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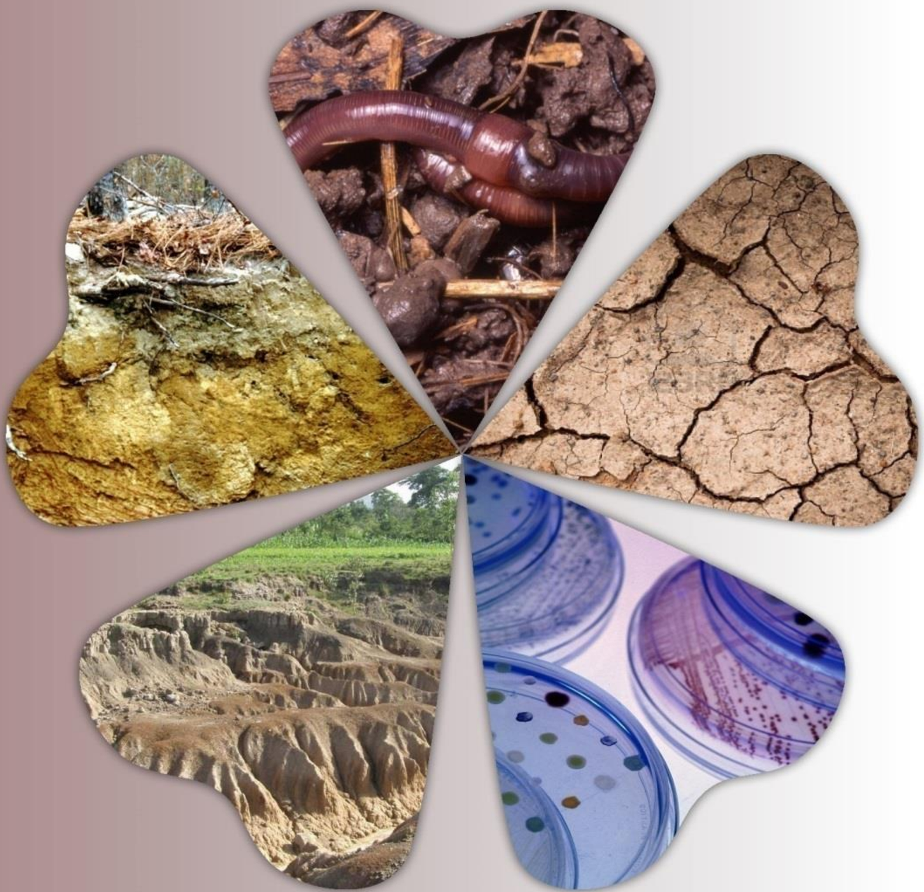

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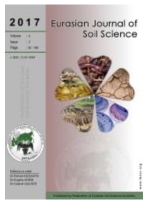

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Comparative potential of *Rhizobium* species for the growth promotion of sunflower (*Helianthus annuus* L.)

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Abstract

Rhizobium besides its nodule formation characteristic with members of Fabaceae family has been recognized for its great root colonizing ability and growth hormone production potential. In addition to nitrogen fixation in legume plants, rhizobia considered as beneficial tools and act as plant growth promoting rhizobacteria (PGPR) with many non-legumes. Present study was elucidated to determine the comparative role of *Rhizbium* sp for growth promotion of sunflower. Rhizobia were isolated from five different legumes (mungbean, barseem, lentil, chickpea, and vegetable pea) and checked for their auxin production efficiency. Rhizobial isolates Cp-4 showed maximum auxin potential (5.37 μg mL^{-1}IAA equivalents).Results showed that inoculation of all rhizobial isolates caused significant increase in growth and physiological parameters of sunflower plants. While prominent results were found with inoculation of mungbean rhizobial isolate Mb-2 which increases the chlorophyll a, N, P, fresh and dry matter of sunflower significantly by 8.34, 4.9, 36, 31, and 34%, respectively in comparison to un-inoculated control plants. Hence, present study concluded that *Rhizobium* sp can be successfully used as PGPR in non-legumes after thorough investigations.

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Keywords: Rhizobium sp, PGPR, auxin biosynthesis, growth, sunflower.

Introduction

Sunflower (*Helianthus annuus* L.) is an oil-seed crop grown in different ecologies of Pakistan. Pakistan is deficient in edible oil and fulfills 65% of its requirement by importing oils of worth 1.5 billion$. Sunflower oil is a rich source of linoleic acid (64%) that minimizes cholesterol level in the coronary arteries and has 40-45% high quality protein. Pakistan produced 13.4% sunflower oil domestically (Shah et al., 2005).

Microorganisms are considered as indicators of soil health. Plant growth promoting rhizobacteria (PGPR) influence the plant ontogeny by several means. It is well established that root exudates produced diverse organic substances important for rhizosphere microbes. Microbial colonization of plant roots influences plant ontogeny in a significant manner. Rhizobacteria stimulate the plant metabolic process and ultimately the plant growth. PGPR affected the plant growth by the production of plant-hormones, antibiotics, vitamins, suppression of plant pathogens and solubilization / mineralization of nutrients to more accessible forms for the plants. The root colonization of plants by PGPR greatly enhances the uptake of nutrients (Chen et al., 2005). Oilseed crops such as sunflower arehyper-accumulator and may be used to decontaminate the polluted soils and PGPR inoculation enhances their ability by number of ways (Kovar et al., 2016). *Rhizobium*, the most studied PGPR responsible for symbiotic nitrogen fixation in legumes may promote the

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growth of non-legumes. Literature confirmed the evidences of growth promotion of cereals, forages and fiber crops by *Rhizobium* sp (Hardoim et al., 2008; Qureshi et al., 2013).

Rhizobium species produced plant hormones such as indole-3-acetic acid (IAA), cytokinins, gibberellins and abscisic acid that regulated the endogenous levels of plant hormones, thus promote plant growth (Roy and Basu, 2004; Chi et al., 2005; Mehboob et al., 2008; Mehboob et al., 2009). Inoculation of *Rhizobium* sp improved the plant growth by solubilization of precipitated / fixed inorganic phosphates and mineralization of organic phosphates by producing organic acids and releasing phosphatase, enhance nutrient uptake and ultimately improve plant health (Biswas et al., 2000; Yanni et al., 2001; Alikhani et al., 2006; Hussain et al., 2009). *Rhizobium* sp promote growth by altering root architecture, producing siderophores and lowering ethylene level (Lupwayi et al., 2004; Madhaiyan et al., 2006; Mehboob et al., 2008; Qureshi et al., 2013) of non-legumes. Present study was designed to evaluate the comparative potential of *Rhizobium* species to promote the growth of sunflower.

Material and Methods

Isolations and purification of *Rhizobium* species

Rhizobium species of mungbean, berseem, chickpea, lentil and vegetable pea were isolated on yeast extract mannitol agar medium (YMA) (Vincent, 1970). The medium was autoclaved for 30 minutes at 121°C temperature and 15 psi pressure. The pouring of medium in petri plates was carried out in laminar air flow cabinets and exposed to UV for half an hour. The surface sterilization of collected nodules of different legumes (mungbean, berseem, chickpea, lentil and vegetable pea) was carried out as reported by Russell et al. (1982). The nodules were crushed separately to obtain suspension and streaked on YMA. The plates were labeled and incubated at 25°C in the incubator for 72 hours. The growth obtained was further purified on fresh plates having YMA containing 10 mL L^{-1} of 0.25% congo red. The purified isolates were preserved at 5± 1°C on slants for further screening.

Determination of auxin biosynthesis

Four purified isolates of each legume were assessed out for the auxin biosynthesis potential. Different isolates were labeled as mung bean (Mb-1, Mb-2, Mb-3, Mb-4), berseem (Br-1, Br-2, Br-3, Br-4), chickpea(Cp-1, Cp-2, Cp-3, Cp-4), Lentil (Lt-1, Lt-2, Lt-3, Lt-4), vegetable pea (Vp-1, Vp-2, Vp-3, Vp-4) during auxin estimation as IAA equivalents. Test tubes containing GPM were sterilized and inoculated with the respective isolates kept un-inoculated control, incubated at 28 ± 2 °C for one week and then centrifuged @1000 rpm for 10 minutes. The supernatants were analyzed colorimetrically using Salkowski reagent at 535 nm (Sarwar et al., 1992). Biochemical tests like congo red, bromothymol blue (BTB), oxidase test and gram reaction were carried out. Isolates having the highest auxin biosynthesis (Mb-2, Br-1, Cp-4, Lt-4 and VP-2) were selected for the pot experiment (Table 1).

Table 1.Auxin biosynthesis potential of isolates of *Rhizobium* species

Macrosymbiont	*Rhizobium species*	Isolates	IAA Equivalents (µg mL^{-1})
Mung bean	*Rhizobium phaseoli*	Mb-1	4.02
		Mb-2	5.11
		Mb-3	4.91
		Mb-4	4.64
Bserseem	*Rhizobium trifolii*	Br-1	4.03
		Br-2	3.33
		Br-3	3.30
		Br-4	3.81
Chickpea	*M. ciceri*	Cp-1	4.40
		Cp-2	4.23
		Cp-3	3.37
		Cp-4	5.37
Lentil	*R. Leguminosarum*	Lt-1	3.10
		Lt-2	3.83
		Lt-3	3.95
		Lt-4	4.20
Vegetable pea	*R. Leguminosarum bv.viciae*	Vp-1	3.81
		Vp-2	4.03
		Vp-3	3.64
		Vp-4	3.30

Preparation of inocula

The broth medium of YEM was prepared and sterilized. The sterilized medium was inoculated by respective isolates and placed on orbital shaker and after gaining optimum growth incubated at 28 ± 2 °C. The well decomposed leaf mold was sterilized and inoculated with broth cultures of *Rhizobium* species. Bacterial inocula were prepared by adding 20% sugar solution and incubated at 28 ± 2°C to enhance the respective bacterial population up to 10^8 CFU $g^{-1}$and applied to seeds as seed coating.

Pot experiment

Pot study was conducted to evaluate the comparative potential of *Rhizobium* species to promote the growth of sunflower using the completely randomized design (CRD). The pre-sowing soil was medium textured having pH 7.88, EC 1.40dS m^{-1}, soil N 0.035 % and available P 7.42 mg kg^{-1} at Soil Bacteriology Section, AARI, Faisalabad with three replication. Uniform fertilizer @ 50 kg N ha^{-1} and 75 kg P ha^{-1} was applied. After one month of sowing, the rhizosphere soil and leaves were collected to assess the IAA equivalents in the rhizosphere (Sarwar et al., 1992) and chlorophyll content as reported by (Arnon, 1949). Data regarding biomass, dry matter, N and P content in plant and post-harvest soil N and available P were determined. Chlorophyll content (a and b) of leaf was determined as reported by Arnon (1949). Soil samples were analyzed for extractable P (Olsen and Sommers, 1982) and soil N by (Bremner and Mulvaney, 1982). Data were subjected to statistical analysis following completely randomized design (CRD) (Steel et al., 1997) and differences among the treatments means were compared by the Duncan's multiple range test (Duncan, 1955).

Results

Lab study was conducted to test the biosynthesis of auxins as IAA equivalents by different rhizobial isolates and isolates i.e. microsymbionts of different legumes showed promising results were selected to assess their sunflower growth promotion potential. Results (Table 1) shown that rhizobial isolates Mb-2, Br-1, Cp-4, Lt-4 and Vp-2 produced auxins i.e. 5.11, 4.03, 5.37, 4.20 and 4.03 µg mL^{-1}as IAA equivalents, respectively. However, rhizobium sp of chickpea produced the maximum IAA production efficacy i.e. 5.37 µg mL^{-1}IAA equivalents.

Results regarding fresh and dry matter yield were presented in Figure 1. Bacterial inoculation of IAA-producing different rhizobial isolates showed positive influence on sunflower growth significantly and enhanced fresh and dry biomass in comparison un-inoculated control. The highest increase in fresh and dry biomass was obtained by Mb-2 inoculation that increased these parameters significantly by 31 and 34% followed by Br-1 i.e. 25 and 22%, respectively than control. The rhizobial isolates Cp-4, Lt-4 and Vp-2 increased these parameters by 23 and 17%, 17 and 7%, 8 and 4%, respectively compared to control.

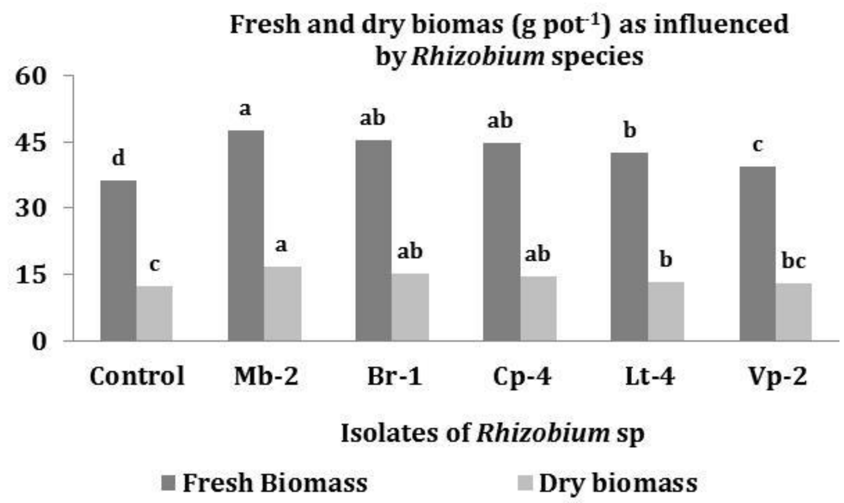

Figure 1. Fresh and dry matter biomass of sunflower as influenced by different treatments.

The data regarding chlorophyll contents and IAA equivalents in the rhizosphere soil presented in Figure 2 and 3. The extent of improvement in chlorophyll contents and IAA equivalents showed variable response with inoculation of different species. The maximum response was observed when inoculation was done with Mb-2 which increased chlorophyll a contents by 1.4 mg g^{-1} (8.34%) significantly while minimum improvement (1.33 mg g^{-1}) in chlorophyll a was noted with Vp-2 than control. In case of chlorophyll b, again Mb-2 gave better response than the rest of isolates under study i.e. 0.643 mg g^{-1} (5%) than control. Rhizobial isolate Mb-2 also gave best performance in case of IAA equivalents and gave higher content of IAA equivalents in the rhizosphere(4.56 µg g^{-1})while the rest of isolates (Br-1, Cp-4, Lt-4 and Vp-2)are statistically non-significant to each other but higher than control. i.e. (4.17, 4.25, 3.98 and 3.82 µg g^{-1}), respectively as compared to control i.e. 3.74.

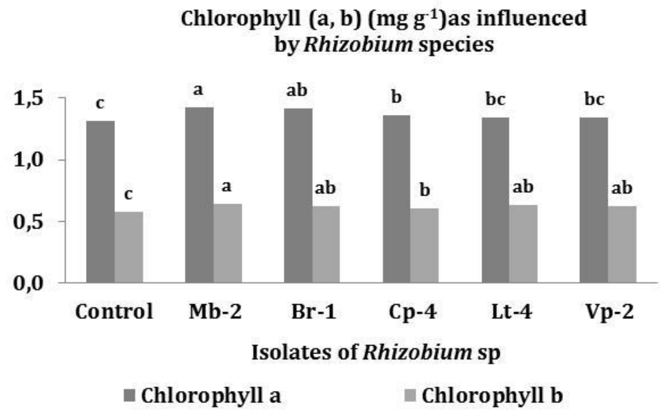

Figure 2. Chlorophyll (a & b) content as influenced by different rhizobium isolates

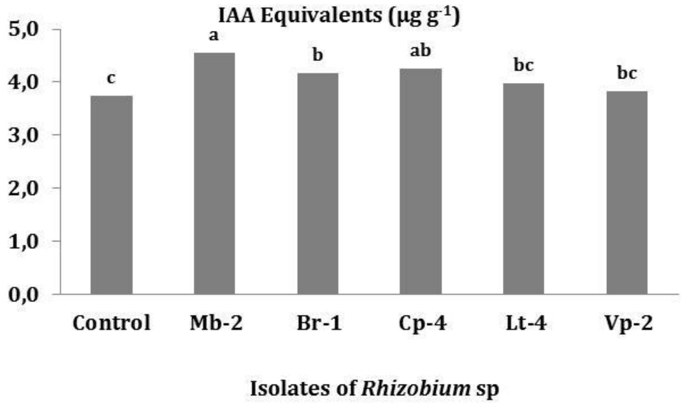

Figure 3. IAA equivalents in the rhizosphere (µg g^{-1}) as influenced by different rhizobium isolates

Data regarding plant N and P content was presented in Figure 4 and 5. Rhizobium sp isolates increased the N and P content significantly and Mb-2 gave much significant increase of 4.9 and 36% in N and P of sunflower plants, respectively than un-inoculated control.Isolate Vp-2 remained least effective. Other rhizobial isolates including Br-1, Cp-4 and Lt-4 enhanced the N and P content by 3 and 27%, 4 and 27, 2.4 and 18%, respectivelythan control. However, response of rhizobial isolates Br-1, Lt-4 and Vp-2 remain at par with each other regarding plant N and P content.

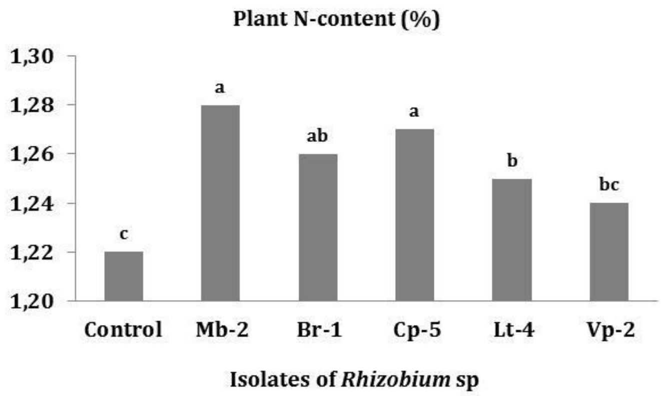

Figure 4. Effect of different treatments on plant N content

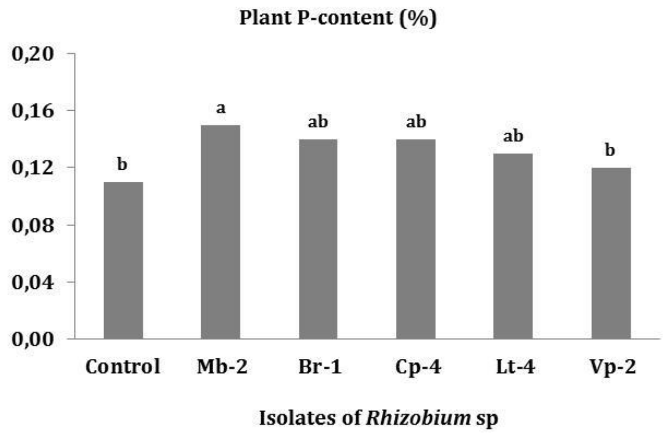

Figure 5. Effect of different treatments on plant P content

Results regarding soil N and available P contents in soil are presented in Figure 6 and 7. Results revealed that inoculation of IAA-producing rhizobial isolates exerts positive influence on soil nutritional status and enhanced soil N and available P content. Likewise, the isolate Mb-2 significantly increased soil N and available P by 11.76 and 13.26%, respectively than un-treated control. However, the effect of Mb-2 on soil N and P levels was non-significant compared to Br-1 and Cp-4.

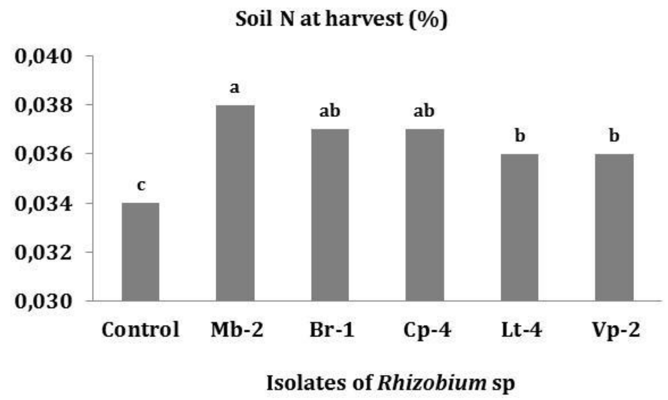

Figure 6. Effect of different isolates on post harvest soil N

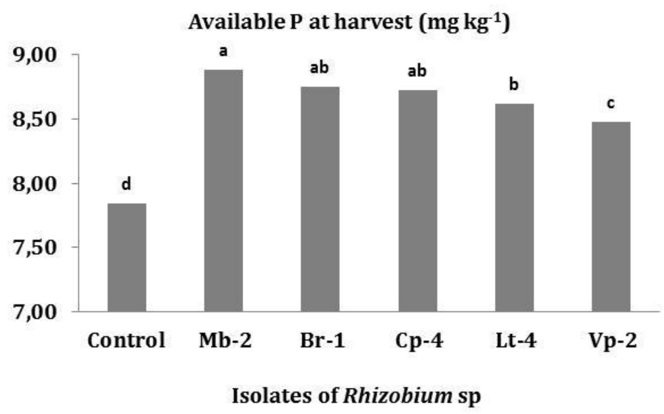

Figure 7. Effect of different isolates on post harvest soil available P content

Discussion

Different *Rhizobium* sp isolated from different legumes are reported to make an association with non-legumes and act as PGPR (Hussainet al., 2009; Mia and Shamsuddin, 2010; Qureshi et al., 2013;Naveed et al., 2015a; Adnan et al., 2016). *Rhizobium* species as PGPR possess various mechanisms which are responsible for stimulating plant growth such as production of phytohoromone, siderophores, cyanide, killing harmful pathogens by lytic enzymes, antibiotics, enhancing micro and macro-nutrients mobilization like phosphate solubalization, quorum-sensing signal interference, organic compounds, inducing systemic resistance, nitrogen fixation, biofilm formation, releasing ACC deaminase and symbiotic relation between plant and microbes (Alamiet al., 2000; Compant et al. 2005; Bhattacharyya and Jha, 2012; Adnan et al., 2016; Jimenez-Gomez et al., 2016).

Present study was conducted to test rhizobium species from five different legumes and for growth promotion of sunflower. Isolates of different species were isolated and checked for auxin biosynthesis potential. The tested isolates produced auxin as IAA equivalents with variable amount on respective media. Our results corroborated the results of Adnan et al. (2016) who isolated rhizobia from nodules of five different summer legumes and found that 47% of isolated rhizobial species were capable of producing IAA.

The isolates having maximum potential of auxin biosynthesis produced fresh and dry biomass of sunflower plants significantly higher than control. Increase in fresh and dry biomass owed to the production of phytohoromones, siderophores, hydrogen cyanide (HCN), solubilization of phosphates (Sessitsch et al., 2002; Hussain et al., 2009;Sachdev et al., 2009; Mia and Shamsuddin, 2010; Naveed et al., 2015a). Furthermore, biocontrol activity of *Rhizobium* sp. i.e.*B. japonicum*, *R. leguminosarum* and *S. meliloti* against *Macrophomina phaseolina*, *Fusarium* sp and *R. solani*in crop plants (Compant et al., 2005; Mehboob et al., 2008; Mia and Shamsuddin, 2010). Our result are in agreement with Alami et al. (2000) that demonstrated significant increase in root and shoot dry matter (50 and 70%, respectively) of sunflower plants in both drought and irrigated conditions when seeds were inoculated with exopolysaccharide (EPS) producing *Rhizobium* sp.

