 for Cu; 7.53–229.30 for Zn. However, the germination indicators, their activity energy and germination duration (Figure 2-4) are higher than those in other types of soils with lower LC. This may be due to the fact that clay soil characteristics are related to the soils possessing high adsorption properties and can bind heavy metals, protecting vegetable products from contamination (Baiseitova and Sartayeva, 2014).

According to modern concepts there are no toxic and non-toxic chemical elements in nature but there are only toxic and non-toxic concentrations. Depending on concentration and metal type, metal ion valence, solubility and duration of exposure, copper and zinc in small amounts contribute to the plant growth and development being essential microelements for them. But high Cu and Zn concentration can suppress them, breaking their vital functions (Black et al., 1999; Gruzdev, 2010).

As the results show, the data obtained confirm the above findings and point to the correlation between the content of heavy metals in soil and the growth activity of test plants. The comparison of maximum LC concentration values of copper and zinc in all test soils with germination, growth and development of lettuce seeds indicators showed the reducing of their concentration in the test soil layers slows down metal toxic effect. However, such correlation is noticeable not in all cases. Such dependence can be traced only in a single soil and it is not observed in other types of soil. It may be explained by natural interrelation between physical and physicochemical properties of the soil and tested culture parameters.

It should also be noted that copper and zinc effect has different focus on the activity for tested plants. Copper and zinc are known to be the most toxic pollutants in excess concentration. According to (STATE STANDARDS 2.2.7.029–99, 1999) copper is assigned to the low class of danger, and zinc to the high one. It is shown in the experiment that when LC (Cu) exceeds LC (Zn) 2–7 times (Table 1), the indices of germination and growth of test cultures are relatively higher (Figure 2-4) than in soils LC (Cu) < LC (Zn).

However, it should be noted that this dependence is not observed in all layers of soil. High indices of test culture germination are observed in the layers 10–15, 20–25 cm in mollic gleyic fluvisol where LC (Cu) exceeds LC (Zn) 0.05–0.06 times, and also in calcic voronic chernozem layers 5, 10–15 cm where the excess is 0.16–0.7 times. Thus, it should be assumed that the ambiguity of the correlation between copper and zinc content in the soil above their LC and active growth of test culture is connected with the complex action of copper and zinc. When combined action of the two metals occurs under unfavorable conditions and in harsh plant doses it may have both strengthening and weakening toxic effects. The synergistic action of zinc and copper may be determined by the location of these elements in the adjacent periodic system. As the authors suggest (Gladkov, 2010), copper possesses particularly high phytotoxicity and in the presence of zinc toxic effect increases sufficiently. That is why such contradictory ambiguous evidence about copper and zinc impact the test cultures and require further and more detailed studies.

Conclusion

The effect of heavy metals contained in the technogenic contaminated soils on plants under controlled conditions is determined, namely:

- soils contaminated by copper and zinc have integrated phytotoxic effect. The combined effect of copper and zinc is manifested as the inhibition or stimulation of lettuce growth processes and is determined, first of all, by the level and nature of contamination, soil properties and biological specificity of the test culture;
- the initial terms of the seed test culture have the potential of nutrients to suppress the effect of heavy metals. However, during later development the dampening effect of metal toxicants becomes stronger;
- there is both inhibition and stimulation of lettuce growth of the vegetative part and root system. Haplic arenosol is characterized by significant inhibition of the test culture, and for calcic voronic chernozem almost all layers showed significant stimulation;
- the seed sensitivity of the test object for copper and zinc is significantly manifested in the vigor testimony. Accoding to this parameter the investigated soils can be arranged in series: a haplic arenosol < mollic gleyic fluvisol < gleyic chernozem < calcic voronic chernozem**;**
- the indicator of seed germination in almost all cases of tested soils is high (7-73 %). However, the duration of the germination rate has the highest rate (0.3-16 %) depending on the type of soil;
- the germination period of seeds is relatively high in the black soils (1.3-7.0 %), but it is much lower in sandy soils (0.2-1.9%).

Evidence-based data provided in the work, are of interest in terms of the methods of bioassay environmental pollution due to the openness and relevance of this issue at the present stage of ecology development. These results confirm the need for agroecological monitoring to prevent possible negative consequences of human activities on the environment.

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