Inoculation of different rhizobial isolates increased the chlorophyll contents of sunflower because due to the microbial production of siderophores andsiderophores bind with Fe and facilitates its availability to plants (Carson et al., 2000; Barry and Challis, 2009; Dimkpa et al., 2009; Naveed et al., 2015a). The bacterial production of siderophores enhanced chlorophyll content and growth of plants due to the selective iron uptake from the solutions of trace elements and also production of siderophores inhibited the free radical formation and substantiated for the oxidative stress and prevented the uptake of heavy metals (Barry and Challis, 2009; Dimkpa et al., 2009; Naveed et al., 2015a). The substantial increase in chlorophyll contents of wheat plants treated with PGPR strains *B. phytofirmans* PsJN was reported by Naveed et al. (2014). A significant increase in plant IAA level of plants might be due to natural IAA-producing capability of selected rhizobial isolates (Khalid et al., 2006: Perrine-Walker et al., 2007; Naveed et al., 2015b). Chi et al. (2005) reported similar findings in which rice seedlings were inoculated with *A.caulinodans* ORS571 and *S. meliloti* 1021 and found substantial increase in levels of gibberellins (GA3) and auxins (IAA) in leaves and leaf sheath of rice plants.

Application of selected plant growth promoters enhanced NP contents of sunflower plants as well as in soil that might be due to associative relation of rhizobia with non-legumes. Microbial inoculation enhanced the nutrient uptake by altering the root system architecture, colonizing roots, producing hormones / siderophores, mobilizing nutrients and also enhanced root exudation (Chalk, 2016; Paungfoo-Lonhienne et al., 2014). Except biological nitrogen fixation, rhizobia also enhance nitrogen uptake in non-legumes such as rice (Biswas et al., 2000). Different isolates belongs to the group of PGPR viz. *Rhizobium*, *Enterobactor*, *Pseudomonas* and *Bacillus* are recognized as P-solubilizer (Shahid et al., 2012). Microbes produced organic acids that responsible for slight decrease in rhizosphere pH and theses organic acids acted like chelates and enhances nutrient uptake. Furthermore, organic acids possess carboxyl and hydroxyl groups which chelate or detach cations (Ca, Mg, Fe, Al) from soil phosphate eventually making it available for plants (Mullen 2005; Shahid et al., 2015).

Some *Rhizobium* sp like *R. leguminosarum* and *S. meliloti* are reported to release riboflavin i.e. vitamin riboflavin converted into lumichromeeither photochemically or enzymatically and motivated root respiration (Dakora et al., 2002; Yang et al.,2009; Qureshi et al., 2013; Naveedet al., 2015a) ultimately facilitating nutrient uptake. Additionally, rhizobia might enhance nutrients availability through release of various chemical compounds like exopolysaccharide, phytohoromones, lipo-chitooligosaccharides (LCO's), siderophores and lumichromes (Mehboob et al., 2008; Mehboob et al., 2009; Naveed et al., 2015a). Biswas et al. (2000) depicted a significant increase (up to 28%) in nutrients (N, P and K) uptake when they applied different rhizobial strains to rice seeds / seedlingscompared to un-inoculated control. Our results are also corroborated with the findings of Shahid et al. (2015) who applied two P-solubilizing bacterial strains (*Alcaligenes faecalis* Ss-2and *Bacillus* sp Ps-5) to sunflower seeds and reported a significant increase in P contents of sunflower plants in comparison to control. Alami et al. (2000) also observed that application of EPS producing rhizobial strains YAS34 increased water and nitrogen nutrition of sunflower under drought. Furthermore, Matiru and Dakora (2004) reported significant increase in P uptake and plant growth was observed through application of different rhizobia to sorghum plants.

Present study concluded that *Rhizobium* species can be successfully used as PGPR after thorough screening and by determining its growth hormone production potential. Present study also presented new horizons about variable response of different crop specific rhizobium species. Furthermore, study concluded that Rhizobium species having growth hormone producing potential might enhance plant growth and development, nutrient uptake and mobilizer.

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Spatial distribution of heavy metals density in cultivated soils of Central and East Parts of Black Sea Region in Turkey

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Abstract

Heavy metal contamination has caused serious environmental and health-related problems around the world. To identify the concentrations and sources of heavy metals, 3400 surface soil samples (0-20 cm depth) were collected from the study area. Subsequently, the concentrations of Cd, Co, Cu, Ni, Pb and Zn in the samples were measured. In order to evaluate natural or anthropogenic sources of heavy metal content and their spatial distribution in agricultural fields of Central and East Parts of Black Sea Region soil geostatistic approach were combined with geographic information system (GIS). GIS technology was employed to produce spatial distribution maps of the 6 elements in the study area. The results showed that the concentration of Ni and Co exceeded its threshold level. The local pollution from Ni was attributed to the natural influences. The concentrations of the other heavy metals are relatively lower than the critical values. The mean values of the heavy metal contents arranged in the following decreasing order: Ni > Zn > Cu >Pb> Co > Cd in the study area. On the other hand, according to distribution ratio of heavy metals in total soil samples, except for Co and Ni distribution in total soil samples, all other heavy metal element exceeded concentration in samples were determined about less than 10% total soil samples. However, in some regions of the study area, the Cd, Cu and Zn contents were also slightly raised, this case possibly stem from excessive P fertilization and field traffic.

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Introduction

Metal accumulation in the soil is largely irreversible and may cause serious environmental problems health-related problems around the world if certain concentration levels are exceeded. The aim of sustainable heavy-metal management in agro-ecosystems is to ensure that the soil continues to fulfill its functions: in agricultural production, in environmental processes such as the cycling of elements, and as a habitat of numerous organisms (Moolenaar, 1999). In view of sustainability, a preventive and monitoring approaches based on predicting spatial and temporal soil heavy-metal contents is therefore promising. Accumulation of heavy metals in arable soils is important because of the potential transfer of heavy metals through crops to animals (feed crops) and humans (food crops and vegetables) (De Temmerman et al., 2003). To this respect Cd, Cu, Co, Ni, and Pb are important elements, not only because of the long term accumulation in humans but also because of the high potential for root uptake and accumulation in above ground plant parts (Saglam et al., 2011; Datsenko and Khimenko, 2016).

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Heavy metal inputs into agricultural soils due to atmospheric deposition and application of commercial fertilizers, animal manure, sewage sludge and pesticides take place at rather slow rate but on large areas (Ali et al., 2017). Hence, it may take decades to detect accumulation trends in soil by repeated sampling. Such contamination may not be of concern in terms of immediate toxicity effects. Yet, it is the ubiquitous character and the increase of heavy metal flows through the soil system that may cause serious problem for soil fertility, ground water quality and food chains. Previous studies indicated that the extent of heavy metal pollution in rural areas varied across time (Pfeiffer et al., 1991) and location (Albasel and Cotteni, 1985), and that increased levels of heavy metals in cultivated soil was related to the intensity of agricultural activities and field traffic (Zheng et al., 2002; Kızılkaya et al., 2004).

Prediction methods to reliably estimate heavy metal distribution in space and time should be based on spatial variability of soil properties. Geostatistical methods that are based on the theory of regionalized variables (Journel and Huijbregts, 1978; Isaaks and Srivastava, 1989; Goovaerts, 1997; Kızılkaya et al., 2011) can provide reliable estimates at the unsampled locations provided that the sampling interval resolves the variation at the level of interest (Kerry and Oliver, 2004).

The objective of this study is determine contents of heavy metal status and physico-chemical properties of soils in agricultural fields of Central and East Parts of Black Sea Region in Turkey using statistics, geostatistics and geographical information system (GIS) techniques, in order to find out heavy metal scale variability and spatial distribution maps and provide valuable information about soil heavy metal pollution for this region.

Material and Methods

Field description of the study area

This study was carried out at arable lands of the central and eastern Black Sea Regions including eight provinces (Sinop, Samsun, Ordu, Giresun, Trabzon, Gümüşhane, Rize, Artvin). Total regions' area is about 5.075.413 ha. However, arable lands selected for this study cover about 32.8 % in total area (Figure 1).

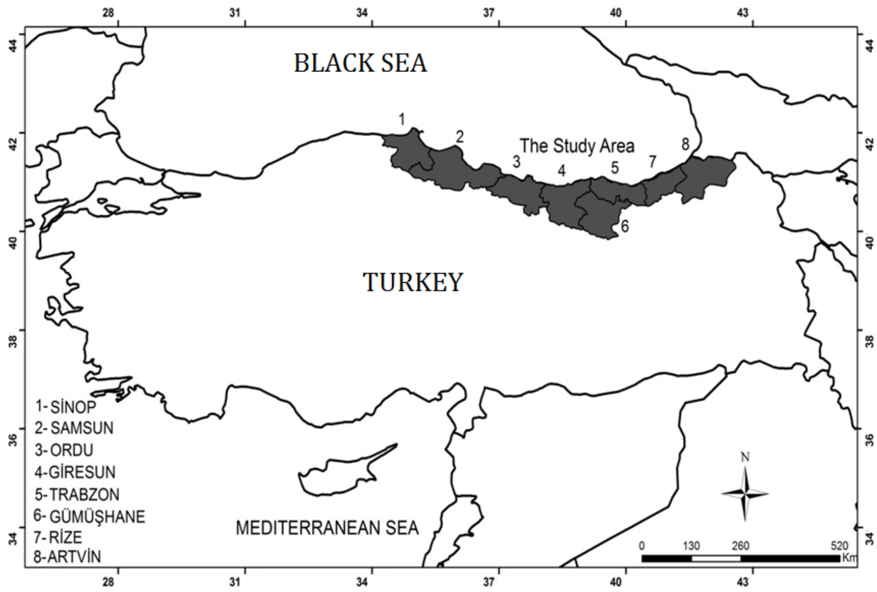

Figure 1. Location map of the study area

The Black Sea Region has a steep, rocky coast with rivers that cascade through the gorges of the coastal ranges. A few larger rivers, those cutting back through the Pontic Mountains, have tributaries that flow in broad, elevated basins. Access inland from the coast is limited to a few narrow valleys because mountain ridges, with elevations of 1525 to 1800 meters in the west and 3000 to 4000 meters in the east in Kaçkar Mountains, form an almost unbroken wall separating the coast from the interior. The higher slopes facing northwest tend to be densely forested. Because of these natural conditions, the Black Sea coast historically has been isolated from Anatolia.

The mild, damp oceanic climate of the coast of Black Sea makes commercial farming profitable. Running from Sinop in the west to Artvin in the east, the narrow coastal strip widens at several places into fertile, intensely cultivated deltas. There are two main deltaic plains in the central Black Sea region which are called Bafra and Çarşamba Plains formed on accumulated sediments depositions carried by the Kızılırmak and Yeşilırmak Rivers in different periods. The Samsun area, close to the midpoint, is a major tobacco-growing region; east of it are numerous citrus groves. East of Samsun, the area around Trabzon is world-renowned for the production of hazelnuts, and farther east the Rize region has numerous tea plantations. All cultivable areas, including mountain slopes wherever they are not too steep, are sown or used as pasture. The North Anatolian Mountains in the north are an interrupted chain of folded highlands that generally parallel the coast. In the west, the mountains tend to be low, with elevations rarely exceeding 1500 meters, but they rise in an easterly direction to heights greater than 3000 meters south of Rize. Lengthy, trough-like valleys and basins characterize the mountains. Rivers flow from the mountains toward the Black Sea. The southern slopes facing the Anatolian Plateau are mostly unwooded, but the northern slopes contain dense growths of both deciduous and evergreen trees.

Black Sea region has a humid subtropical climate with high and evenly distributed rainfall the year round. At the coast, summers are warm and humid, and winters are cool and damp. The Eastern Black Sea coast receives the greatest amount of precipitation and is the only region of Turkey that receives high precipitation throughout the year. The eastern part of that coast averages 2500 millimeters annually which is the highest precipitation in the country. Snowfall is quite common between the months of December and March, snowing for a week or two, and it can be heavy once it snows (Ozturk, 2011).

Soil sampling

Soil samples were obtained from the study area between 2008 and 2012. The sites divided into 2.5 x 2.5 km grid squares. Total 3400 soil samples were collected from surface (0-20 cm) depth of each grid intersection point by taking into consideration of research area's size and geographical positions (Figure 2). The samples were transported to the laboratory. The soil samples were crumbled gently by hand without root material. These samples were used to determine physico-chemical and heavy metal status of soils.

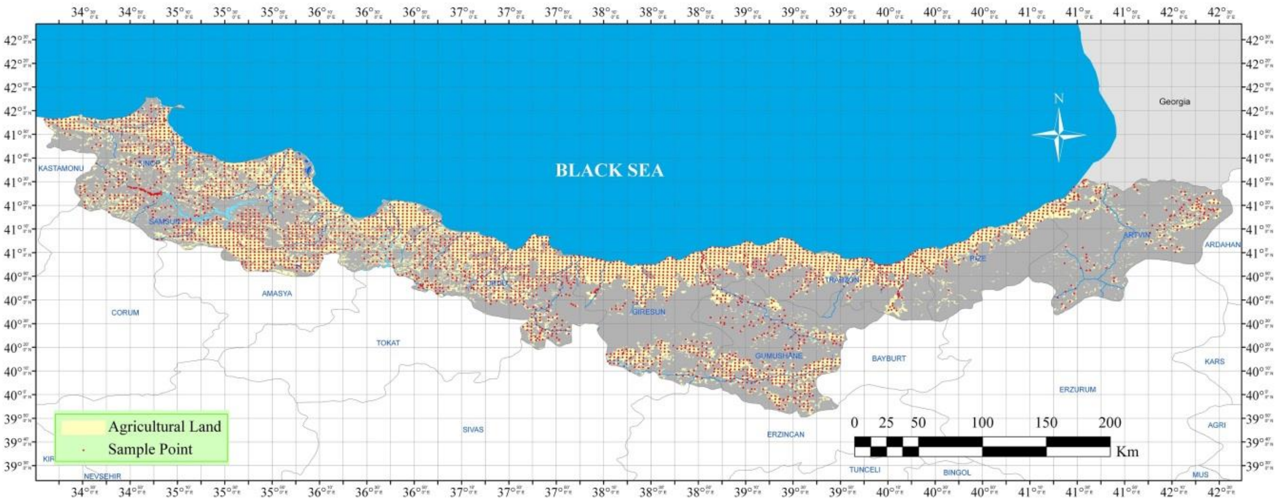

Figure 2. Map of soil sample points in the study area

Soil physico-chemical analyses

Physico-chemical analyses were conducted on air-dried samples stored at room temperature and from which crop residues, root fragments and rock larger than 2 mm in diameter had been removed. The particle size composition was determined by a hydrometer, the pH and electrical conductivity (EC) in a paste (the water to soil ratio was 1:2.5), the $CaCO_3$ by the volumetric method, the total nitrogen (N) by the Kjeldahl method, the available phosphorus (P) in a 0.5 M $NaHCO_3$ extract, and the exchangeable potassium (K), calcium (Ca), sodium (Na) and magnesium (Mg) by the 1 N ammonium acetate extraction method. The organic matter content was determined by the method of oxidation in an oxygen flow with $K_2Cr_2O_7$ (Rowell, 1996). Available soil micronutrients (Fe, Cu, Zn, Mn) were determined on each sample (Lindsay and Norvell, 1978).

Total heavy metal concentrations

The total heavy metal (HM) concentration for investigated each heavy metal elements was measured using the following procedure. The soil samples were preliminarily dried in a thermostat at 110°C for 34 h. They were sifted using a sieve with a mesh of 0.074 mm. The weighed soil samples were placed into a mixture of 12 M HCl and 14 M HNO_3 (the solution to soil ratio was 10:1). The soils were heated on a hot plate to 120°C. The heating stopped after a reddish gas was removed. The soils were dried. The HCl and HNO_3 mixture (10 ml) was added to each sample; then the samples were filtered through Whatman filters. A blank test was made to check the quality of the reagents. Three standards were used for the determination of each metal. The samples for the HM contents were analysed by the method of atomic absorption on a Perkin Elmer A400 spectrophotometer (Kloke, 1980).

Statistical and geostatistical analyses

The descriptive statistics of soil properties including sample mean, minimum, maximum and coefficients of variation were calculated. Moreover, in order to determine six heavy metal distributions and to produce their maps, inverse distance weighted (IDW) interpolation method that is one of the spatial analysis tools of geographic information systems (GIS-ArcGIS 10.0v) was used.

Results and Discussion

Soil physico-chemical properties

Physical and chemical properties that have been taken into consideration in this study showed variability as a result of dynamic interactions among natural environmental factors (degree of soil development and leaching processing etc.) and human activities such as fertilization management. Soil chemical and physical properties considered in this study are pH, EC, soil organic matter, total $CaCO_3$ content, total N, available P, exchangeable K, exchangeable Ca, exchangeable Na, exchangeable Mg, available Mn, available Zn, available Fe, available Cu and soil texture. The descriptive statistics as minimum, maximum, mean, and coefficients of variation of physic-chemical properties surface soil samples were presented in Table 1.

Table 1. Descriptive statistics of the soil physico-chemical properties studied.

Properties	Mean	Min.	Max.	S.D.	C.V., %	Skew.	Kurt.	n
Sand,%	41.62	1.61	91.98	16.66	40.04	0.08	-0.71	3400
Clay,%	29.98	2.49	79.23	13.67	45.60	0.33	-0.49	3400
Silt,%	28.39	1.10	65.78	7.96	28.03	0.38	0.97	3400
pH	6.28	3.14	8.50	1.33	21.24	-0.60	-0.98	3400
EC, dS m^{-1}	0.47	0.05	3.08	0.29	62.67	2.86	14.70	3400
$CaCO_3$,%	4.48	0.10	58.80	7.76	173.10	2.59	7.82	3400
SOM,%	3.35	0.30	12.91	1.74	52.02	1.41	2.86	3400
AP, mg kg^{-1}	19.02	0.09	226.47	28.52	149.91	3.12	12.06	3400
TN, %	0.20	0.01	0.88	0.09	47.80	1.45	3.77	3400
K, $cmol_c$ kg^{-1}	0.57	0.02	4.65	0.45	78.93	2.60	12.04	3400
Ca, $cmol_c$ kg^{-1}	217.19	1.45	908.50	141.01	64.92	0.68	0.42	3400
Mg, $cmol_c$ kg^{-1}	29.18	1.66	224.64	28.58	97.94	2.67	9.63	3400
Na, $cmol_c$ kg^{-1}	5.70	0.47	77.61	5.94	104.12	4.09	25.45	3400
Fe, mg kg^{-1}	36.56	0.75	278.32	35.16	96.19	1.96	5.43	3400
Cu, mg kg^{-1}	2.55	0.03	29.31	2.20	86.05	2.89	17.29	3400
Zn, mg kg^{-1}	1.26	0.03	25.98	1.98	156.75	5.05	36.42	3400
Mn, mg kg^{-1}	31.12	0.10	227.98	29.47	94.70	2.36	7.24	3400

SOM: Soil organic matter, AP: Available phosphorus, TN: Total nitrogen, S.D: Standard deviation, C.V: Coefficient of variation

The values of pH in soil samples widely ranged between 3.14 and 8.50 that called from strong acid to moderately alkali soil reaction, whereas electrical conductivity had a minimum value of 0.05 dS m^{-1} and a maximum value of 3.08 dS m^{-1}. Most of the all sampling points have low $CaCO_3$, with the exception of 5.4% of total soil samples which have more than 20% $CaCO_3$. The mean values of organic matter and $CaCO_3$ content (%) were 3.35 and 4.48. As for macronutrient element of samples, available P and exchangeable K showed high variation between minimum and maximum values. Total N varied between 0.01 and 0.88 and the average value of total N was 0.20. The mean values of Ca, Mg, and Na concentration were found 217.19, 29.18 and 5.70 $cmol_c\ kg^{-1}$, respectively. In addition, Table 1 shows statistical distribution of micronutrient elements (Fe, Cu, Zn and Mn) concentration of samples. According to limit values reported by Lindsay and Norvell (1978), Fe and Cu were found in sufficient amounts in all of the soil samples and their mean values are 36.56 and 2.55 mg kg^{-1}, whereas all samples were insufficient in respect to available Zn content and its mean value is 1.26 mg kg^{-1}. Besides, 12% of samples were insufficient in respect to available Mn and it has high variation between minimum and maximum values (0.10-227.98).

Heavy metal concentrations in cultivated soils

The data indicate that not only the basic soil properties show great variation but also the heavy metal content in soils. Table 2 shows maxima, minima, means, variance, standard deviations and coefficient of variation of the total heavy metal (cadmium, cobalt, copper, nickel, lead, and zinc) contents. Total concentrations of heavy metals ranged as follows: Cd (0.04-34.65), Co (1.52-97.50), Cu (2.60-308.13), Ni (2.19-1063.99), Pb (3.65-444.91) and Zn (0.81-552.89) mg kg^{-1}. In Table 3, maximum permitted values of heavy metal concentration in agricultural soils that have been evaluated by Kloke (1980) are shown. In all cases, soil samples from the area studied had higher maxima values of heavy metal concentrations than those permitted from the Anonymous (2001). Ni and Co concentrations are higher than maximum permitted values in 25% and 73% of soil samples, respectively whereas, only less than 3% of soil samples have high Cd, Pb, Zn and Cu concentration.

Table 2. Maxima, minima, means, variance, standard deviations (SD), coefficient of variation (CV), Skewness and Kourtosis of the heavy metals studied (n=3400).

	Cu (mg kg^{-1})	Zn (mg kg^{-1})	Ni (mg kg^{-1})	Cd (mg kg^{-1})	Pb (mg kg^{-1})	Co (mg kg^{-1})
Min.	2.60	0.81	2.19	0.04	3.65	1.52
Max.	308.13	552.89	1063.99	34.65	444.91	97.50
Mean	44.73	57.51	62.15	1.26	29.27	25.57
Kurt.	5.91	42.76	50.91	112.71	116.75	5.61
Skew.	1.94	4.60	5.87	9.13	8.88	1.35
CV	65.58	60.65	133.50	145.22	72.15	38.72
SD	29.33	34.88	82.97	1.84	21.12	9.90
Variance	860.34	1216.5	6884.3	3.37	445.99	98.00

Table 3. Maximum permitted values of heavy metal concentration in agricultural soils that have been evaluated from Anonymous (2001).

Heavy metals	Maximum permitted values (mg kg^{-1})
Cd	3
Co	20
Cu	140
Ni	75
Pb	300
Zn	300

Spatial distribution of heavy metals

One of the spatial analysis tools of geographic information systems (GIS) is geostatitical analysis module. In order to determine 6 heavy metal distributions and create their maps in the study area, ArcGIS 10.0v interpolation model called IDW was used. Many researchers indicated that this technique is a suitable interpolation method for general assessment purposes and a potential timesaving alternative to current survey methods for generating distribution maps (Lo and Yeung, 2002; Roberts et al. 2004; Dogan et al. 2013). The interpolated maps of heavy metal elements including Cd, Co, Cu, Ni, Pb and Zn concentrations are illustrated in Figures 3-8. As seen from the maps and Figures, almost all heavy metal element concentration was found as low level except for Ni and Co concentrations in the study area. Almost the same results were detected by Saglam et al. (2011) in Çarşamba Delta Plain in order to evaluate natural or anthropogenic sources of heavy metal content and their spatial distribution in agricultural fields.

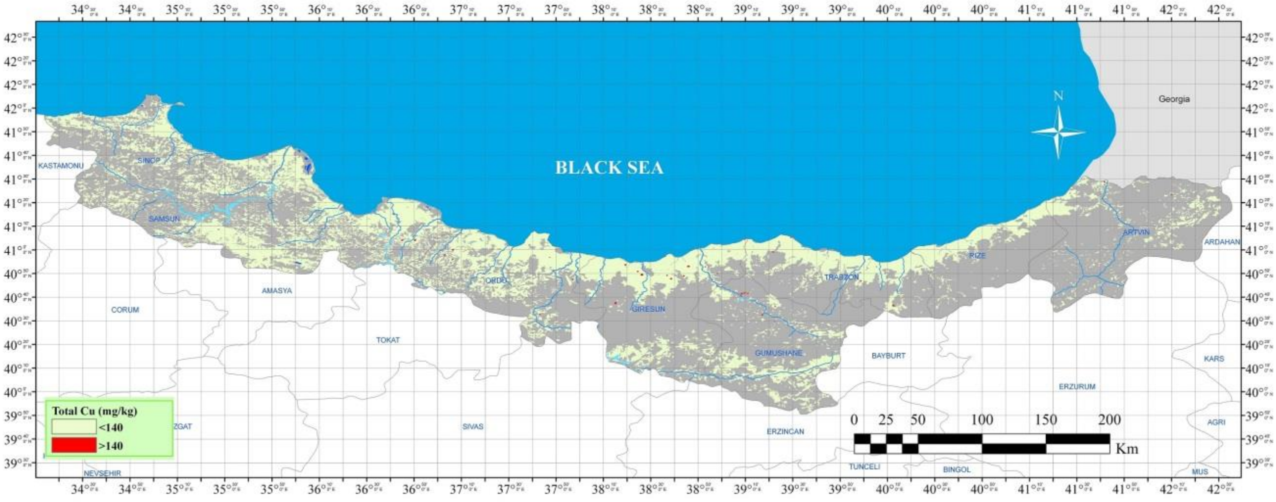

Figure 3 - Interpolation mapping of cupper in the study area

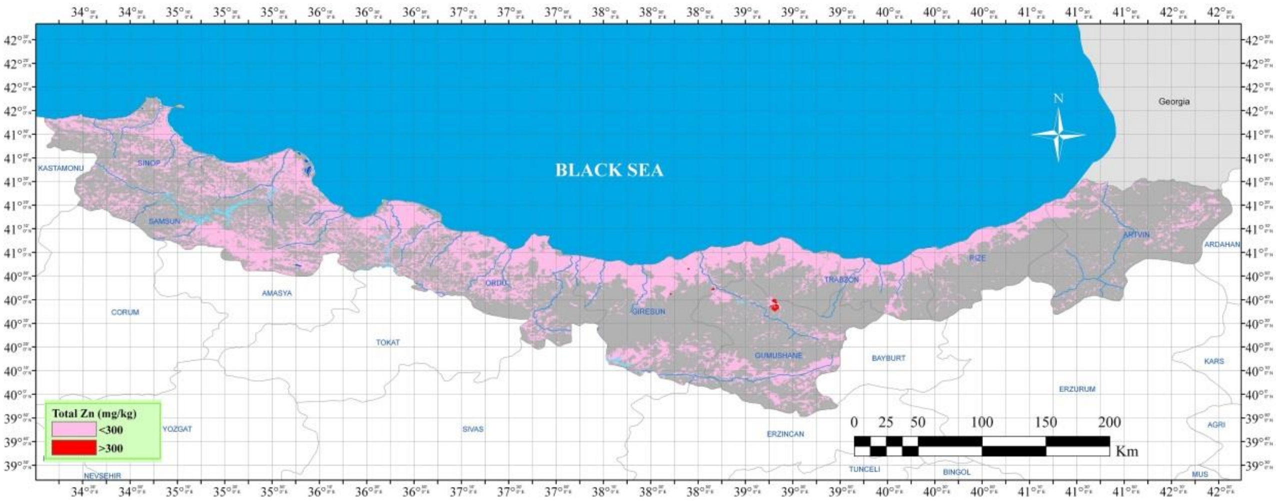

Figure 4 - Interpolation mapping of zinc in the study area

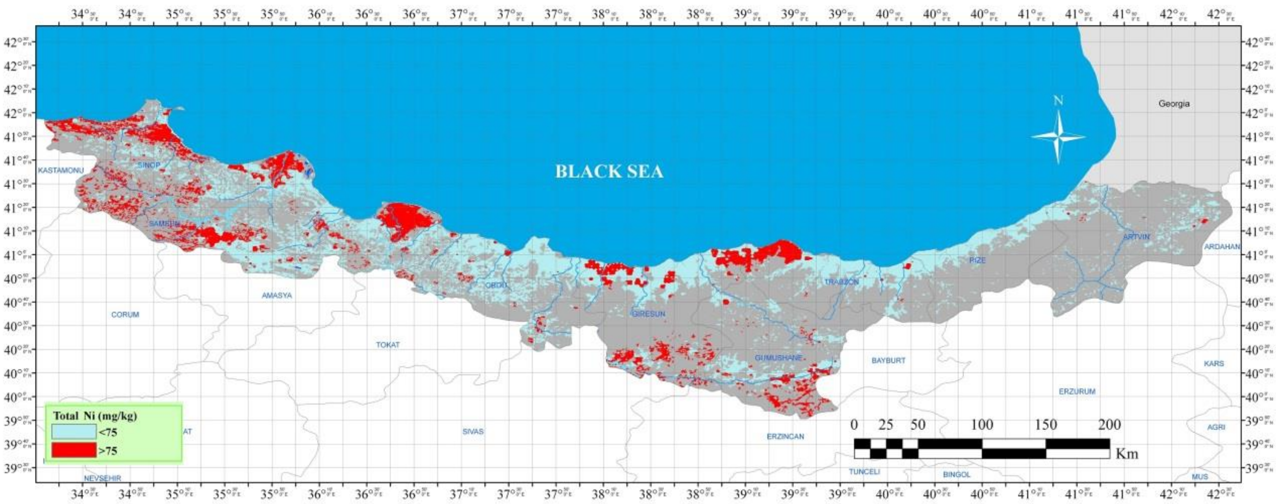

Figure 5. Interpolation mapping of nickel in the study area

Distribution of the total sample numbers for each eight provinces was given Table 4. In order to assess the results for these provinces, the Regulation for the Control of Soil Pollution (Anonymous, 2001) was taken into consideration for threshold level heavy metals presented in Table 3. Results also show that cupper concentrations exceeded the threshold level in soils taken from Samsun, Ordu, Giresun, Gümüşhane, Trabzon and Artvin. However, sample amount and ratio are lower than 2%. In addition to that, it was not found exceeded the threshold level in soils taken from Sinop and Rize provinces. The zinc and lead concentrations of arable land soils in all provinces were found to be under the threshold levels or less than 1%.

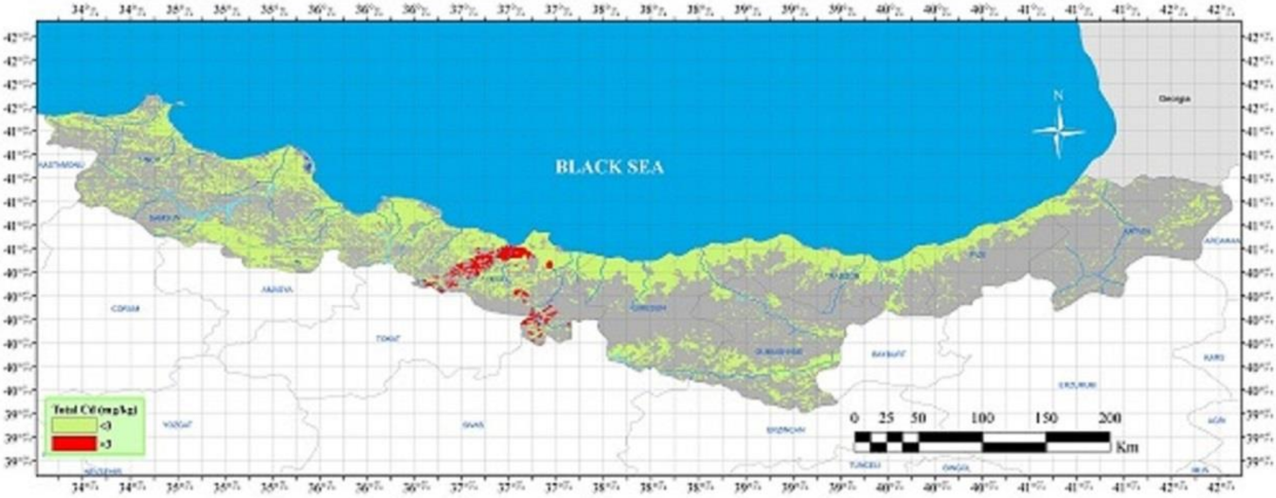

Figure 6. Interpolation mapping of cadmium in the study area

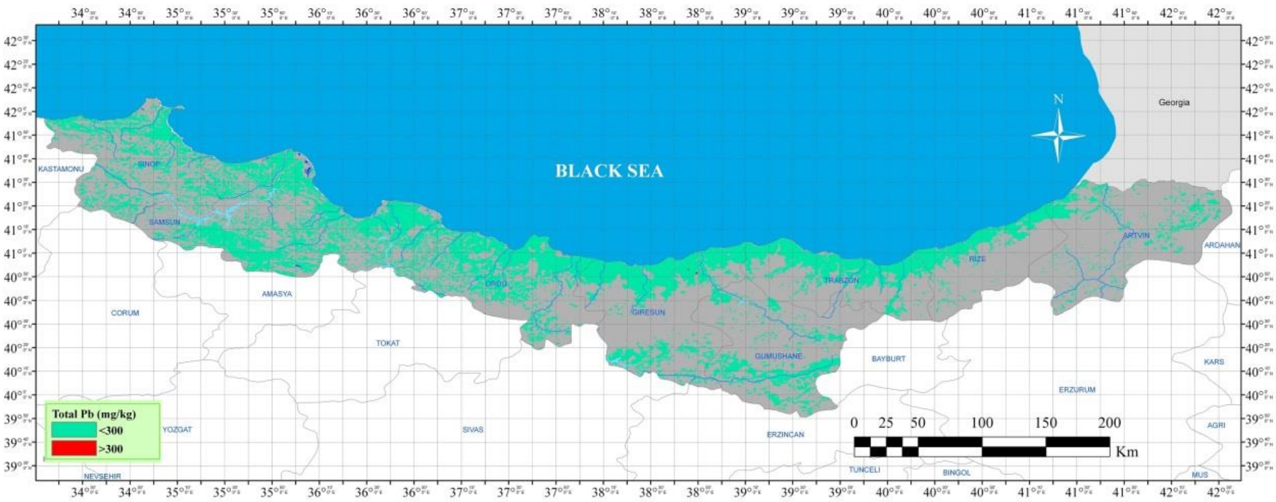

Figure 7. Interpolation mapping of lead in the study area

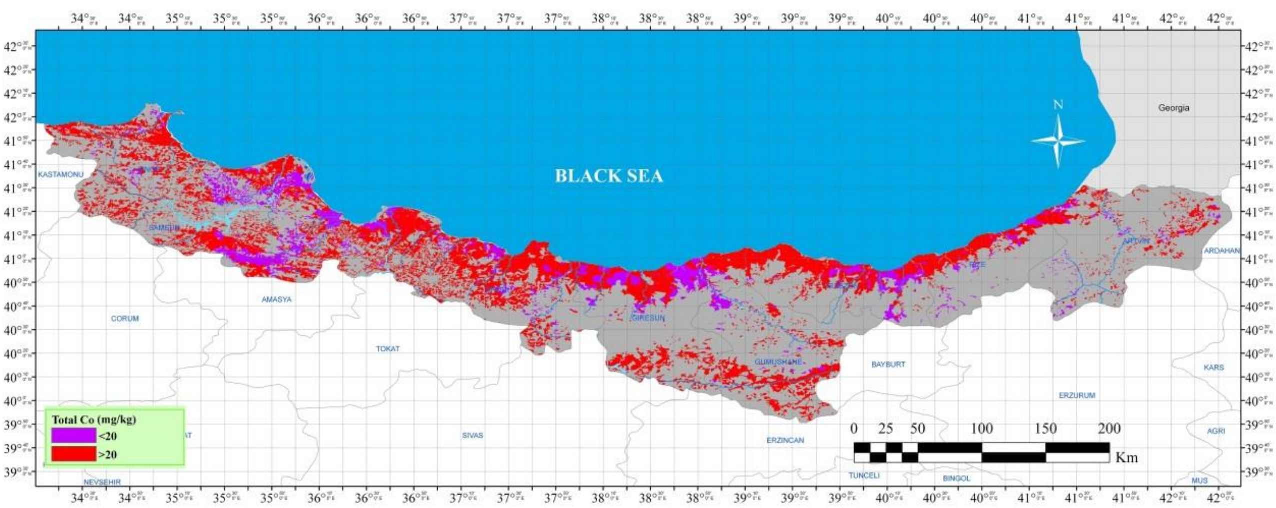

Figure 8. Interpolation mapping of cobalt in the study area

Table 4. Total sample number for each provinces' arable land and exceed threshold level of sample number and ratio for each heavy metal (HM).

HM	Provinces and their total sample number															
	Sinop		Samsun		Ordu		Giresun		Gümüşhane		Trabzon		Rize		Artvin	
	432		889		596		466		319		371		159		168	
	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%
Cu	--	--	7	2	9	2	14	3	6	2	6	2	--	--	2	1
Zn	--	--	--	--	--	--	5	1	4	1	2	1	--	--	--	--
Ni	241	56	300	34	36	6	98	21	90	28	78	21	2	1	11	7
Cd	--	--	11	10	98	16	--	--	--	--	--	--	--	--	--	--
Pb	--	--	--	--	--	--	4	1	--	--	--	--	--	--	--	--
Co	375	87	571	64	478	80	318	68	256	80	259	70	113	71	123	73

Moreover cadmium concentration were not determined exceeded the threshold level in soils taken from all studied provinces except for Samsun and Ordu including less than 16%. Nickel concentration exceeded limited level in soils taken from all investigated arable land soils. But it shows very variable from province to province. The highest common distribution of nickel concentration that is over limited value were determined in cultivated soils of Sinop whereas, only 1% of the total samples located in Rize province was found exceed threshold level. Cobalt is the same as nickel and it is also very common in the study soil samples. On the other hand, it doesn't show very variable and about more 65% soil samples taken from all provinces exceeded limited level.

Conclusion

The present study examined the distribution and spatial parent of heavy metals in the agricultural fields located in Central and East Parts of Black Sea Region using statistics, geostatistical analysis and geographic information system to attain the natural and anthropogenic effects such as industrial effluents, agricultural activities etc. on heavy metal pollution. These region soils have generally acidic reaction and their pH values are low and very low (5.5-4.5) due to high precipitation and leaching process (Ozyazici et al., 2011). As expected, solubility of heavy metal concentration increased with a decrease in pH (Martinez and Motto, 2000). 31% of the total soil samples (1092) have low pH (< 5.5). Except for Co and Ni distribution in total soil samples, all other heavy metal elements were determined about less than 10% in soil samples and they have not common places in the study area. Therefore, natural contents of heavy metals in arable soils depend primarily on its geogenic source from the parent materials in the area. However, changes in composition are possible during transport of heavy metal containing dust particles and, for some elements, also agricultural practices (Kloke, 1980). Particularly, the Cd, Cu and Zn contents were slightly raised, possibly due to excessive P fertilization, wrong fertilizer addition to low pH soil like ammonium sulphate (to increase acidity leading to high solubility) and field traffic. In addition it was found only Ni concentration over the threshold level. It is thought that this high level of Ni concentration dose not stems from the contaminations, but that this result is related to the parent material of the soils that were formed from magmatic rocks which include high amounts of nickel.

Such studies could help validate procedures of spatial predictions that have limited measured data. This may be suitable for many problems and contributes to the knowledge of the content in soil monitoring where heavy metal changes are relatively for large area such as agricultural soils of the Central and East Part of the Black Sea Region.

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Estimation of Soil loss by USLE Model using GIS and Remote Sensing techniques: A case study of Muhuri River Basin, Tripura, India

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Abstract

Soil erosion is a most severe environmental problem in humid sub-tropical hilly state Tripura. The present study is carried out on Muhuri river basin of Tripura state, North east India having an area of 614.54 Sq.km. In this paper, Universal Soil Loss Equation (USLE) model, with Geographic Information System (GIS) and Remote Sensing (RS) have been used to quantify the soil loss in the Muhuri river basin. Five essential parameters such as Runoff-rainfall erosivity factor (R), soil erodibility Factor (K), slope length and steepness (LS), cropping management factor (C), and support practice factor (P) have been used to estimate soil loss amount in the study area. All of these layers have been prepared in GIS and RS platform (Mainly Arc GIS 10.1) using various data sources and data preparation methods. In these study DEM and LISS satellite data have been used. The daily rainfall data (2001-2010) of 6 rain gauge stations have been used to predict the R factor. Soil erodibility (K) factor in Basin area ranged from 0.15 to 0.36. The spatial distribution map of soil loss of Muhuri river basin has been generated and classified into six categories according to intensity level of soil loss. The average annual predicted soil loss ranges between 0 to and 650 t/ha/y. Low soil loss areas (<25 t/ha/y) have been recorded under very densely forested areas and intensely plantation (mainly Rubber plantation) area. The high rate (>70 t/ha/y) of soil erosion was found along the main course of Muhuri River.

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Introduction

Soil erosion may be simply defined as the detachment and transportation of soil (Tideman, 1996). Natural or geological soil erosions do not occur at constant or consistent rates. Semi-arid and arid soils, which lack protective plant covers, may erode naturally at rates averaging 10-50 times greater than those for humid climate soils (Miller and Donahue, 1990). Asia has the highest soil erosion rate of 74 ton/acre/yr. (El-Swaify, 1997) and Asian rivers contribute about 80 % per cent of the total sediments delivered to the world oceans and amongst these Himalayan rivers are the major contributors (Stoddart, 1969). The soil erosion process is modified by biophysical environment comprising soil, climate, terrain, ground cover and interactions between them. Important terrain characteristics influencing the mechanism of soil erosion are slope, length, aspect and shape (Ganasri and Ramesh, 2016). Universal Soil Loss Equation (USLE) is the most widely applied empirical models for estimating the soil loss which was developed by Wischmeier and Smith (1965). Tripura is predominantly a small hilly state (Bera and Namasudra, 2016). Soil erosion is the common recurring phenomena of this state. Muhuri river basin lies in the southern- most part of the state. The

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catchment area of Muhuri river basin is 614.54 sq.km within Tripura and within Indian Territory the total length of the river is 59 km. The objective of the present study is to assess the amount of soil loss of Muhuri river basin by USLE method with the help of remote sensing and Geographical Information System techniques.

Material and Methods

Location of the study area

The Present study was conducted at Muhuri river basin in Tripura (Figure 1). Latitudinal and longitudinal extent of the basin are between 23°6'59" N to 23°25'16" N and 91°26'46"E to 91°44'35" E respectively. The maximum portion of Muhuri river basin lies in the South Tripura districts. It originates from the Deotamura hill range and there after it flow towards eastern direction, then enters into Bangladesh. The climate of the Muhuri river basin is under the influence of south west monsoon. The average annual rainfall is 335.27 cm and maximum humidity was noticed in the month of June.

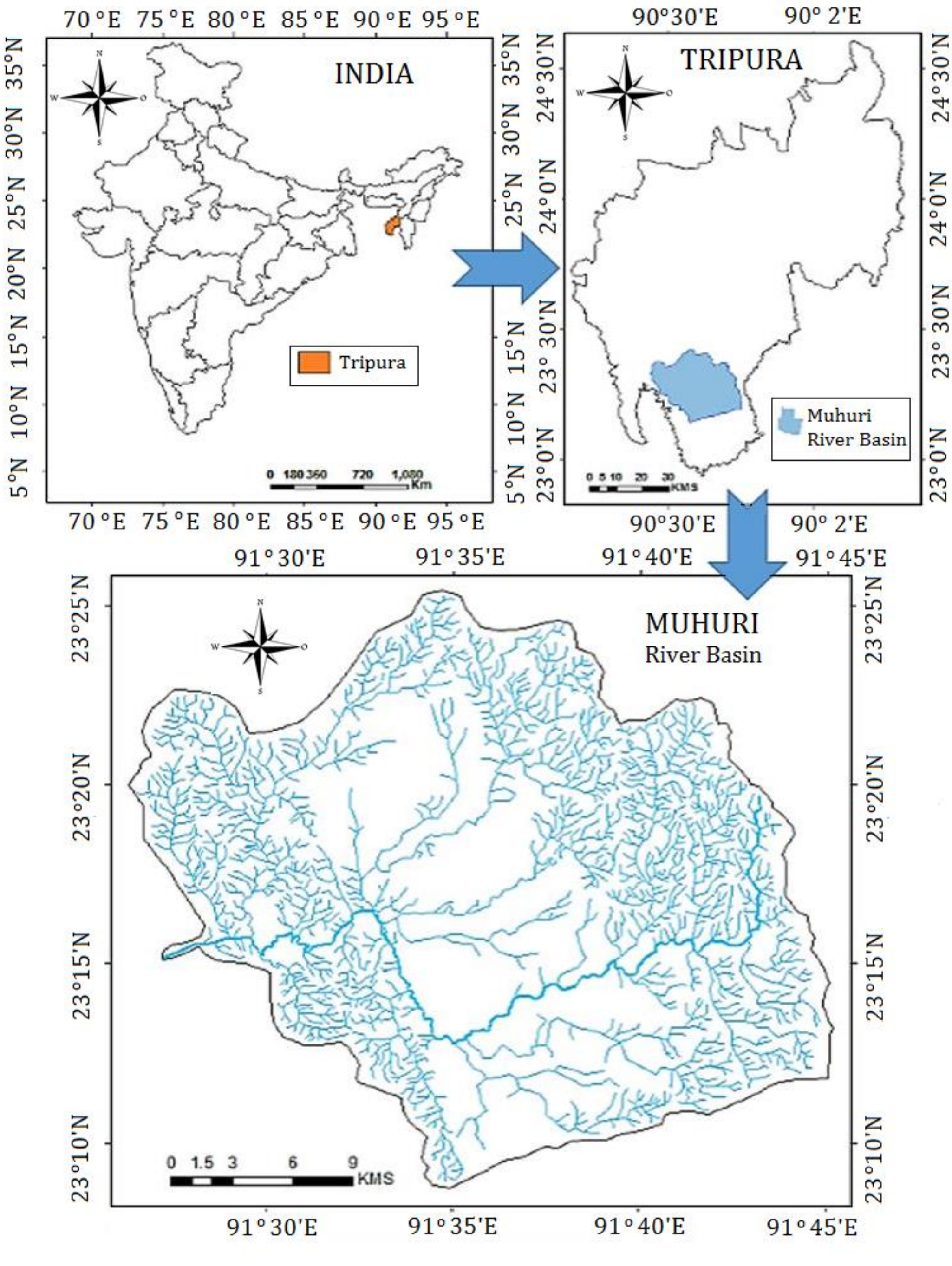

Figure 1. Location of the study area

Various types of materials have been used for the calculation of soil loss within the Study area. Those data are mainly, Rainfall data From Indian Meteorological Department (IMD), Soil Data from NBSS & LUP, ASTER DEM and LISS data.

The USLE soil loss equation is:

$$A=R\times K\times LS\times C\times P$$

Where, 'A' is the average annual soil loss; R is rainfall-runoff erosivity factor; K is Soil- erodibility factor; L is Slope-length factor; S, the slope-gradient factor; c, cropping-management factor and P is support practice factor.

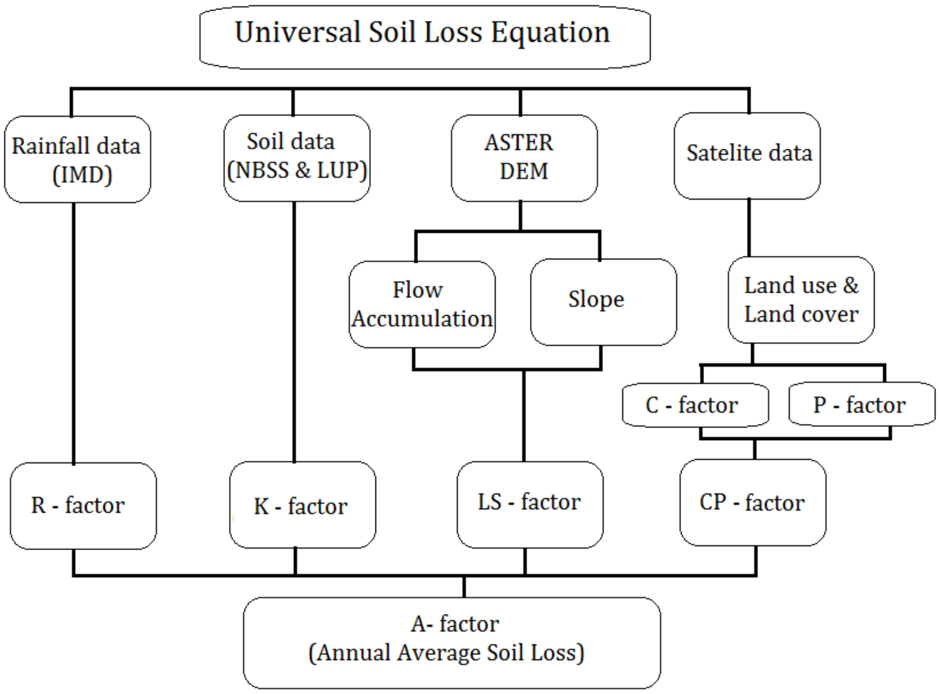

Figure 2. Methodological flow chart for the preparation of soil loss assessment map

Rainfall erosivity (R) factor

The erosivity factor of rainfall (R) is a function of the falling raindrop and the rainfall intensity, and is the product of kinetic energy of the raindrop and the 30-minute maximum rainfall intensity (Pandey et al., 2007). But in Indian context that kind of detailed meteorological data is less available. Therefore, G. Singh's (1981) empirical equation has been used for estimating annual and seasonal R factors in Indian context. The annual erosion index was as follows:

$$R_a = 79 + 0.363*P$$

Where, R_a is the average annual Rainfall erosivity factor (mt ha-cm^{-1}); and P is the Rainfall in mm. In the present study, R was computed by analyzing the rainfall data available from six rain-gauge stations (Udaipur, Amarpur, Belonia, Subroom, Bagafa and Sonamura) located in the Muhuri river basin and its adjoining area. Spatial distribution of R Factors data in the study area is estimated using inverse distance weighting (IDW) method of interpolation. In this IDW interpolation method, 10 years rainfall data for 6 rain gauge stations in and around the Muhuri river basin area were considered. The calculated R factor is given in Table 1.

Table 1. Average annual rainfall (mm) and calculated *R* value for the selected stations.

SL No.	Station	Average Annual (2001-2010)		SL No.	Station	Average Annual (2001-2010)	
		Rainfall (mm)	R-Factor			Rainfall (mm)	R-Factor
1	Udaipur	2220.46	885.03	4	Sabroom	2496.17	985.11
2	Amarpur	2144.88	857.59	5	Bogafa	2226.9	887.36
3	Belonia	2205.38	879.55	6	Sonamura	2072.71	831.39

Soil erodibility factor (K)

On the basis of the Geo-pedological map (Figure 4) of the National Bureau of Soil Survey and Land Use Planning (NBSS & LUP), Govt. of India, Soil erodibility index factor (K) values of different soil types of Muhuri river basin have been estimated and there after the soil erodibility map of the study area has been prepared by plotting the K values of each map unit. Here K factor is rated '0' to '0.36', where '0' indicates the vulnerability rate of soil erosion is less and '0.36' is the indication of high vulnerable rate of soil erosion by water. Based on salient characteristics of different soil types a detailed table has been prepared and calculated K values of surface soil was also computed.

Table 2. Geo-pedological characteristic and computed K values

Map unit	Relief type	Soil Taxonomy	K Value
LRSH1	Low relief Structural hills and ridges	Fine loamy Typic Dystrochrepts, coarse loamy Typic Udorthents, fine loamy Hapludalfs	0.24
LRSH2	Low relief Structural hills and ridges	FineTypic Dystrochrepts, Fine loamy Typic Dystrochrepts, Fine loamy Typic Paleudults	0.24
LRSH3	Low relief Structural hills and ridges	Fine loamy Typic Udorthents, Fine loamy Typic Haplumbrepts, Fine loamy Umbric Dystrochrepts	0.24
LRSH4	Low relief Structural hills and ridges	Loamy skeletal Umbric Dystrochrepts, Fine loamy Typic Dystrochrepts	0.24
LRSH5	Low relief Structural hills and ridges	Coarse loamy Typic Udorthents, Fine loamy Umbric Dystrochrepts, Fine loamy Typic Dystrochrepts	0.24
FTDH6	Flat topped Denudation hill	Fine loamy Typic kandiudalfs, Fine loamy Aquic Dystrochrepts, Fine Typic Dystrochrepts	0.15
UPLM7	Undulating plains with low mounds and narrow valleys	Fine loamy Typic Dystrochrepts, Fine loamy Typic Epiaquepts, Coarse loamy Typic Dystrochrepts	0.16
UPLM8	Undulating plains with low mounds and narrow valleys	Fine loamy Typic Dystrochrepts, Fine loamy Aquic Dystrochrepts, Fine loamy Oxyaquic Dystrochrepts	0.16
UPLM9	Undulating plains with low mounds and narrow valleys	Fine loamy Typic Dystrochrepts, Fine loamy Oxyaquic Dystrochrepts, Coarse loamy Typic Udorthents	0.16
UPLM10	Undulating plains with low mounds and narrow valleys	Fine Typic kandiudults, fine silty over sandy Aquic Dystrochrepts, Coarse loamy Typic Udorthents	0.16
IHV11	Inter hill valley	Fine loamy Aquic Dystrochrepts, Coarse loamy Fluventic Dystrochrepts	0.36
FP12	Flood plain	Fine Aquic Dystrochrepts, Fine Oxyaquic Dystrochrepts, Fine Aquic Dystrochrepts	0.34
FP13	Flood plain	Fine Typic Epiaquepts, Fine loamy Aeric Epiaquepts	0.34

Source: Through the review of literature (Ghosh et al. 2013) and NBSS and LUP, Bangalore

Topographic Erosivity Factor (LS)

Topographic Erosivity Factor (LS) has been considered as one of the most important model parameters in USLE analysis. When the slope length increases, the soil erosion by water also increases as due to the greater accumulation of surface runoff. Slope gradient and slope length factor is calculated from the flow accumulation and slope values. Finally the Topographic Erosivity Factor (LS) map has been derived using the following formula in ArcGIS spatial analysis raster calculator function.

LS = power (Flow Accumulation *cell size/22.13, 0.4) * power (sin(slope) * 0.01745) / 0.09, 1.4) * 1.4

Crop management factor (C) and conservation supporting practice factor (P)

Cropping management factor is be considered according to the USLE and RUSLE, with which cropping pattern determines the amount of erosion process (Vinay et al. 2015). C factor map was prepared on the basis of land use-land cover map of the study area. The land use land cover of the Muhuri river basin was classified with six major types of land use-land cover classes. Satellite image was processed for extracting these six land use-land cover classes using supervised classification method and there after the land use-land cover map was reclassified based on their estimated C-factor value for the generation of the Crop management factor (C) map.

During the field visit, it was found that soil conservation practice are not adopted in the area, so, for this study the P factor values are assumed as 1 for the entire Muhuri river basin. C and P factors are treated together as CP (biological erosivity) factor. The C factor and P factor were assigned as per Table 3.

Table 3. Computed CP values for Muhuri river basin area

Land-Use/Land Cover Class	C Factor	Researchers/Author/Source	P Factor value	CP Factor
Dense forest	0.008	Kumar and Kushwaha, (2013)		0.008
Forest plantation	0.02	Kumar and Kushwaha, (2013)	1	0.02
Moderately Dense forest	0.04	Ghosh et al.(2013)		0.04
Degraded forest	0.06	Ghosh et al.(2013)		0.06
Agricultural land	0.34	Devatha et al.(2015)		0.34
Fallow/ Wasteland	0.6	Biswal (2015)		0.6

Results and Discussion

Rainfall erosivity (R)

The annual average rainfall erosivity factor (*R*) for the years 2001 to 2010 was found to be in the range of 863.44 to 926.43 mt ha-cm^{-1}. Within the Muhuri river basin area the highest value (887.36 mt ha-cm^{-1}) of annual R factor was observed in Bagafa station when the total average annual rainfall was 2226.9 mm and the lowest value (879.55 mt ha-cm^{-1}) of annual R factor was found to be in Belonia meteorological station when the total rainfall was 2205.38 mm.

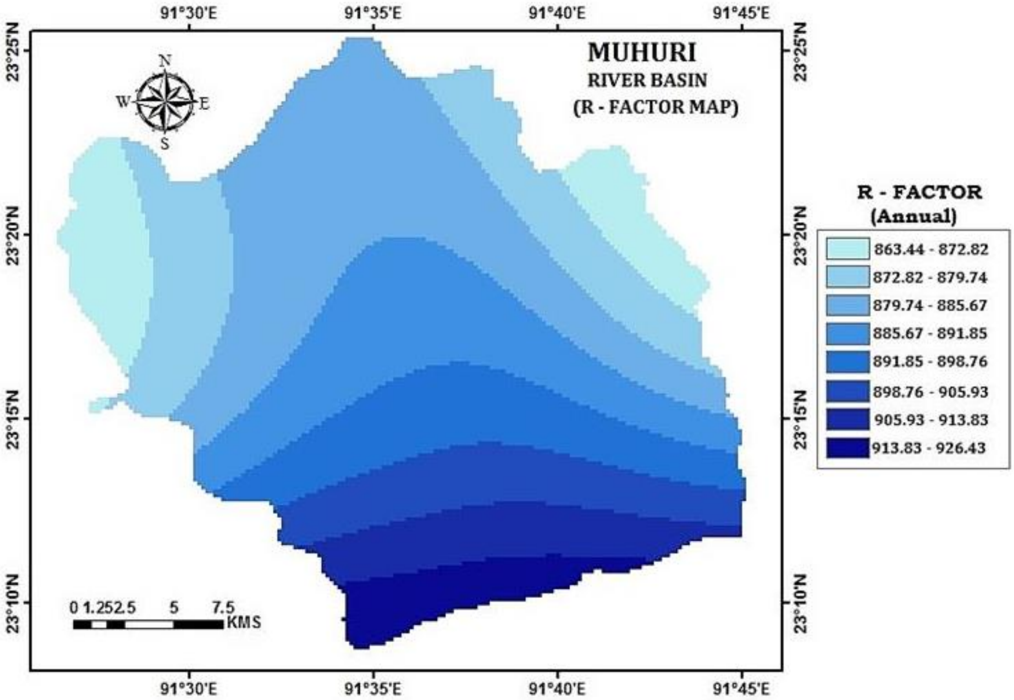

Figure 3. Spatial distribution of *R* factor

Soil erodibility (K)

Soil erodibility is an important index, which help to evaluate the soil vulnerability to erosion. Spatial distribution of surface soil K values in Muhuri river basin has shown in Figure 5 and Table 2. From the study (K factor map) it has been found that, In low relief areas like alluvial plains, an inter-hill valley and flood plains region, the K value is become significantly high which is ranges from 0.34 to 0.036. Soil erodibility of flood plain is comparatively high because soils texture of flood plains lying along Muhuri river course were generally loamy sand to sandy loam texture in nature and organic matter content was also very low, which making them more susceptible to erosion. In high relief area like structural hill and Denudation hill, the K value is comparatively less, it generally ranges from 0.24 to 0.15.

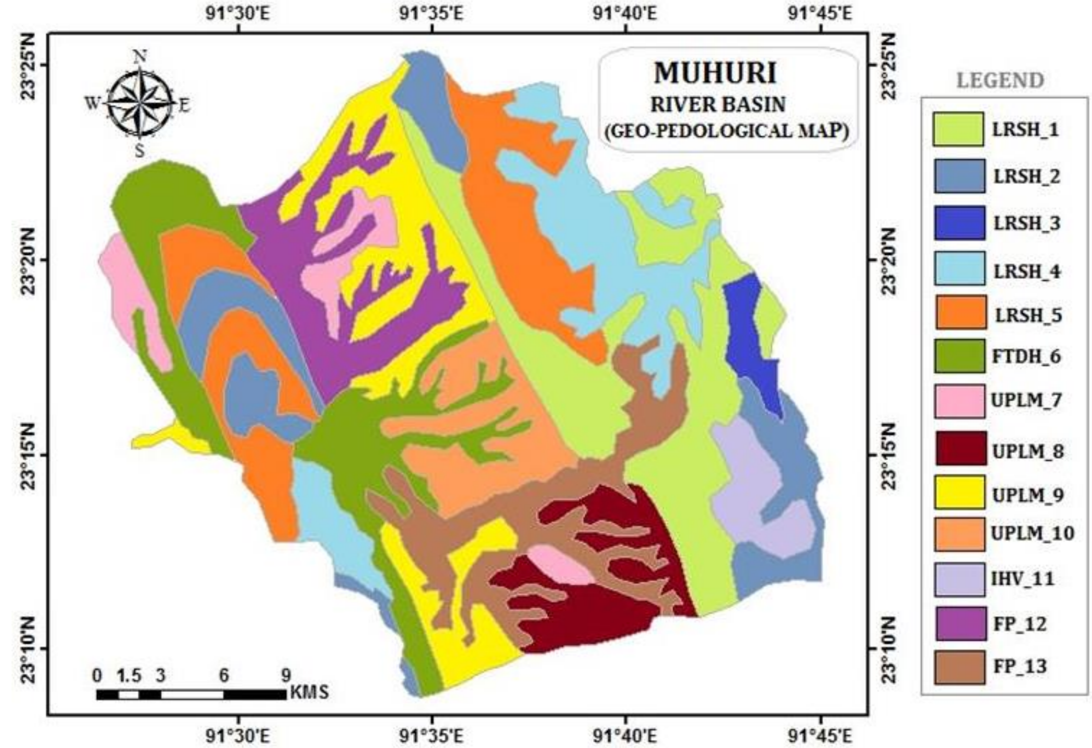

Figure 4. Geo-pedological map of Muhuri river Basin

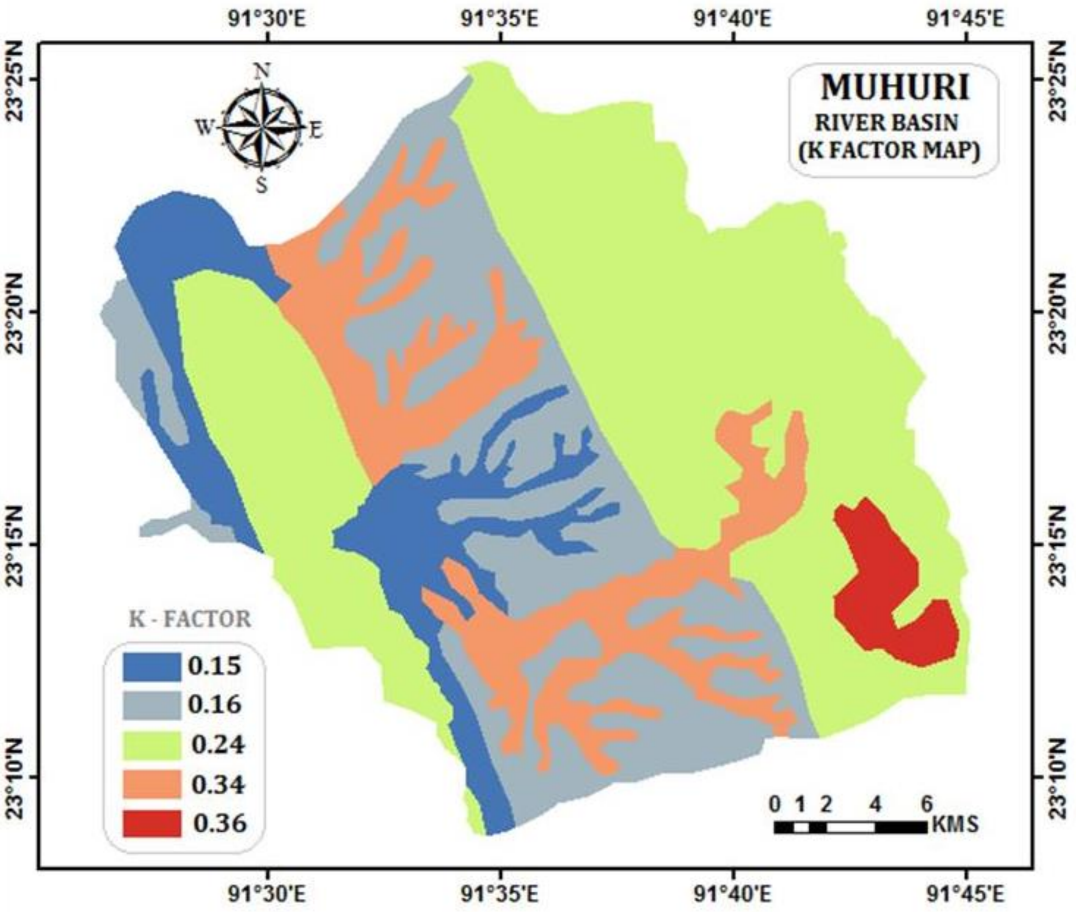

Figure 5. Spatial distribution of K factor of Muhuri river Basin

Topographic erosivity (LS Factor)

The slope length factor (L) and slope steepness factor (S) mainly reflect the effect of Topography on erosion (Yildirim, 2012). Slope length is defined as the horizontal distance from the point of origin of overland flow to the point where either the slope gradient decreases enough that deposition begins, or runoff is concentrated in a defined channel (Renard et al., 1997; Wischmeier and Smith, 1978). Slope steepness reflects the influence of slope gradient on erosion. In general, an increase in the L and/or S factor produces higher overland flow velocities and correspondingly greater erosion (Ozsoy et al., 2012) Topographic Erosivity factor (LS) factor of Muhuri river basin has been calculated by considering the flow accumulation and slope factor extracted from DEM. From the analysis, it is observed that the Topographic Erosivity factor in Muhuri river basin has been found to be in the range of 0 to 50 (Figure 6).

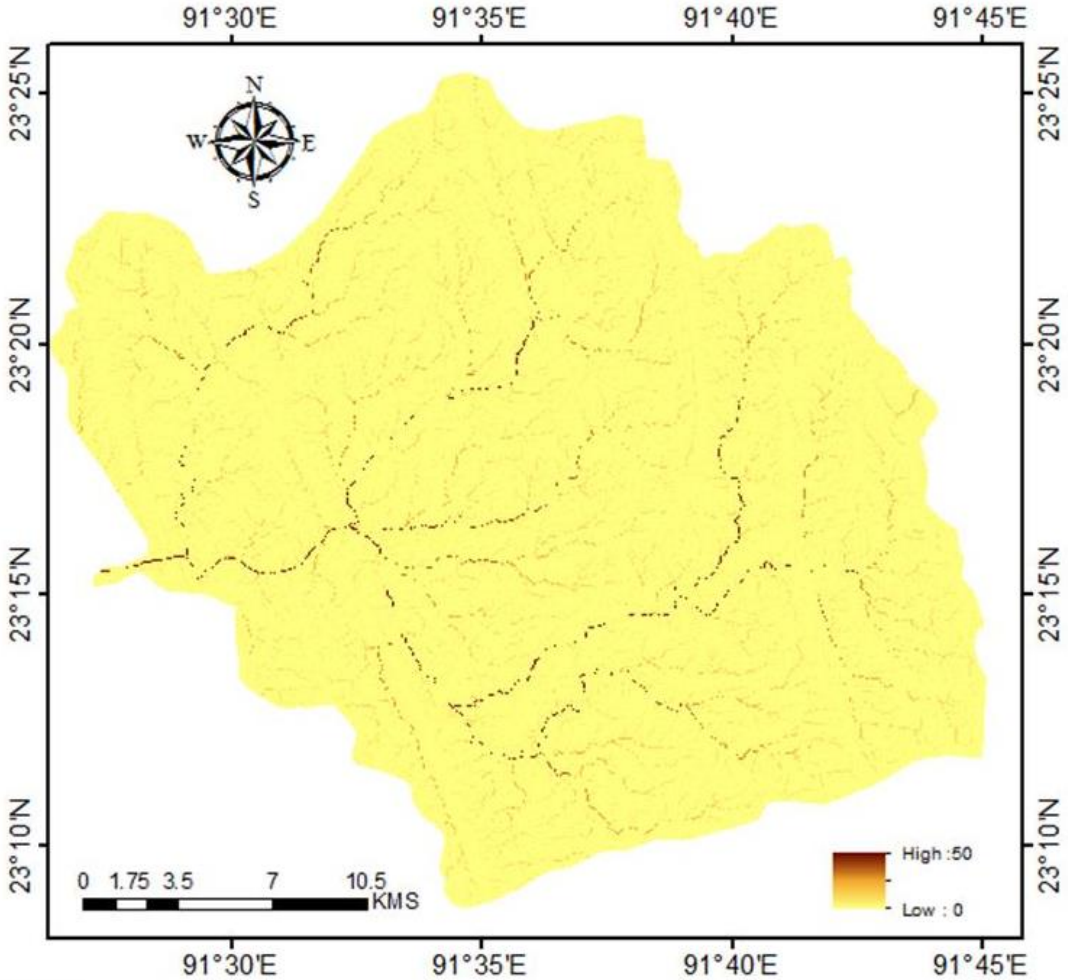

Figure 6. LS factor of Muhuri river Basin

C P factor

The cover management factor (C) is a crucial factor to the erosion because it is a readily managed condition to reduce erosion (Renard et al. 2011). Soil loss is very sensible to land cover in addition to relief (Chatterjee et al., 2014). In the present study area almost 65 % area is under dense and degraded forest. C factor is less significant when land use and land cover area comprises maximum percentage of natural vegetation and plantation crops. The value of which ranges from ‘0’ in water bodies to slightly greater than ‘1’ in barren land (Toy et al., 2002). The CP factor values in the study area vary from 0 to 0.6. The lower CP factor values (0.008-0.02) are mostly seen in the eastern most part of the basin where maximum potion of land use and land cover is dominated by dense forest and densely rubber plantation. However, the agricultural areas which occupy the central part of the basin have moderate CP factor values (Figure 7). The high CP factor value (0.34 – 0.6) was found along the main course of Muhuri River and the Waste land and barren land.

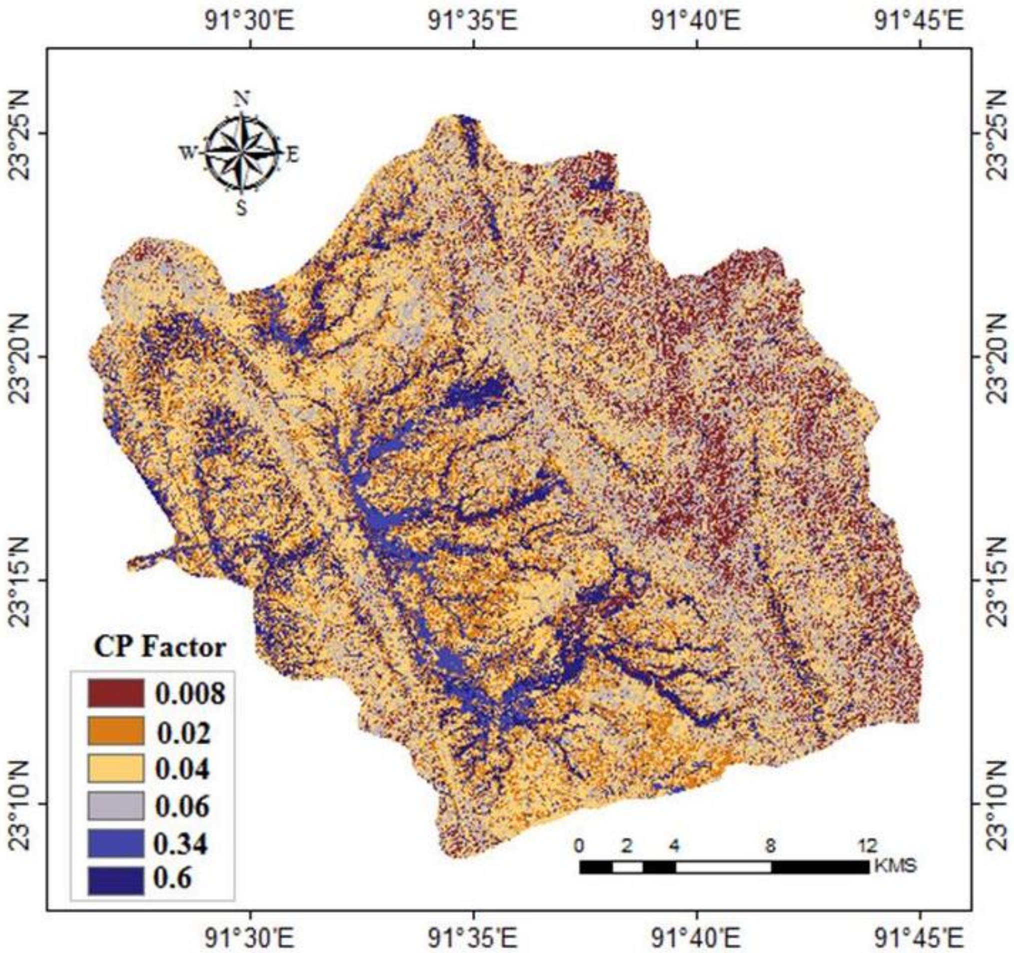

Figure 7. CP factor map of Muhuri river Basin

Average annual soil loss (A factor)

The average annual soil erosion potential (A) has been computed by multiplying the developed raster data from each factor (A= R K L S C P) of USLE analysis. The final 'A' factor map displays the average annual soil loss potential of the Muhuri river basin is shown in figure 8. Results shows that the study area has gentle slope so the erosion loss is obtained with low rate and it is within acceptable limit. Predicted average annual soil loss of Muhuri river basin has been classified into six erosion intensity classes (Table 4) to assess erosion potential severity. The average annual predicted soil loss ranges between 0 to and 650 t/ha/y. Negligible soil loss areas (<5 t/ha/y) have been recorded under very densely forested areas and low soil loss (5-10 t/ha/y) was found manly intensely plantation (mainly Rubber plantation) area and degraded forest area. Soil erosion rate was predicted moderately high (10-25 t/ha/y) for agriculture, which needs proper soil conservation measures to reduce erosion. The high rate (>70 t/ha/y) of soil erosion was found along the main stream and along the Lunga (valley) portion of the basin, because of moderate slope value and the high slope length and steepness factor. According to erosion risk classes it is observed that 80-90 % area is under negligible to slight class whereas only 20 -10 % area is under moderate to extremely high class.

Table 4. Different classes of soil erosion

Soil loss classes (t/ha/yr)	Erosion Intensity Type
0-5	Negligible Erosion
5-10	Low Erosion
10-25	Moderate Erosion
25-70	Moderately high Erosion
70-100	High Erosion
>100	Extremely high Erosion

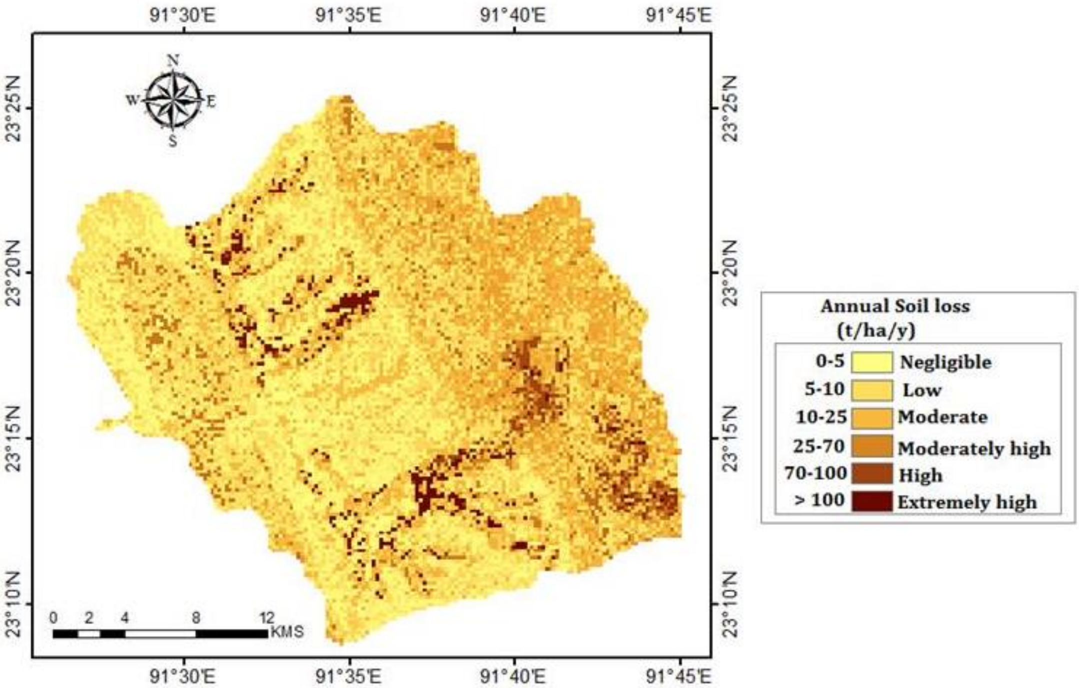

Figure 8. Spatial distribution of Average annual soil loss (A factor) map of Muhuri river Basin

Conclusion

The study was done to address and quantify the soil loss problem in Muhuri river basin of Tripura. Geographic Information System (GIS) and Remote Sensing are emerging most effective tools for analyzing spatial distributed information in a vast area now a days. The use of the USLE model integrated to GIS and RS is an effective tool than the time consuming conventional methods for assessing the soil loss vulnerability in a basin's scale. The all USLE parameter R, K, LS, C and P factor maps were combined together for creating the annual average soil loss map of the Muhuri river basin area. The output results shows that the LS factor varies from 0 to 50; CP value in the study area varies from 0.008 to 0.6 and K value is observe in between 0.15 to 0.36. Average annual soil loss risk in the study area is moderately high from the acceptable limit. The methods and the predicted amount of soil loss and its spatial distribution of the basin described in this study which are useful to formulate and further implement conservation program that will reduce soil loss from the basin.

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Sodium-resistant plant growth-promoting rhizobacteria isolated from a halophyte, *Salsola grandis*, in saline-alkaline soils of Turkey

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Abstract

Phytoremediation is an expanding field of research in environmental studies due to the benefits of its cost effectiveness and environmental friendliness. The use of this technology in saline and alkaline soils can be a promising approach because soil salinity inhibits crop growth and causes tremendous yield losses in many regions of the world, especially in arid and semi-arid regions. However, little is known about the plants that can be applicable in the phytoremediation of saline soils and role of their rhizobacteria in the phytoremediation processes. In this study, we examined sodium (Na) uptake by the halophyte *Salsola grandis* and screened Na resistant rhizobacteria inhabiting in an extremely saline soil environment. *S. grandis* could uptake Na at the value of 15447 mg·kg^{-1} and transported Na to stem and leaves from roots. We found that 50 out of the 131 strains were Na resistant and 8 out of these 50 strains contributed to the growth of *S. grandis*. Using 16S ribosomal RNA sequencing, we determined these eight strains to be within the genera *Arthrobacter* spp. and *Bacillus* spp. Moreover, four of the eight strains (A22, WP5, B14, AP20) showed traits of being both siderophore and indole-3-acetic acid producers. Therefore, these eight strains appear to be suitable candidates for plant growth-promoting rhizobacteria of *S. grandis*.

Keywords: *Arthrobacter, Bacillus*, phytoremediation, *Salsola*, salinity in soil.

Introduction

The introduction of irrigation systems has raised agricultural productivity in dry and semi-dry regions. Although only 18% of the cultivated area is irrigated globally, 40% of global food production comes from irrigated agriculture (Siebert et al., 2005). In these areas soil salinization is a major problem and leads to low agricultural productivity. Soil salinity is a worldwide problem affecting about 20% of the world's cultivated land and nearly half of all irrigated land (Zhu, 2001). According to the FAO/UNESCO soil map of the world, approximately 1.5 million hectares of land in Turkey are suffering from salinity and sodicity problems (Kendirli et al., 2005).

Phytoremediation has attracted rising attention as a suitable technology that can be applied not only in polluted but also in saline soil remediation because of its low economic cost and environmentally friendly

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process (Qadir et al., 2002, 2003). Some reports suggest that the harvested plant, if not plowed back to the same soil, can contribute significantly to the removal of salts such as sodium (Na) from salt-affected soils (Ravindrana et al., 2007; Qadir et al., 2007). The genus *Salsola* (Chenopodiaceae) comprises about 120 species; they are widespread herbaceous or shrubby halophytic plants, especially in the brackish grounds of the moderate and subtropical regions of Europe, Asia, Africa and North America (Tundis et al., 2008). *Salsola soda*, typically grows in coastal regions and saline conditions, was used as a companion plant because of its high accumulation of Na (Colla et al., 2006; Zuccarini, 2008). In 1999, *Salsola grandis*, a species closely related to *S. soda*, was reported as a new species of genus *Salsola* (Freitag et al., 1999). This plant is an annual halophytic plant growing in open habitats with sparse vegetation where local semi-desert climate creates an extreme saline soil habitat (eg. pH over 9 and Na over 3500 mg·kg^{-1}) that forms on highly clayey salt-containing marly substrate, in Nallıhan Region, Ankara Province, Turkey. To the best of our knowledge, there is no information on how this plant manages abiotic stress factors and survives in such a harsh environment and also no previous report about its Na uptake from soil.

Various soil microorganisms were shown to tolerate unwanted elements in soil using various mechanisms including exclusion, active removal, biosorption, precipitation, and extra or intracellular bioaccumulation (Silver, 1996; Whiting et al., 2001; Lasat, 2002; Gadd, 2004). These processes are known to effect the solubility and the bioavailability of toxic elements, such as heavy metals, to plants and therefore interactions between hyperaccumulator plants, metals and plant growth-promoting rhizobacteria (PGPR) have gained increasing attention for the last two decades (Turgay and Bilen, 2012). PGPR are free-living soil bacteria that can either directly or indirectly facilitate rooting and growth of plants. These bacteria serve the functions of N-fixation, production of siderophores, solubilization of minerals like phosphorus and synthesis of phytohormones such as indole-3-acetic acid (IAA). To date, some bacteria such as *Klebsiella* sp. D5A showed the high plant growth promoting activity on the glycophytic crop in saline–alkaline soils (Liu et al., 2014; Liu et al., 2016). Likewise, many studies have been published on beneficial effects of bacterial application on growth of wheat under salt stress (Ashraf et al., 2004; Sadeghi et al., 2012; Upadhyay et al., 2012; Chakraborty et al., 2013; Paul and Lade, 2014).

According to Ruppel et al. (2013), "micro-organisms use similar approaches to cope with oxidative stress and it is likely that microorganisms use the same mechanisms to alleviate salt stress effects in halophytes as in other tolerant plants. However, little is known about the effect of PGPR on the salt accumulation in halophytic plants. The application of PGPR may prove useful when developing strategies to facilitate plant growth in saline soils. Therefore, in the present study, we studied Na-resistant rhizobacteria isolated from the rhizosphere of a halophytic plant, *S. grandis,* and a halotolerant plant, wheat (Triticum aestivum). The aims of this study were to explore Na-resistant indigenous PGPR on *S. grandis*, and determine the effect of these isolates on the salt accumulation in *S. grandis*.

Material and Methods

Sampling sites and soil properties

The seeds and five seedlings (2-3 month old) of *Salsola grandis* Freitag, Vural and N. Adıgüzel (Amaranthaceae) were collected from harsh ecological conditions which is including raw gypsum and salt-containing marly substrate located at 28km east-southeast of Nallıhan region (40°05'29.57"N, 031°36'10.26"E) in Ankara Province in November 2015 and in April 2016, respectively. The seeds collected were stored in paper bags at 4°C until use and the seedlings were used to isolate bacteria from rhizosphere soil on the next day after sampling. For the greenhouse experiment, soil was collected from a depth of 0-20 cm in a non-agricultural area (38°20'08.6"N, 033°59'06.5"E) located at the campus of Aksaray University, Aksaray Province, Turkey. This soil was air-dried and stored in room conditions prior to soil analysis and the pot experiment. The soil characteristics are outlined in Table 1.

Pot experiment

We conducted a pot experiment, setting up *S. grandis* and *Triticum* spp., in triplicate for 82 days on a soil collected from Aksaray district to examine Na uptake of *S. grandis*. *Triticum* spp. (wheat) was used as control to compare the Na uptake with *S. grandis*. Five seedlings (82 days old) of *S. grandis* per pot and three seedlings (82 days old) of wheat per pot were grown at a greenhouse (Ankara University, Ankara, Turkey). The analysis of the Na concentration in *S. grandis* and wheat was performed as follows. Harvested plants were washed carefully using tap water and then distilled water, and then stem-leaves and roots were separated. After the measurement of the fresh weight, all plant samples were dried at 60°C, and their dry weight was measured. Then, the plants were ground and dry ashing was conducted in a muffle furnace at temperatures of 500 ± 50°C for approximately 20 h. The ash was dissolved in 10 M of nitric acid (HNO_3) and the extract solution was analyzed using ICP-OES (Optima 2100 DV; Perkin Elmer, Inc., Shelton, CT, USA) (Kacar and Inal, 2010).

Table 1. Soil properties collected from Aksaray and Nallıhan. The soil from Aksaray was used at both of pot experiment and conical tube one.

		Aksaray	Nallıhan
pH (H_2O)		8.36	9.34
EC	(dS/m)	2.37	1.46
Total Nitrogen	(%)	0.40	0.15
Organic Materials	(%)	5.42	0.73
$CaCO_3$	(%)	40.3	8.45
Phosphate	(mg kg^{-1})	8.19	5.12
Exchangeable Na	(mg kg^{-1})	746	3538
Exchangeable Ca	(mg kg^{-1})	5501	5374
Exchangeable Mg	(mg kg^{-1})	2282	354
Exchangeable K	(mg kg^{-1})	402	290

Isolation of *rhizobacteria* from Salsola grandis and wheat

Bacteria were isolated from 0.5 g of rhizosphere soils of *S. grandis* and wheat taken from the pot experiment mentioned above and that of naturally growing *S. grandis* taken from Nallıhan. The soil samples were mixed with 5 ml of sterile distilled water with a sufficient vortex and then diluted to 10^{-3}, 10^{-4}, and 10^{-5}. We then placed 50 µl of the resulting soil solutions onto plates of nutrient agar and of potato dextrose agar adjusted to pH 7.0. The plates were then incubated at 25°C for 3 days.

Screening of Na-resistant bacteria

Potato dextrose broth was adjusted to pH 7.0 using 1M potassium hydroxide (KOH) and the media with Na (750 mM) and without Na (0 mM) were prepared we then divided 5 ml of the media into 15-ml centrifugation tubes. Na concentration was set referring to Hotchkiss (1923). Individual bacteria were inoculated to each tube and incubated at 25°C for two days. After incubation, the optimal density for bacterial growth was measured at 600 nm. The strains which grew to more than half the number of those growing in the control (0 mM Na) tubes were selected as Na-resistant bacteria.

Screening of PGPR

In order to handle high number of strains in experimental condition, a sterile conical tube was set to test 50 strains with 4 replications at the same time in terms of microbes-Na uptake association. Saline soil collected from Aksaray was sieved (<2 mm) and autoclaved at 121 ^{0}C for 120 min, and then 20 g of the sieved soil was put into a 50-ml conical centrifuge tube treated with 0.5 ml mineral nutrient solution (N, 100 mg kg^{-1}; P, 100 mg kg^{-1}; K, 125 mg kg^{-1}). The bacterial suspension was adjusted to an optimal density of 1.0 at 600 nm. It was then inoculated with 1 ml bacterial suspension into each tube. The seeds were soaked in 0.5% of sodium hypochlorite (NaOCl) for 2 min for brief surface-sterilization and then rinsed using distilled water. Seeds were then individually transplanted into the tube and we prepared four replications for each bacterial isolate. The seedlings were grown in the plant growth chamber (15°C / 25°C night/day temperature, 55% relative humidity and 12 hours photo period) and harvested at 35 days of the plant development. The yield of shoots and roots was determined based on fresh and dry weight. Moreover, the Na concentration in the harvested plant was also measured using ICP-OES. The data obtained were statistically analyzed using Student's *t*-test to compare the plant growth between seedlings with and without each bacterial species ($P <$ 0.05).

Biochemical assay of PGPR properties

Determination of IAA

The cultures were grown for 4 days at 25°C in IAA production media (30 g of glucose, 2g of beef extract, 3g of $CaCO_3$, 1mM (final concentration) of tryptophan, pH 7, 1 L of distilled water) and centrifuged at 10000*g* for 10 min. The IAA was determined in 300 µl of supernatant using 1.2 ml of Salkowski's reagent at 535 nm (Acuña et al., 2011).

Determination of phosphate solubilization

The media developed by Pikovskaya (1948) was used for quantitative estimation of tri-calcium phosphate solubilization by the isolates. Strains selected as PGPR were inoculated to the media and incubated at 25°C for 7days. As evaluation of P solubilization, we used following index; - No clear zone, ± detectable of the clear zone but very weak activity, + detectable of clear zone.

Siderophore production

Chrome azurol S (CAS) medium was prepared using a slightly modified method of Schwyn and Neilands (1987) and Pérez-Miranda et al. (2007). The medium for 100 ml of overlay was prepared as follows: CAS 6.04 mg, hexadecyltrimetyl ammonium bromide (HDTMA) 7.3 mg, Piperazine-1,4-bis(2-ethanesulfonic acid) (PIPES) 3.04 g, and 1 ml of 1 mM $FeCl_3 \cdot 6H_2O$. Siderophore detection was achieved after 10 mL overlays of this medium were applied over the agar plates containing cultivated microorganisms to be tested for siderophore production. The CAS-blue agar changed to light orange or yellow if bacteria produce the siderophore. − No color change, + change the color, ++ color change detected in all over the medium.

16S rRNA analysis of selected bacteria

To identify the isolated microorganisms, the nucleotide sequences of their 16S rDNA were investigated using molecular techniques. DNA was extracted using 100 µl of 2 × CTAB buffer [2.0% (w/v) CTAB; 1.4 M NaCl; 100 mM Tris-HCl (pH 8.0); 20 mM EDTA (pH 8.0)]. After vortexing, the sample solution was incubated for 30 min at 60°C. An equal volume of chloroform was added to the sample solution and mixed to emulsify the mixture. The mixture was centrifuged at 15300*g* for 20 min at room temperature, and the supernatant was transferred to a new tube. A 2.5 times volume of ethanol was added and the mixture was incubated at room temperature for 20 min, then centrifuged at 15300*g* for 20 min at 4°C. The resulting DNA pellet was rinsed with 70% ethanol and dried. Each dried DNA pellet was dissolved in 50 µl sterilized distilled water. The 16S rRNA gene (700 bp) of each isolate was amplified by a T100™ Thermal Cycler (Bio-rad, CA, USA), with 1 cycle of 95°C for 5 min, 30 cycles of 94°C for 30 s, 58°C for 30 s and 72°C for 1 min and a final extension at 72°C for 7 min. The 40-µl polymerase chain reaction (PCR) mixtures contained 1.5 µl DNA extract, 1.5 µl of 10 mM each primer; 800F (GGATTAGATACCCTGGTA) and 1500R (TACCTTGTTACGACTT), 10 µl of 5×FIREPol® MasterMix (SolisBioDyne, Tartu, Estonia). The nucleotide sequences of PCR-amplified fragments were determined by PCR-direct sequencing. The nucleotide sequences determined were compared with those in GenBank. All sequence data, including newly obtained and retrieved sequences, were aligned using the computer program BioEdit (available at http://www.mbio.ncsu.edu/BioEdit/bioedit.html). Distance-based phylogenetic trees were generated using the model of Jukes and Cantor (1969) and a neighbor-joining algorithm (Saito and Nei, 1987). The topology of phylogenetic trees was evaluated by bootstrap resampling (1000 replicates). Clustal W, provided by the DNA Data Bank of Japan (available at http://www.ddbj.nig.ac.jp/Welcome-j.html), was used for the analyses.

Results

Pot experiment

The pot experiment was conducted to confirm the Na uptake and allocation by *S. grandis*. The Na uptake by *S. grandis* was compared with wheat in this study. The mean dried weight after the growth period of 82 days was 12.4 mg and 6.36 mg for *S. grandis* and wheat, respectively. The growth of stems and leaves in *S. grandis* were higher than those of wheat, but root biomass showed the opposite result (Table 2). Under Na uptake, *S. grandis* transported high amounts of Na to the stem and leaves from the roots (Table 2), whereas wheat retained Na in the roots.

Table 2. Na uptake by *Salsola grandis* over the short-term cultivation (82 days)

	Dry biomass (mg)		Na accumulation (mg kg^{-1})	
	Stem & Leaves	Root	Stem & Leaves	Root
S. grandis	12.3	0.16	15447	1459
Wheat	1.73	4.63	346	1563

Isolation and screening of Na-resistant bacteria

The population density of rhizosphere bacteria averaged to 5.38 $\times 10^6$, 3.17 $\times 10^6$ and 1.10 $\times 10^7$ cfu g^{-1} in the rhizosphere soil of wheat (Aksaray soil), *S. grandis* (Aksaray soil) and *S. grandis* (Nallıhan soil), respectively.

A total of 131 strains—50, 50 and 31 strains from rhizosphere of wheat, *S. grandis* (Aksaray soil) and *S. grandis* (Nallıhan soil), respectively—were found during the screening of Na-resistant bacteria. In the screening of Na-resistant bacteria, a total of 36 strains were able to grow more than 80% above the growth levels of the control in the medium with 750 mM NaCl. 14 strains were grown in the medium with 750 mM of NaCl than the control (Figure 1).

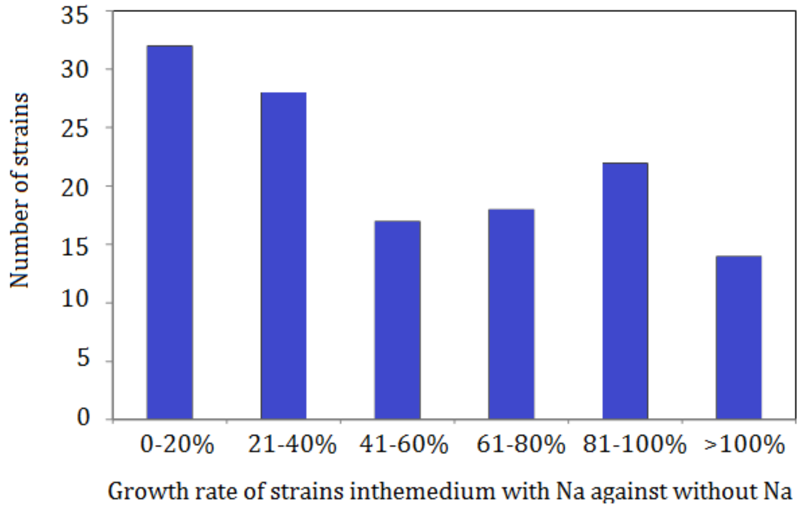

Figure 1. Relative frequency of Na-resistant bacteria from rhizosphere soil

Screening of PGPR

The plant growth promotion effect of the strains was shown in Figure 2 and Table 3. Total eight strains of bacteria isolated from the rhizosphere of *S. grandis* (strains AP20, B14, A22, B2 and A3) and wheat (strains WN4, WP18 and WP5) enhanced the growth of *S. grandis*, but little variation was observed in bacterial effectiveness among parameters (Table 3). Differences between treatments in the dry weight of stem and leaves were statistically greater than the control in all cases except for strain AP20. The strains A3 and A22 showed significant increases in the fresh weight and dry weight, and means of dried stem and leaves of *S. grandis* in tubes inoculated with these strains increased 15.5% and 17.4%, respectively, above the non-bacterial control. Moreover, the mean dried root weight increased 53.9% and 59.2% in strains A3 and A22, respectively, relative to the non-bacterial control. There was no significant difference between the control and other bacterial treatments (Table 3).

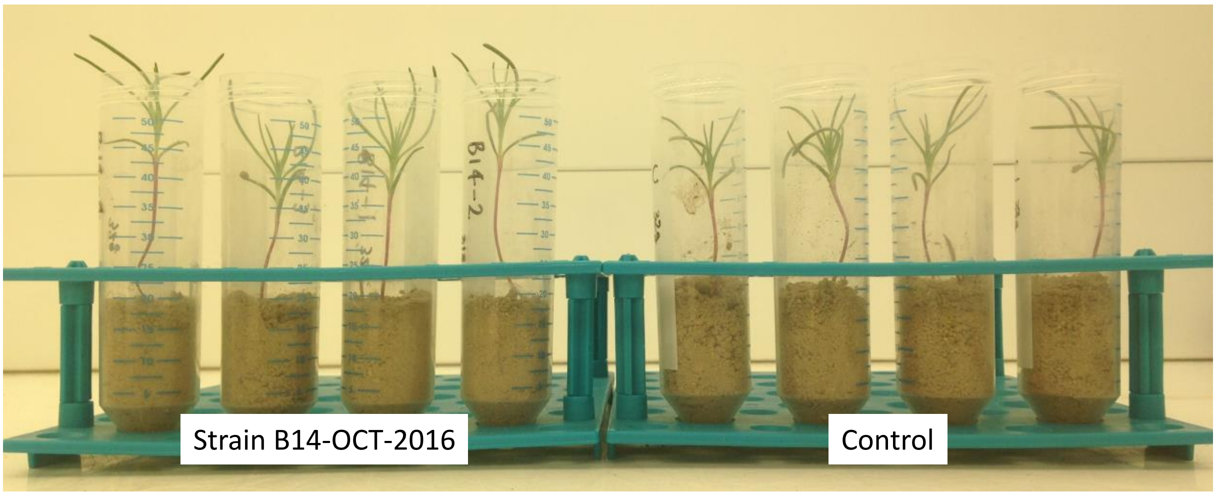

Figure 2. Growth of *Salsola grandis* which was inoculated with strain B14-OCT-2016 (left) and uninoculated control (right)

For PGPR traits, all strains showed a negative activity on the phosphate solubility, but there was a positive activity on the siderophore and IAA production in most strains. Strains A22, WP5, B14 and AP20 produced more than 19 mg L^{-1} of IAA after the 4-day incubation (Table 4). All strains were either siderophore producers or IAA producers. Four strains out of the eight strains (A22, WP5, B14, AP20) showed both traits of siderophore and IAA producers.

Table 3. Growth and Na uptake of *Salsola grandis* by inoculating bacterial strains. These values showed means ± standard deviation. Asterisks indicate significant differences from the control values (*t*-test, $P < 0.05$)

	Fresh weight (mg)		Dry weight (mg)		Length (cm)		Na conc. in plant
Strain No.	Stem & Leaves	Root	Stem&Leaves	Root	Stem& Leaves	Root	(mg kg^{-1})
AP20	194.2 ± 13.3 *	16.9 ± 3.35	24.8 ± 2.3	6.23 ± 1.5	9.38 ± 0.28 *	8.40 ± 1.93	25424 ± 2659
WP18	207.6 ± 21.3 *	36.2 ± 11.5 *	26.8 ± 1.4 *	11.5 ± 1.0	8.40 ± 0.44	8.75 ± 2.19	23529 ± 422
WP5	209.5 ± 18.7 *	34.7 ± 8.28 *	27.8 ± 4.9 *	15.6 ± 7.7	8.80 ± 0.42	10.6 ± 0.84 *	21867 ± 1581
B14	192.8 ± 23.3 *	19.4 ± 7.15	27.2 ± 2.4 *	11.7 ± 4.1	8.85 ± 0.44	8.63 ± 0.85	26081 ± 3464
WN4	233.3 ± 40.3 *	19.7 ± 5.05	30.2 ± 1.3 *	10.9 ± 2.4	9.03 ± 0.62	10.6 ± 1.33 *	27634 ± 2479
A3	206.7 ± 17.6 *	32.2 ± 7.74 *	29.8 ± 4.2 *	14.5 ± 1.9 *	8.73 ± 0.76	8.83 ± 0.99	23638 ± 3142
A22	233.6 ± 12.5 *	28.0 ± 2.19 *	30.3 ± 1.4 *	15.0 ± 2.3 *	9.43 ± 0.96	8.27 ± 1.01	25390 ± 3834
B2	207.4 ± 41.9	32.6 ± 6.25 *	26.1 ± 5.2 *	16.5 ± 4.0 *	8.40 ± 0.71	6.50 ± 1.62	21984 ± 851
Control	163.4 ± 11.5	15.9 ± 4.88	25.8 ± 3.1	9.42 ± 2.1	8.10 ± 0.89	7.92 ± 1.73	24562 ± 2115

Table 4. PGPR traits of Na resistant bacteria. +; producing siderophore and solubilizing P, -; no siderophore production and P solubilization, ±; weak P solubilization.

Strains	Siderophore	P solubilization	IAA (mg L^{-1})
A3	+	±	2.34
WN4	+	-	2.55
WP5	++	-	19.0
A22	+	-	17.7
B14	+	-	21.7
B2	+	-	1.61
AP20	+	-	18.7
WP18	-	-	33.4

16SrRNA sequencing analysis

The 16S rRNA partial gene sequences of the eight strains were compared with those of the bacterial sequences in GenBank. Strains AP20, WP18, WP5, B14, WN4, A22 and B2 exhibited high sequence similarities to those of genus *Arthrobacter*, as shown by the constructed phylogenetic dendrogram (Figure 3). The highest sequence similarity of AP20 (GenBank accession no. LC176874), WP18 (GenBank accession no. LC176871), WP5 (GenBank accession no. LC176876), B14 (GenBank accession no. LC176872), WN4 (GenBank accession no. LC176873), A22 (GenBank accession no. LC176870) and B2 (GenBank accession no. LC176875) was found with *Arthrobacter globiformis* (GenBank accession no. KF923428), *A. pascens* (GenBank accession no. X80740), *A. sulfureus* (GenBank accession no. KF923457), *A. nitroguajacolicus* (GenBank accession no. FM213395), *A. agilis* (GenBank accession no. X80748), respectively. In addition, Strains A3 (GenBank accession no. LC176877) was closely related with *Bacillus amyloliquefaciens* (GenBank accession no. KX369577).

Discussion

In the present study, we revealed that *Salsola grandis* was able to uptake Na from soil and located it in aboveground plant tissues in higher amounts. Moreover, eight new PGPR, seven strains of *Arthrobacter* spp. and one strain of *Bacillus* sp., were isolated from rhizosphere soils.

S. grandis was reported as a new species of genus *Salsola,* and it is an annual halophytic plant which is distributed in Nallıhan region as the region has semi-arid climate conditions and clayey soil environments (Freitag et al., 1999). However, there has been no previous report about the Na uptake from soil by this plant. It is said that the halophytes are associated with a correlation between cation accumulation and plant succulence (Maimaiti et al., 2012), and *S. grandis* is also succulent. Moreover, *Salsola soda*, a relatively closely related species, is also able to uptake Na at the value of around 45,980 mg kg^{-1} (Hasanuzzaman et al., 2014). Thus *S. grandis* also has a high potential to uptake Na. In this study, we revealed that *S. grandis* is able to uptake Na, as shown in Table 2. We tried to show the sodium accumulation of *S. grandis* in comparison with that of wheat. Wheat (*Triticum aestivum* L.) is moderately salt-tolerant and one of the most important staple crops in the world (Maas and Hoffman, 1977). Moreover, it occupies 32.8% (7860,000 ha in 2015) in the cultivated land in Turkey. The comprehensive surveys conducted on salt tolerant crops indicated that wheat can tolerate and grow with no yield loss up to salinity level of 6–8 dS m^{-1} EC corresponding 60–80 mM NaCl.

(Maas and Hoffman, 1977; USDA-ARS, 2005; Munns et al., 2005), which is very close to the level of Nallıhan soil, 9.3 dS m^{-1} EC and 60 mM NaCl, in the present study. Besides, the survival of any plant species under such a stress condition depends primarily on the ability of the plant to develop adaptive response within their own rhizosphere and phyllosphere, which can be achieved by the aid of specific rhizosphere microorganism. Many studies indicate that plants in different living areas contain different microorganisms (Berendsen et al., 2012). Therefore, in the present study, we tried to isolate bacteria from the rhizosphere of both *S. grandis* and wheat to monitor how free-living rhizospheric bacteria of a salt tolerant (wheat) and a salt accumulator (*S. grandis*) plant respond with high salinity.

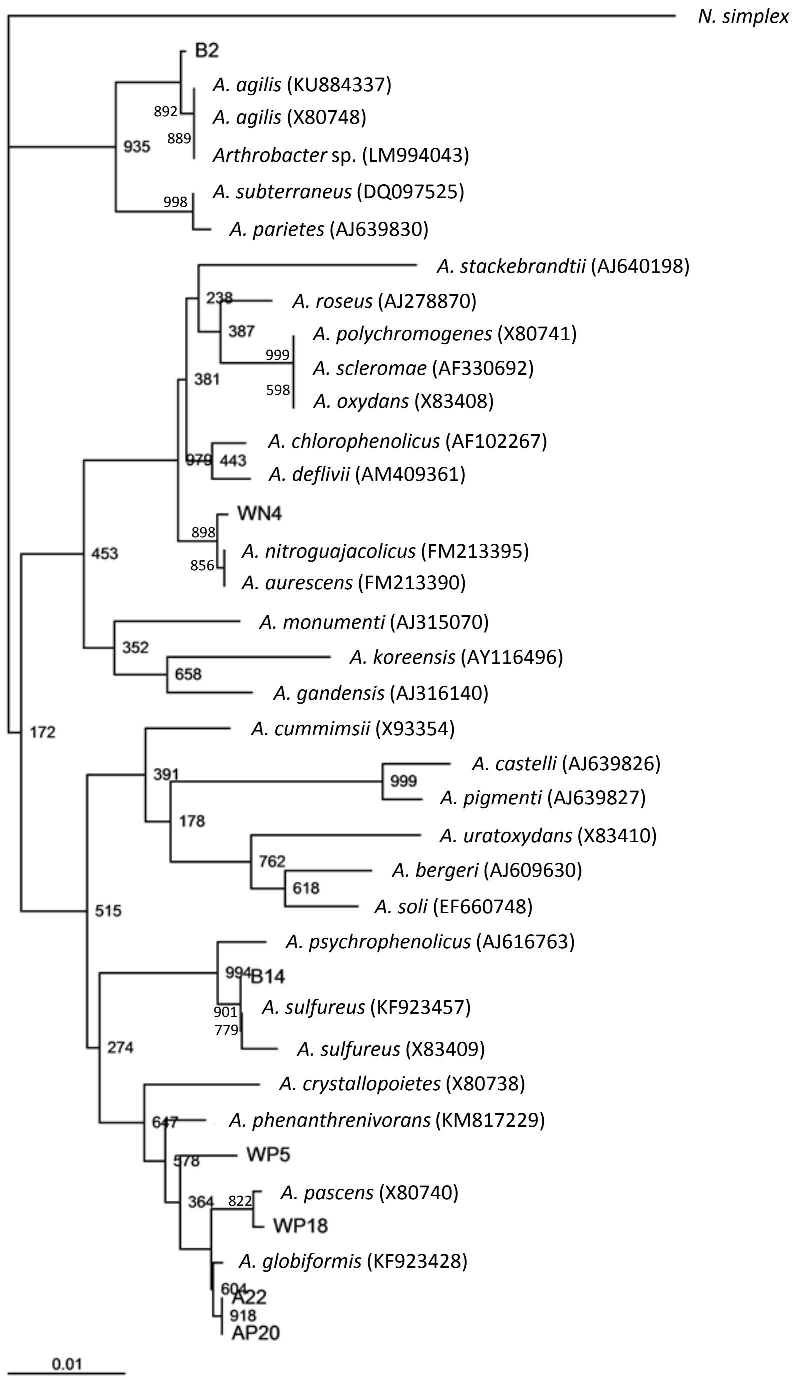

Figure 3. Phylogenetic relationships of strains AP20, WN4, B14, A22, WP5, WP18 and B2 isolated in this study and related species. The phylogenetic tree of 16S rRNA sequences was generated by the neighbor-joining method. The tree was tested for support by performing bootstrap resampling (1000 replicates). Bootstrap values are given at branch points, and the accession numbers of each sequence employed are in parentheses. Bars indicate a relative distance of 0.01 in the 16S rRNA-based tree.

All bacteria isolated and screened as PGPR were able to grow under the medium with 750 mM of NaCl. In the sequencing analysis, seven out of eight strains belonged to genus *Arthrobacter*. Strains of *Arthrobacter* species are among the most frequently isolated indigenous aerobic bacterial genera found in soils. Moreover, there are some reports that indicate *Arthrobacter* spp. has high levels of resistance to not only a variety of toxic metals (Hanbo et al., 2004; Megharaj et al., 2003; Henn et al., 2009) but also NaCl (Upadhyay et al., 2009, 2012). In this study, thus, we suggest that the high isolation of *Arthrobacter* spp. was caused by the high levels of Na stress. In the process of screening, there were many more resistant bacteria in the rhizosphere soil of *S, grandis* than in that of wheat as the frequency was 42.6%, 77.4%, 18% in Aksaray (rhizosphere soil of *S. grandis*), Nallıhan (rhizosphere soil of *S. grandis*), Aksaray (rhizosphere soil of wheat), respectively. The salt concentration might become higher in both soils surrounding of *S. grandis* because of the Na uptake from soil and the non-Na-resistant bacteria might be eliminated from the rhizosphere soil. Therefore, Na-resistant bacteria may be more easily isolated from *S. grandis* than from wheat.

IAA production is an important trait of PGPR (Goswamia et al., 2014), as this phytohormone enables plants to develop their root systems. There are only few reports about IAA production by *Arthrobacter* species (Forni et al., 1992; Siddikee et al., 2010). Our results are in line with these previous findings and indicated that *Arthrobacter* spp. had various types of species with production of IAA even in the genus *Arthrobacter*. Therefore, *Arthrobacter* species in the present study can be considered as potential PGPR. Isolated strains A22, WP5, B14, WP18 and AP20 produced IAA in the presence of L-Tryptophan. Moreover, strains A22, WP5, B14 and AP20 also produced siderophore. The production of siderophores is important and possible to bind with the available form of iron Fe^{3+} in the rhizosphere (Ahmad et al., 2008). This suggests that these strains enhance the growth of *S. grandis* as a result of their enhanced IAA and siderophore production.

We previously expected that Na-resistant PGPR might increase Na uptake of *S. grandis* from soil which may facilitate phytoremediation of saline soils. In this study, however, inoculation with Na-resistant PGPR (AP20, B14, WN4 and A22) did not change Na uptake from soil significantly but they showed higher Na uptake compared to un-inoculated control within 35 days laboratory experiment (Figure 2 and Table 3). This may be attributed to short experimental growth period in the present study. Therefore, experimental studies with longer period should be conducted for a better understanding of the association between Na-resistant PGPR and *S. grandis*.

In conclusion, eight strains appear to be suitable candidates for PGPR of *S. grandis*. These strains belong to genus of *Arthrobacter* and *Bacillus* and this is the first report on the Na-resistant PGPR of *S. grandis*. These PGPR candidates on *S. grandis* have possibility of achievement to remove salt from saline soils using microbe assisted phytoremediation.

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Biochar amendment improves soil fertility and productivity of mulberry plant

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Abstract

Biochar has the potential to improve soil fertility and crop productivity. A field experiment was carried out at the experimental field of Bangladesh Sericulture Research and Training Institute (BSRTI), Rajshahi, Bangladesh. The objective of this study was to examine the effect of biochar on soil properties, growth, yield and foliar disease incidence of mulberry plant. The study consisted of 6 treatments: control, basal dose of NPK, rice husk biochar, mineral enriched biochar, basal dose + rice husk biochar and basal dose + mineral enriched biochar. Growth parameters such as node/meter, total branch number/plant, total leaf yield/hectare/year were significantly increased in basal dose + mineral enriched biochar treated plot in second year compared with the other fertilizer treatments. In second year, the total leaf yield/hectare/year were also 142.1% and 115.9% higher in combined application of basal dose + mineral enriched biochar and basal dose + rice husk biochar, respectively, than the control treatment. The soil properties such as organic matter, phosphorus, sulphur and zinc percentage were significantly increased with both the (mineral enriched and rice husk) biochar treated soil applied with or without recommended basal dose of NPK than the control and only the recommended basal dose of NPK, respectively. Further, the lowest incidences of tukra (6.4%), powdery mildew (10.4%) and leaf spot (7.6%) disease were observed in second year under mineral enriched biochar treated plot than the others. The findings revealed that utilization of biochar has positive effect on the improvement of soil fertility and productivity as well as disease suppression of mulberry plant.

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Introduction

Biochar is a source of organic fertilizer that is receiving attention by researchers all over the world (Lehmann et al., 2003). The process of biochar production is known as pyrolysis and it results in a very stable carbon (C)-rich material not only capable of improving physical and chemical soil properties but also increasing soil C storage on a large scale (Sohi et al., 2010; Kookana et al., 2011). Among soil organic amendments, biochar is considered as a more stable nutrient source than others (Chan et al., 2007). Organic C content in biochar has been reported up to 90% depending upon its feedstock, which enhances C sequestration in soil (Lehmann and Joseph, 2009).

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Different types of biochar can be used for improving soil properties and increasing agricultural production. This includes rice-husk and mineral enriched biochar. Application of biochar to soil help improve yield and its components of different crops. A study conducted by Agboola and Moses (2015) showed that the growth and yield of soybean [*Glycine max* (L.) Merr.] was increased due to addition of rice husk biochar. Likewise, soil properties such as soil pH, organic C, soil nitrogen (N), calcium (Ca), magnesium (Mg), potassium (K) and sodium (Na) changes due to the application of rice husk biochar in soil. Similarly, Gebremedhin et al. (2015) reported that biochar significantly increased grain and straw yields of wheat (*Triticum aestivum* L.) by 15.7 and 16.5%, respectively, over the NPK application.

Biochar amendment may not only improve soil properties but also suppress disease infestation on various crops. Mercado-Blanco and Bakker (2007) found that biochar addition to the potting medium of strawberry (*Fragaria* sp) plants suppressed foliar diseases caused by fungi having *Pseudomonas* in several crops. Several studies observed that biochar amended soil help proliferate plant growth promoting rhizobacteria/fungi (PGPR/F) which are known to promote plant health in addition to plant growth either by directly controlling plant pathogens or through the potentiating of plant systemic resistance responses against diseases. Elad et al. (2011) demonstrated that addition of biochar to soil increased beneficial microorganism populations that promote plant growth and improve resistance against biotic stresses. However, the impact of biochar on mulberry (*Morus alba*) plant production with common disease incidence and changes in soil physical and chemical properties is not known yet in Bangladesh. Therefore, the focus of this investigation was to quantify the response of rice husk and mineral enriched biochar on changes in soil properties, yield components and diseases incidence of mulberry plant. We hypothesize that mulberry yield will be the highest and incidence of common mulberry disease will be the lowest in mineral-enriched biochar treatment compared with other treatments. Likewise, soil physical and chemical properties will be changed due to long-term application of biochar in soil.

Material and Methods

Experimental location

The experiment was conducted at the experimental field of Bangladesh Sericulture Research and Training Institute (BSRTI), Rajshahi, Bangladesh (24° 22’ 29” North and 88° 37’ 3.84” East). On the basis of Agro-Ecological Zone (AEZ), BSRTI, Rajshahi falls under the Active Ganges Floodplain-10 and High Ganges River Floodplain-11.

Soil condition

The soil of the experimental plot was mainly loamy in nature, having alkaline characteristics with pH ranging from 8.2 to 8.6 in water. As a consequence of this alkalinity, the soil was poor in K and available P. Both C and N levels were low in uncultivated as well as in the cultivated plots. N level was not in balance with C. This was more prominent in the farm area where mulberry was cultivated for years. Toxic metals were present in traces, but they were well below the harmful levels. The average two years pre-treated basic physicochemical properties of the experimental soil are presented in Table 1.

Table 1. Average two years data of the experimental soil before applied the treatments

Soil pH in H_2O	Nitrogen (%)	Phosphorus ($mgkg^{-1}$)	Potassium ($cmol\ kg^{-1}$)	Sulphur ($mgkg^{-1}$)	Zinc ($mgkg^{-1}$)	Organic matter (%)
8.4	0.1	13.9	0.1	12.6	0.9	1.1

Sample plant material

Mulberry plant was used as a sample planting material for this experiment and it is perennial, deep rooted and hardy in nature. Due to these characteristics, mulberry is grown in a wide range of soil and agro-climatic conditions. The mulberry variety BM-9 and square high bush (plant to plant and row to row distance is 3ft and plant height is 1.5 ft) plantation system was used for this study.

Taxonomy of mulberry plant

Mulberry belongs to the genus *Morus*. Mulberry is highly heterozygous and out breed in nature Vijayan et al. (2012). Different workers from different corners of the world classified the genus *Morus* L. in different ways. Linnaeus (1753) recognized seven species under the genus *Morus* L. presently of which only five, namely *Morus alba*, *Morus nigra*, *Morus rubra*, *Morus. india* and *Morus tartarica* fit in to the circumscription of the genus. Based on female inflorescence and fruit character, French Botanist Bureau (1873) recognized five species, 19 varieties and 11 sub-varieties of the genus *Morus*.

Experimental condition

In this experiment, three-years old and high-bush plantation system mulberry garden was used. In Bangladesh, generally four commercial silkworm rearing seasons is followed in a year. On the basis of silkworm rearing seasons mulberry garden was pruned four times in a year each after three months interval. In this experiment, the pruning was done for two years from January 2014 to December 2015.

Experimental design

The experiment was arranged in a randomized complete block design with three replications, where the plot size was 8 m long × 3 m wide. Each plot had 20 plants and fertilizer management was done for each replicated plot. The same experiment was repeated for two years.

Treatments

There were six treatments for this experiments which are as follows:

(i) Control
No fertilizer was applied.

(ii) Recommended basal dose
The recommended basal dose of BSRTI is 305 kg N, 105 kg K and 66 kg P/hectare/year with four split doses each after three months interval.

(iii) Biochar (Rice husk)
Only the rice husk biochar was applied at a time @ 1 t/hectare/year. In this case, the 2nd dose was applied after one year interval.

(iv) Biochar (Mineral enriched)
Only the mineral enriched biochar was applied at a time @ 1 ton/hectare/year. In this case, the 2nd dose was applied after one year interval.

(v) Recommended basal dose + Biochar (rice husk)
BSRTI recommended basal dose at four split doses each after three months interval in a year and 1 t rice husk biochar/hectare/year at a time was applied for mulberry cultivation.

(vi) Recommended basal dose + Biochar (mineral enriched)
BSRTI recommended basal dose at four split doses each after three months interval in a year and 1 t mineral enriched biochar/hectare/year at a time was applied for mulberry cultivation.

Chemical properties of biochar

Biochar is the C-rich solid product resulting from the heating of biomass in an oxygen-limited environment. Due to its highly aromatic structure, biochar is chemically and biologically more stable compared with the organic matter from which it is made. Generally, the properties of biochar vary widely, depending on the source of biomass used and the conditions of production of biochar (Lehman and Joseph, 2009). In our experiment, two types of biochar such as rice husk and mineral enriched biochar were used, which were collected from China. The chemical properties of the applied biochar are provided in Table 2.

Table 2. Properties of rice husk and mineral enriched biochar used in the experiment

Elements	Rice husk biochar	Mineral enriched biochar
pH	8.0	8.9
Total N (%)	1.7	2.1
Total P (%)	0.2	0.5
Total K (%)	0.2	1.1
OC (g/kg)	54.0	67.0
Total Na (%)	0.2	0.6
S (mg/kg)	0.2	0.4
Fe (mg/L)	7.8	8.7
Ca (mg/L)	213.0	273.0
Cu (mg/L)	0.1	0.1
Al (mg/L)	0.9	1.0
Mn (mg/L)	4.4	5.1
Ash (%)	50.3	39.7

Experimental procedure

The treatments were randomly assigned in the experimental plot for each replication. In each year and every case according to the treatment, all the fertilizers such as basal, rice husk biochar, mineral enriched biochar, basal + rice husk biochar and basal + mineral enriched biochar were applied 15 days after pruning (DAP) on the mulberry plant when sprouting was started. Both the biochar (rice husk and mineral enriched) were applied through surface application method in the root zone area of plant and was then incorporated into soil. In flooding system three times irrigation per crop season @ 1.5 to 2.0 acre inch water per irrigation at an interval of 15-30 days was done and other intercultural practices such as digging cum weeding and insect-pest control were done as per requirement for each cropping season.

Data collection

According to the treatments data were collected for the growth parameters, percentage of disease incidence and soil properties for each two years.

Growth parameters

For determining the growth impact data were collected for the following parameters:

(i) Node/meter/plant

The number of node/meter was determined by counting the number of node/meter manually for each of the plant each after 90 days of pruning.

(ii) Total branch number/plant

The number of branch/plant was determined by counting the number of branch manually for each of the plant each after 90 days of pruning.

(iii) Total branch height/plant (cm)

All the branch height for a plant was measured by the measuring tape and adding each after 90 days of pruning

(iv) Total leaf weight/plant (g)

All the green leaves of a plant except shoot were weighted using a weighting balance each after 90 days of pruning.

(v) Total leaf yield (t/hectare /year)

At maturity of leaf (after 80 days of pruning) the total green leaf yield/ha/year was determined by the following formula:

$$\text{Leaf yield (t / hectare / year)} = \frac{\text{Leaf weight (g) of m}^2 \text{ plant x Number of crop season / year x 10000 m}^2}{1000 \times 1000}$$

Disease incidence

The occurrence of disease incidence/plant was recorded for three consecutive years after 60–65 days of pruning by randomly selecting 10 plants in each replication. Foliar diseases such as powdery mildew (*Phyllactinia corylea*) and leaf spot (*Pseudocercospora mori*) and tukra (*Meconellicoccus hirsutus*) were recorded during this period. Studies on percent disease incidence (PDI) with respect to powdery mildew, leaf spot and tukra diseases were recorded and the data during both the years were pooled and analyzed. The percent disease incidence (PDI) was calculated following the formula suggested by Rai and Mamatha (2005).

$$\text{Percent Disease Incidence (PDI)} = \frac{\text{Number of diseased leaves on each plant}}{\text{Number of total leaves on each plant}} \times 100$$

Soil analysis

Soil was collected from approximately the top 55 cm depth of the experimental plots. According to the treatments for each cropping year, the collected soil sample was homogenized, air-dried for 72 hours, ground and sieved to pass a 2 mm sieve. The initial soil pH was determined before incubation experiment in deionised water using a soil-to-solution ratio of 1:2.5. Soil organic matter content was determined by (Walkley et al., 1934) multiplying the percent value of organic C with the conventional Van-Bemmelen factor of 1.724 (Piper, 1950). The N content of the soil sample was determined by distilling soil with alkaline $KMnO_4$ solution (Subhaiah and Asija, 1956) and the distillate was collected in 20 ml of 2% boric acid solution with methylred and bromocresol green indicator and titrated with 0.02 N H_2SO_4 (Podder et al., 2012). Soil available S (mg kg^{-1}) was determined by calcium phosphate extraction method with a spectrophotometer at

535 nm (Petersen, 1996). The soil available K was extracted with 1N NH_4OAC and determined by an atomic absorption spectrometer (Biswas et al., 2012). The available P of the soil was determined by spectrophotometer at a wavelength of 890 nm. The soil sample was extracted by Olsen method with 0.5 M $NaHCO_3$ as outlined by Huq and Alam (2005) and the Zn in the soil sample was measured by an atomic absorption spectrophotometer after extracting with DTPA (Soltanpour and Workman, 1979).

Statistical analysis

Statistical analysis was conducted using Genstat 12.1 for Windows (Lawes Agricultural Trust, UK) and Statistix 10 software. Treatment means were separated by the least significant difference (LSD) at the 0.05% level of significance (Steel and Torrie, 1984). Mulberry plant growth and composition data were analyzed by a one-way analysis of variance (ANOVA) for the main effect of mulberry plant growth and Sigma Plot 12.5 version was used for representing the growth results in a figure form. Disease parameters were statistically analyzed and mean values were evaluated by DMRT test.

Results

Effect of fertilizer application on bulk soil properties

The effect of treatment application on the soil properties after harvesting of mulberry leaf are presented in Table 4. The addition of different fertilizer as a soil amendment significantly ($P < 0.001$) increased organic matter, P, S and Zn contents in soil. In contrast, only organic matter ($P < 0.001$) and S ($P < 0.05$) contents were significantly increased for the cropping season and the effect of season × treatment was also significant for S ($P < 0.01$) and Zn ($P < 0.001$) contents in soil (Table 3). The highest level of organic matter (2.0%) was found in the second year due to the application of mineral enriched biochar @ 1 t/hectare/year, the highest level of P (30.7%) was obtained in the second year for rice husk biochar @ 1 t/hectare/year, the highest S content 9.3% was found in second year for mineral enrichedbiochar @ 1 t/ hectare//year + recommended basal fertilizer dose and the highest Zn content was recorded 1.8% in the second year for rice husk biochar @ 1 t/hectare/year (Table 4). The soil properties such as pH, N and K level were not influenced by the cropping season, treatment and season × treatment effect (Table 3).

Table 3. Level of significance for the main and interaction effect on season and treatment for soil properties

Sources of variation	Soil pH	Organic matter (%)	N (%)	P (%)	K (%)	S (%)	Zn (%)
Season	ns	***	ns	ns	ns	*	ns
Treatment	ns	***	ns	***	ns	***	***
Season × treatment	ns	ns	ns	ns	ns	**	***

Table 4. Effect of different fertilizers on soil properties by year after leaf harvest in mulberry plant

Treatments	pH		Organic matter (%)		N (%)		P (%)		K (%)		S (%)		Zn (%)	
	1st year	2nd year	1st year	2nd year	1st year	2nd year	1st year	2nd year	1st year	2nd year	1st year	2nd year	1st year	2nd year
Control	8.2	8.3	1.1	1.1	0.1	0.1	18	17.7	0.2	0.2	8.9	8.4	1.1	1.0
Basal fertilizer	8.2	8.1	1.2	1.2	0.1	0.1	28.5	28.2	0.2	0.2	9.0	9.2	1.4	1.4
Rice husk biochar	8.3	8.3	1.1	1.1	0.1	0.1	30.5	30.7	0.2	0.2	8.6	8.9	1.8	1.8
Mineral enriched biochar	8.2	8.4	1.9	2.0	0.03	0.05	27.2	27.3	0.2	0.2	7.3	7.5	0.8	0.8
Basal fertilizer + rice husk biochar	8.2	8.4	0.7	0.8	0.03	0.04	21.3	21.6	0.2	0.2	7.9	8.1	0.7	0.7
Basal fertilizer + mineral enriched biochar	8.3	8.4	0.8	0.8	0.03	0.05	23.2	23.4	0.2	0.2	9.1	9.3	0.5	0.5

Effect of fertilizer application on growth and yield

Node/meter

The two-way interaction between season and fertilizer treatment was significant ($P < 0.05$) for node/meter of mulberry plant (Table 5). Among the two cropping seasons, the highest number of node/meter (24.5) was found in the second year for the fertilizer treatment of mineral enriched biochar @ 1 t/hectare/year with the recommended basal dose of BSRTI (Table 6). In contrast, the control had the lowest number of node/meter (18.5) in the second year compared with other treatments (Figure 1).

Table 5. Significance levels from for the main and interactive effect on season and treatments for growth and yield contributing characteristics of mulberry plant

Source of variation	Node Per meter	Total branch number per plant	Total branch height per plant (cm)	Total leaf weight per plant (gm)	Total leaf yield/ ha/yr (mt.)
Season	***	***	***	***	***
Treatments	***	***	***	***	***
Season ×Treatments	*	n.s.	***	***	***

Table 6. Average two years values of growth and yield contributing characteristics for fertilizer treatments

Treatments	Node per meter		Total branch number per plant		Total branch height per plant (cm)		Total leaf weight per plant (gm)		Total leaf yield/ ha/yr (mt)	
	1st year	2nd year	1st year	2nd year	1st year	2nd year	1st year	2nd year	1st year	2nd year
Control	20.0	18.5	8.7	8.3	686.4	697.9	336.6	320.5	21.5	20.5
Basal	20.3	22.5	10.3	10.7	788.0	785.0	411.9	422.5	26.4	27.0
Biochar (Rice husk)	20.2	22.7	11.3	12.7	898.0	916.3	433.6	550.6	27.7	35.0
Biochar (Mineral enriched)	21.0	23.2	12.3	14.0	935.7	1229.0	446.3	648.6	28.6	41.5
Basal + Biochar (Rice husk)	21.2	23.5	13.0	16.3	1006.3	1230.0	447.2	692.1	28.6	44.3
Basal + Biochar (Mineral enriched)	23.5	24.5	15.8	17.2	1121.3	1361.3	486.2	775.8	31.1	49.6

Total branch number per plant

Our results showed that the effect of cropping year and fertilizer treatments were significantly ($P < 0.001$) increased the total branch number per plant (Table 5). The average highest total branch number per plant (17.2) was found in 2nd year for the biochar (Mineral enriched) @ 1 t/hectare/year with recommended basal dose of BSRTI treated plot as compared to others fertilizer treatments. (Table 5, Table 6, Figure 2).

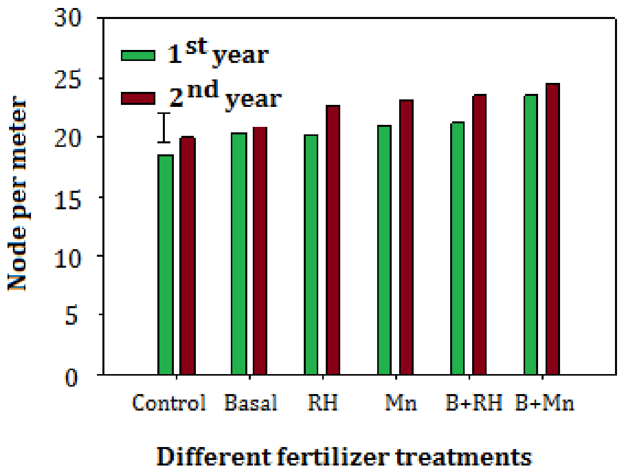

Figure 1. Effect of various fertilizer treatments on node per meter of mulberry plant. Vertical bar represent LSD ($P \geq 0.05$) for season and treatment interaction. RH indicates rice husk biochar, Min indicates mineral enriched biochar and B means BSRTI recommended basal fertilizer

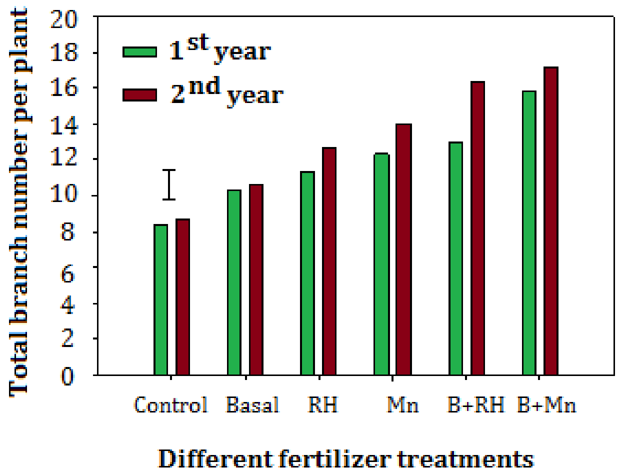

Figure 2. Effect of various fertilizer treatments on total branch number per plant. Vertical bar represent LSD ($P \geq 0.05$) for season and treatment interaction. RH indicates rice husk biochar, Min indicates mineral enriched biochar and B means BSRTI recommended basal fertilizer

Total branch height per plant (cm)

Interestingly, the effect of cropping season, treatments application and interactive effect of different fertilizer treatments and cropping seasons on the total branch height per plant were significantly ($P < 0.001$) changed with various amendments (Table 5). However, the highest average total branch height per plant was obtained 1361.3 cm in 2nd year due to application of biochar (Mineral enriched) @ 1 t/hectare/year with recommended basal dose of BSRTI followed by the rest of the treatments (Table 5, Table 6, Figure 3).

Total leaf weight per plant (gm)

Interestingly, the total leaf weight per plant was significantly (P < 0.001) increased by the seasons, treatments and season × treatments (Table 5). The maximum average total leaf weight per plant was recorded 775.8 gm in 2nd year for the treatment of biochar (Mineral enriched) @ 1 t/ hectare/year with recommended basal dose (@ 660 kg urea, 330 kg TSP and 210 kg MP per /hectare/year) of BSRTI as compared to other treatments. (Table 5, Table 6, Figure 4).

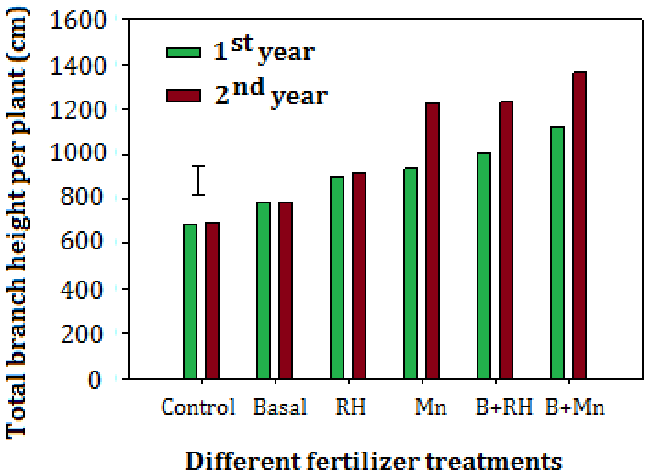

Figure 3. Effect of various fertilizer treatments on total branch height per plant. Vertical bar represent LSD ($P \geq$ 0.05) for season and treatment interaction. RH indicates rice husk biochar, Min indicates mineral enriched biochar and B means BSRTI recommended basal fertilizer

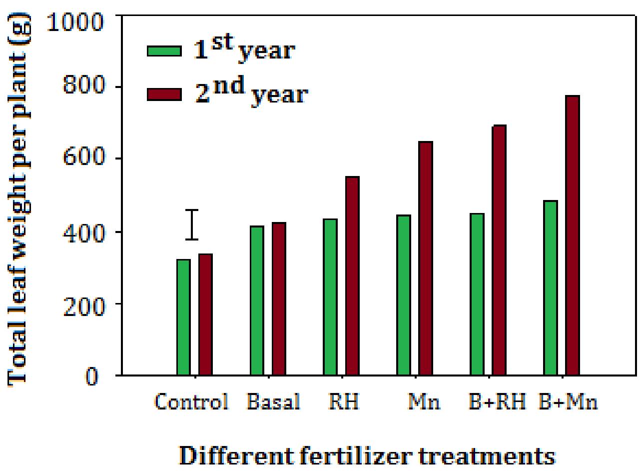

Figure 4. Effect of various fertilizer treatments on total leaf weight per plant. Vertical bar represent LSD ($P \geq$ 0.05) for season and treatment interaction. RH indicates rice husk biochar, Min indicates mineral enriched biochar and B means BSRTI recommended basal fertilizer

Total leaf yield/ha/yr (mt.)

According to the results of the present study among the two cropping years the total leaf yield per hectare per year was significantly (P<0.001) influenced by the cropping seasons, treatments and season × treatments (Table 3). However, application of biochar (Mineral enriched) @ 1 t/hectare//yr with recommended basal dose (@ 660 kg urea, 330 kg TSP and 210 kg MP per hectare/year) of BSRTI gave the maximum average total leaf yield per hectare per year 49.6 mt. in 2nd year followed by rest of the treatments. On the contrary, the lowest average total leaf yield per hectare per year was 20.5 mt. in 2nd year for control treatment (Table 5, Table 6, Figure 5).

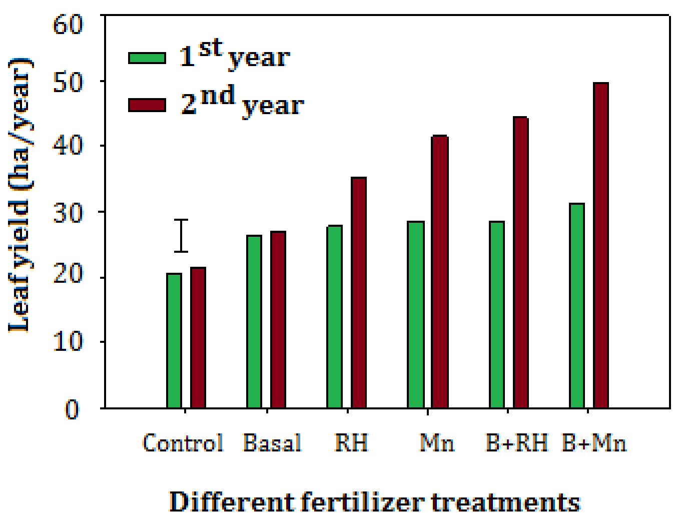

Figure 5. Effect of various fertilizer treatments on leaf yield of mulberry plant. Vertical bar represent LSD ($P \geq$ 0.05) for season and treatment interaction. RH indicates rice husk biochar, Min indicates mineral enriched biochar and B means BSRTI recommended basal fertilizer

Incidence of common mulberry diseases under organic fertilizers management

Common mulberry diseases regulated by organic fertilizer management. In general, mineral rich biochar has fewer incidences of common diseases as compared to other fertilizer management practices. The incidence percentage of Tukra disease was highest in control treatment for the first year. However, lowest incidence was found both in mineral enriched biochar as well as combination of recommended basal dose plus mineral enriched biochar. Moreover, incidence of Tukra disease in first year did not differ significantly (P > 0.05) by the basal, rice husk biochar and recommended basal dose plus rice husk enriched biochar fertilizer treatments (Table 7). Similarly, in second year the highest occurrence of Tukra disease (17.1%) also found for the control treatment and lowest incidence was observed for the mineral enriched biochar as well as combination of recommended basal dose plus mineral enriched biochar. However, in second year the Tukra disease incidence was not also differ significantly (P > 0.05) by the basal, rice husk biochar and basal dose plus rice husk biochar fertilizer treatments (Table 7).

Table 7. Average two years incidence percentage of common diseases under different fertilizer treatments

Treatments	Mean diseases incidence percentage					
	Tukra		Powdery mildew		Leaf spot	
	1st year	2nd year	1st year	2nd year	1st year	2nd year
Control	12.1^{a}	17.1^{a}	17.1^{a}	20.1^{a}	13.4^{a}	15.6^{a}
Basal dose	9.8^{b}	10.2^{b}	13.8^{b}	11.0^{b}	11.5^{bc}	12.5^{b}
Biochar (Rice Husk)	9.3^{b}	9.4^{b}	12.5^{bc}	10.2^{b}	11.8^{ab}	10.8^{c}
Biochar (Mineral enriched)	6.4^{c}	7.1^{c}	10.4^{c}	7.9^{c}	7.6^{d}	7.9^{d}
Basal + biochar (Rice Husk)	9.5^{b}	10.5^{b}	11.3^{c}	10.0^{b}	10.0^{c}	10.2^{c}
Basal + biochar (Mineral enriched)	6.1^{c}	7.2^{c}	10.8^{c}	8.3^{c}	10.3^{bc}	9.1^{c}

In case of Powdery mildew disease in first year the maximum incidence percentage was found (17.1%) in control treatment. In contrast, the minimum occurrence of powdery mildew disease was recorded both for the mineral enriched biochar as well as combination of recommended basal dose plus mineral enriced biochar treatments. Similarly, in first year the basal, rice husk biochar and recommended basal dose plus rice husk biochar treatments also did not significantly (P > 0.05) differ (Table 7). Likewise, in second year the highest occurrence of powdery mildew disease was also observed for the control treatment but the lowest incidence was recorded for mineral enriched biochar as well as combination of recommended basal dose plus mineral enriched biochar. The rest of the treatments like basal, rice husk biochar and basal dose plus rice husk biochar were not significantly (P > 0.05) differ for the powdery mildew occurrence percentage (Table 7).

The highest incidence of leaf spot disease was found in control as well as rice husk biochar in first year. In contrast, the lowest occurrence of leaf spot disease was observed only for the mineral enriched biochar but the other treatments like basal, basal plus rice husk biochar and basal plus mineral rice biochar were not significantly (P > 0.05) differ among them (Table 7). In second year regarding the incidence percentage the maximum occurrence of leaf spot disease was also recorded for control treatment and minimum occurrence was found for the mineral enriched biochar treatment. However, the treatments basal, basal plus rice husk enriched biochar and basal plus mineral enriched biochar were not significantly (P > 0.05) differ for the incidence percentage of leaf spot disease (Table 7).

Discussion

Changes of soil nutrient concentration response to different fertilizers

Soil nutrient availability increased due to biochar amendment within soil. Biochar application improved soil nutrient availability by rising of soil pH, organic matter, phosphorus, sulphur and zinc contents percentage in within soil (Table 4). Though the soil pH was not significantly increased but the average soil pH was increased due to the soil treated with only the biochar (mineral enriched and rice husk). Our results indicate that most of soil chemical properties increased due to biochar amendment within soil except nitrogen and potassium. Similarly, a study conducted by Oguntunde et al. (2004) found that the soil treated with biochar significantly increased soil pH, electrical conductivity (EC), exchangeable Ca, Mg, K, Na and P in compared to the control soil. They also found that the relative K change observed up to 329% while organic C and total N decreased by 9.8% and 12.8%, respectively. They recommended that organic C and total N were highly correlated ($P < 0.01$) and both the parameters significantly ($P < 0.05$) depended on clay contents in soils. In addition due to the dominating effect of biochar residues while it reduction in soil organic C and total N contents depend on the effects at the severe fire during pyrolysis the soil pH, available P, electrical conductivity, base saturation, exchangeable K, Ca, Mg and Na were significantly higher in biochar treated soil than the control soil. Likewise, Dume et al. (2016) found that biochar increased the soil pH, electric conductivity (EC), cation exchange capacity (CEC), organic carbon (OC), organic matter (OM), total nitrogen (TN), exchangeable cations and available phosphorus of the soil. They speculated that in biochar had a displacement of exchangeable acidity and high buffering capacity consequentially soil pH increased, high organic matter contents and enhanced decomposition rate when added biochar in soil in terms enriched the soil organic matter and also biochar attributed the improvement of soil pH which ultimately reduce the activity of Fe and Al resulting the phosphorus availability increased in soil due to the Biochar treated soil.

Our speculation is that biochar directly contents high amount of soluble nutrients (macro and micro), ash containing organic matter and some exchangeable elements. Besides, biochar itself has a cat ion exchange and high buffering capacity with high decomposition rate of organic matter. As a results the soil treated with the biochar both as a single dose or combined application with recommended basal dose of BSRTI absorbed maximum nutrients as per requirements, organic matter decomposition rate increased resulting improved the soil status and significantly increased the soil properties than the other treatments.

Biochar amendments enhances mulberry plant growth and yield

Biochar amendments along with BSRTI recommended basal dose improved the growth and yield of mulberry plant. Our findings showed that the total leaf yield per hectare per year were respectively increased 142.1 % and 115.9 % in addition of 1 ton mineral enriched biochar and 1 ton rice husk biochar in soil respectively with BSRTI recommended basal dose of NPK than the control treatment. However, there was no study available in literature about combined application of biochar and inorganic fertilizer for growth and yield of mulberry plant. Some studies available in literature for combined application of inorganic fertilizer and biochar. Igarashi (2002) reported that rice husk biochar application increased the yield of maize, soybean and peanut in Indonesia. Likewise, Lehmann et al. (2006) found that addition of high rates of biochar in the tropical environment have been associated with increase plant uptake of P, K, Ca, Zn and Cu. This could be due to the fact that increased nutrient uptake from combined biochar and inorganic fertilizer can result mulberry plant growth and yield. Likewise, biochar (mineral enriched and rice husk) with combination of recommended basal dose of BSRTI may more capable to improve the growth and development of mulberry plant. It could be due to the reason that biochar (mineral enriched and rice husk) contents most of the plant nutrients (macro and micro). Regardless of that our biochar amendment increased some of the soil properties like organic matter, sulphur, zinc and phosphorus content in soil which may be improve the soil texture, water holding capacity, soil aggregation, soil moisture retention. This causes improved nutrient uptake status by mulberry plants. As a result, mulberry plant growth and yield increased due to biochar amendment.

Effect of time on biochar decomposition

Biochar addition along with BSRTI recommended basal dose increased the mulberry leaf yield in the second year comparatively to the first year. Our findings showed that biochar treated plot along with BSRTI recommended basal dose produced highest leaf yield 49.6 metric ton/hectare/year in 2nd year which was comparatively high than the 1st year leaf yield 31.1 metric ton/hectare//year. Likewise, Major et al. (2010) found that a single application of 20 t ha^{-1} biochar to a Colombian savanna soil resulted in an increase in maize yield by 28 to 140% as compared with the unamended control in the 2nd to 4th years after application. They stated that decomposition rate of biochar can increased in 4th year as compared to 2nd year that results increase in maize production. However, our speculation is that combined application of biochar with BSRTI, recommended basal dose provides a suitable habitat for a large and diverse group of soil microorganisms in respect of point in time. This could be due to the reason that microbial decomposition of biochar and nutrients availability comparatively high in second year than the first year. As a result the growth and development of mulberry plant was comparatively advance in second year than the first year in biochar treated plot with BSRTI recommended basal dose of NPK fertilizers than the others.

Application of biochar in soil reduces common diseases in mulberry plant

Biochar application to soil diminishes the incidence of common diseases in mulberry plant. Our findings showed that the use of mineral enriched biochar within soil reduced about 50% the occurrence of common mulberry diseases (Table 7). This reduction of diseases happen could be due to the availability of all essential macro and micro nutrients within mineral rich biochar. Our speculation can be verified by Huber and Grahan (1999). They stated that essential nutrients are vital for plant growth, development, soil microbial activities and most important factors for diseases control. They also mentioned that all the essential nutrients can affect disease severity of plant.

Further, common diseases like Tukra, Powdery mildew and Leaf spot were occurred simultaneously both two years in our mulberry plant. Tukra, powdery mildew and leaf spot occurrence was significantly high in control where no inputs were applied. In first year significantly the lowest average Tukra, Powdery mildew and Leaf spot disease incidence percentage were 6.1, 10.4 and 7.6 respectively under mineral enriched biochar and in second year the lowest average incidence percentage were 7.1, 7.9 and 7.9 respectively. Mineral enriched biochar may be due to increased levels of soil microbial activity leading to increased competition and antagonism in the rhizoshere may be contributing factors for reduction of Tukra, Powdery mildew and Leaf spot disease incidence.

Very few studies have addressed for the potential of Biochar soil amendment to impact plant resistance to disease pathogen. However, similarly Elad et al. (2010) found that the severity of diseases caused by necrotrophic (*Botrytis cinerea*) and biotrophic (*Oidiopsis sicula* (originally referred to according to its teleomorph name: *Leveillula taurica*)) foliar pathogens in pepper and tomato was significantly reduced in biochar-amended treatments. Likewise, Harel et al. (2012a; 2012b) found that Biochar addition to the potting medium of strawberry plants suppressed foliar diseases caused by fungi having very different infection strategies: necrotrophic (grey mould, *Botrytis cinerea*), semi-biotrophic (anthracnose, *Colletotrichum acutatum*), and biotrophic (powdery mildew, *Podosphaera apahanis*). Our speculation, Biochar influence microbial populations which increase in beneficial microorganisms that directly protect against pathogens by producing antibiotics, by out-competing the pathogens, or by grazing on the pathogens. In addition, chemical compounds in the residual tars that are added to the soil with the biochar may have direct toxic effects on soil pathogens which is more or less closely related with the findings of Graber et al. (2010). They identified a number of biochar compounds that are known to adversely affect microbial growth and survival. These include ethylene glycol and propylene glycol, hydroxy-propionic and butyric acids, benzoic acid and o-cresol, quinones (recorsinol and hydroquinone), and 2-phenoxyethanol. Low levels of these toxic compounds could suppress sensitive components of the soil microbiota thereby resulting in proliferation of resistant microbial communities. Graber et al. (2010) also isolated a number of bacteria with high 16S rRNA gene sequence identity to known biocontrol agents, induced resistance agents and growth promoters (15 out of 20 total isolates) from the root zone of biochar-amended pepper plants where promotion of plant growth and induction of systemic resistance against fungal foliar diseases occurred simultaneously (Elad et al., 2010; Graber et al., 2010). Besides, Bernardet and Bowman (2006) also reported that biochar strongly induced the bacteroidetes-affiliated genus flavobacterium, which is widely distributed in nature; commonly possess an arsenal of extracellular enzymes such as proteinases and chitinases which enable them to degrade bacteria, fungi, insects, and nematode constituents. Our another rumor that the applied biochar improved soil nutrient and produces direct antibiotic against micro organisms, which reduced the common diseases incidence. Similarly, Graber et al., (2010) reported biochar improved plant nutrition and Microorganisms which excel at degrading toxic organic contaminants generally are more resistant to a variety of toxic organic compounds. Also, antibiotic and volatile organic compound producers are often resistant to a multitude of antibiotics (Nodwell, 2007; Laskaris et al., 2010). Antibiotic producers (*Pseudomonas mendocina* and *P. aeruginosa* strains) were identified in biochar-amended soil (Graber et al., 2010).

Conclusion

This study demonstrated that the application of biochar in soil either with or without BSRTI recommended basal dose of N P K fertilizers can improve soil fertility and productivity, decrease the disease incidence and increase the leaf productivity in mulberry plant. The mulberry leaf production, soil properties and disease incidence rate was significantly differed from first year to second year due to addition of Biochar in soil either with or without BSRTI recommended basal dose. However, the highest leaf yield was found in combined application of Biochar (mineral enriched) with BSRTI recommended basal dose. Comparatively the lowest incidence of powdery mildew, tukra and leaf spot disease were found in second year than first year. Further study will be conducted for wheat plant to quantify the effect of biochar for soil fertility and crop productivity.

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Glyphosate, 1,1'- dimethyl-4,4'-bipyridinium dichloride and Atrazine induces changes in Soil organic carbon, bacterial and fungal communities in a tropical alfisol

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Abstract

The increasing use of agrochemicals for weed control has raised concerns about their ecotoxicological effects on soil micro-biota communities and soil functions which serve as indicators of soil quality. Thus, this study was conducted to evaluate the effects of continuous field applied herbicides glyphosate, paraquat, atrazine and their combined forms over a period of five years on soil organic carbon, bacterial and fungal population in Akure, Ondo State Nigeria. Soil samples from farmer's field which have been exposed to continuous herbicide application were collected and analysed for physio-chemical properties, organic carbon, total bacterial and fungal population. Simultaneously, soil samples designated as control were collected from adjacent fields with no history of herbicide application and analysed. Results showed a significant (P=0.05) 86% and 128% increase in bacterial population from glyphosate and atrazine treated fields respectively and 42% decrease in paraquat and Glyphosate + paraquat fields when compared with the untreated field. A significant 35% decrease in fungal population was observed in fields applied with atrazine and a further 10% decrease in fungal populations in all herbicide treated fields irrespective of herbicide type and combinations when compared with the untreated field. These changes also correlates with the abundance of beneficial microbes such as *Pseudomonas aeruginosa, Pseudomonas fluorescens, Proteus mirabilis, Aspergillus flavius* with a probable influence on plant growth promotion and potentials for biodegradation of persistent herbicides. SOC, SOM and pH was significantly (P=0.05) increased in atrazine and atrazine + paraquat treated fields when compared with the untreated fields and other herbicide treatments.

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Introduction

Herbicides use are of primary concern in recent times due to their increasing application into the soil ecosystem. Intensively cultivated farmlands by farmers in south-western Nigeria are periodically treated with herbicides to combat weed infestation. In recent years, large quantity of pre and post-emergent herbicides have been consistently used to impede the activity of weeds which compete with grown crops for space, water, nutrients and ultimately affect crop yield (Varshney et al., 2012). A proportion of herbicides introduced as pre- or post-emergence weed killer have great residual activity in soil, which are ecologically destructive (Ayansina and Oso, 2006; Riaz et al., 2007; Pandey et al., 2007). Whereas, it is expected that herbicides applied should be of good efficacy and also pose minimum deleterious effects to crop and soil ecosystem (Hoerlein, 1994). Herbicides are extraneous to soil component pools, and these actions causes'

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changes in the catalytic efficiency and behaviour of soil enzymes (Sannino and Gianfreda, 2001), which induces microbial functions in the soil-plant environment. Various studies have stated that herbicides causes qualitative and quantitative change in enzyme activity (Sebiomo et al., 2011, Xia et al., 2012). The action of these herbicides in the soil is becoming increasingly important since they could be leached, in which case groundwater is contaminated and could persist on the top soil (Sebiomo et al., 2011 cited Ayansina et al., 2003). When applied, there are possibilities that these herbicides may exert certain effects on non-target organisms, including soil microorganisms (Simon-Sylvestre and Fournier, 1980). Many of the active ingredients in herbicides such as Glyphosate – Isopropylamine salt, Propanil – 3'3'-dichloropropionanile, 2, 4 – D acid – 2, 4-dichlorophenoxy acetic acid etc are persistent soil pollutants, whose impacts may persist for decades and adversely affect soil properties and soil biota (Shaner and Leonard, 2001). There is a growing concern that herbicides may not only affect target weeds but also the microbial communities present in soils and the performance of important soil functions (Hutsch, 2001). A study conducted by El-Ghamry et al., (2001), documented both positive and deleterious effect of herbicides on soil functions and activities of soil microbes.

Microorganisms degrade different kinds of carbonaceous substances including accumulated herbicides in soil to derive their energy and other nutrients for their cellular metabolism (Das et al., 2012). This reaction could favourably influence soil functions, the transformations of nutrients and soil biota activity. However, the interaction between the herbicides, soil functions and microorganisms could be dependent on the type of herbicides, soil type and microorganisms present (Nongthombam et al., 2008). Hence, the need to study the impact of herbicides on soil microbiota communities and address issues surrounding the environmental impacts of herbicide use. Glyphosate [N-(phosphonomethyl) glycine] is a broad-spectrum, non-selective, post-emergence herbicide that controls most of the annual and perennial weeds through inhibition of aromatic amino acids biosynthesis involved in protein synthesis (Battaglin et al., 2005). It also inhibits 5-enolpyruvylshikimic acid synthase *via* the shikimic acid pathway (Franz et al., 1997), which is ubiquitous in microorganisms (Bentley, 1990) that link primary and secondary metabolism. Glyphosate also acts as a competitive inhibitor of phosphoenolpyruvate, which is one of the precursors to aromatic amino acid synthesis. Soil and climate conditions affect glyphosate persistence in soil as it has been reported that the half-life of glyphosate in soil ranges between 2 and 197 days (NPIC, 2010). However, recent findings has disputed this with research now showing that a typical glyphosate field half-life of around 47 days in soil can increase up to 22 years becoming difficult to biodegrade due to strong complexes with metal ions (Jayasumana et al., 2014). 1,1'- dimethyl-4,4'-bipyridinium dichloride (Paraquat) is stable in acidic or neutral solutions, but is hydrolyzed at pH > 12 (Wauchope et al., 1992). It undergoes photolysis in aqueous solution to form N- methybetaine of isonicotinic acid, and subsequently methylamine hydrochloride (Slade, 1965). Paraquat is highly persistent in the soil environment, with reported field half-lives of greater than 1000 days (Wauchope et al., 1992). The reported half-life for paraquat in one study ranged from 16 months (aerobic laboratory conditions) to 13 years (field study) (Rao and Davidson, 1980), while Atrazine is a pervasive environmental contaminant (Cox, 2001). It is strongly persistent and is one of the most significant water pollutants in rain, surface, marine, and ground water (Wiegand et al., 2001). Its persistence (it has a half-life of 125 days in sandy soils (Wiegand et al., 2001)) and mobility in some types of soils because it is not easily absorbed by soil particles means it often causes contamination of surface and ground waters. The micro-biota community and enzymatic activities are sensitive to agrochemicals and have been regarded as potential indicators for measuring the degree of soil fertility, soil pollution and alterations in microbial communities (Kalia and Gosal, 2011; Bacmaga et al., 2015; Borowik et al., 2016). There is a growing need to preserve and monitor soil quality and the micro-biota community as they are considered as an indicator of soil health and pollution. However, recent studies on herbicides effect on soil microbial communities are short term, pot experiments and screen house based (Bacmaga et al., 2015; Borowik et al., 2016) and empirical findings from these studies may not provide a realistic evaluation of the effects of herbicides on soil microbes in field conditions.

This study was conducted to determine the long term effects of single and dual field applied herbicides (glyphosate, paraquat and atrazine) on soil organic carbon and selected soil microbial population (bacteria and fungi), of a 5 years intensively cultivated farm land. This study is expected to provide better understanding of the possible long term response of these selected microbes to different herbicides under field conditions.

Material and Methods

Study site

The present study was conducted in November, 2015 on a farmer's field located near the poultry section of the Teaching and Research Farm of the Federal University of Technology Akure, Ondo state, Nigeria. The location lies between Latitude 7°20'N and Longitude 5°30'E, a bimodal rainfall pattern, with a long rainy season, usually between March and July and a short rainy season, usually extending from September to early November, after a short dry spell in August and a longer dry period from December to February. The Soils at the experimental site are alfisol classified as clayey skeletal oxic-paleustaif. Alfisols are a soil order in USDA soil taxonomy formed in semiarid to humid areas, typically under a hardwood forest cover, moderately leached soils with a clay-enriched subsoil and relatively high native fertility (USDA, 2003). Annual daily average minimum and maximum temperature are 28.10°c and 32.0°c, while the relative humidity ranges between 75% - 85%.

Experimental layout

The experiment was laid out in plots set-up as completely randomized design, and each plot measured (3 x 5) m^2. The dominant weed species on these plots includes, *Chromolaena odorata, Euphorbia heterophylla, Tridax procumbens, Cyperus esculentus, Cyperus rotundus* etc. Seven different plots; Control (no herbicide applied plot), three singly and three dual applied (Pre and Post emergence) herbicide treatments were identified and mapped out based on the herbicides application information collected from the farmers through an informal unstructured interview method. The herbicides used by the farmers on the plots were Paraquat, Glyphosate and Atrazine. The fields were periodically sown to maize, cassava, yam, vegetables and were reportedly sprayed with these herbicides as pre and post emergent herbicides at manufacturer's recommended rates Paraquat, (200g/l), Glyphosate (360g/l) and Atrazine (500g/l) and half of recommended rates for combined dosage for over five years.

Soil sampling and bioassay

Soil sampling was in accordance with the general methods for soil microbiological study. Moist rhizosphere soil sample were collected from a depth of (0-5) cm from each plot. Each plot was divided into 5 sub-plot, and from each sub-plot, five sub samples were collected randomly. These samples were thoroughly mixed to form a composite sample. The composite samples were homogenized, sieved through 0.2 mm sieve to remove stone and plant debris, and were analyzed. The effect of the different herbicides applied on the soil were analyzed in response to changes in organic carbon, bacteria population, fungal population and identification and soil chemical properties with respect to control treatment in triplicates.

Soil physico-chemical determination

The soil particle size analysis was done using standard hydrometer method described by Gee and Bauder (1986), while the particle fraction was calculated using the formulae and the textural classes described by Okalebo et al. (2002). Soil pH was determined in 1:2.5 (Soil: water) and KCl solution (1:1) using glass electrode pH meter. Total nitrogen in the soil was analyzed using Kjeldahl method (Bremner, 1960). Available phosphorus was extracted using Olsen's extract while the P in the extract was determined by the use of spectrophotometer. Exchangeable cation (K, Ca, Mg) were extracted with 1 N Ammonium Acetate K in the extract was determined by flame photometry, Ca and Mg were determined by Atomic Absorption Spectrometer (AAS). Soil organic carbon (OC) in different herbicides treated and control soil samples were determined by partial oxidation method (Walkley and Black, 1934) through titration against 1N $(NH_4)_2Fe(SO_4)_2.6H_2O$ using diphenylamine indicator.

Microbial enumeration

Nutrient agar (NA) was used for the enumeration of total heterotrophic bacteria by the pour plate method. Incubation was done at 30°C for 24 - 48h. Potato dextrose agar (PDA) was used for enumeration, isolation of fungi and incubation was at 25°C for 48h. Bacterial isolates were characterized based on cultural characteristics, staining reactions and biochemical reactions. Identification was thereafter made with reference to Bergey's manual of systemic bacteriology and fungal isolates were characterized as described by Barnett and Hunter (1972).

Statistical analysis

The data were statistically analyzed using a one-way analysis of variance (ANOVA) and means separated using Tukey's HSD test by SPSS Statistical package20th edition.

Results and Discussion

Effects of herbicides on soil chemical properties

The effects of herbicide application on soil chemical properties are presented in Table 1. The soil textural analysis indicates that the soil was a sandy loam (Sand 70. 79%; Silt 16.22%, and clay 12.98%). Results from all treatments indicate that there was no significant (P=0.05) difference with respect to soil pH, however atrazine treated plots recorded the highest statistical soil pH (6.55) values while glyphosate + paraquat treated plots recorded the lowest values (4.96). The atrazine treated plot recorded a 14.3% increase in pH values when compared to the control which makes the soil on this plot to be slightly acidic and in a range where most plant nutrients are available for uptake. The increase in soil pH could become disadvantageous as this could increase the persistence of some herbicide due to restricted hydrolysis associated with high pH levels and increase in exposure period by soil microbes thereby causing mortality and a decrease in microbial biomass carbon (Abbas et al., 2014). Atrazine + paraquat treated plots and singly treated atrazine plots increased soil organic matter when compared with other treatments and the control (untreated plots). SOM values of (5.53 and 5.50%) respectively were recorded in these plots which was significantly (P = 0.05) higher than other treatments. Paraquat treated plots (1.05%) recorded the lowest SOM values while the control (untreated) plot had values of (3.98%). Soil available P was significantly (P=0.05) higher in Atrazine + Glyphosate treated plot (49.93mg/kg) while paraquat treated plots recorded the lowest value of available P (6.77mg/kg). There was no significant (P=0.05) difference observed across all treatments with respect to total soil nitrogen (N). However, Atrazine + paraquat treated plots recorded higher statistical values of total N (0.62%) while the untreated plot (control) recorded the lowest values (0.48%). Exchangeable cations was significantly (P=0.05) increased across all herbicide treatments, Untreated plot (control) recorded the highest values of calcium (2.07cmol/kg) and magnesium (1.07cmol/kg), while glyphosate and atrazine + paraquat both recorded the lowest calcium and magnesium values (0.53 and 0.27cmol/kg) respectively. Na was increased in glyphosate treated plot (1.78cmol/kg) which recorded the highest Na values but wasn't significantly (P = 0.05) different from other treatments. Extractable K was also increased in glyphosate treated plot (2.61cmol/kg), however this wasn't significantly (P=0.05) different from other treatments. Cation exchange capacity (CEC) was higher and significantly (P=0.05) different in untreated (control) plot (7.64) when compared with other treatments.

Table 1. Chemical properties of herbicides treated soils at (0 – 15 cm) depth

Treatments	pH (H_2O)	OM (%)	P (mg/kg)	N (%)	Ca	Mg	K	Na	CEC
					(cmol/kg)				
Atrazine	6.55a	5.50a	38.19a	0.55a	1.53a	0.77ab	2.40a	1.61a	6.91a
Glyphosate	5.26b	1.62c	12.83b	0.52a	0.53b	0.27b	2.61a	1.78a	5.35ab
Paraquat	5.40b	1.05c	6.77c	0.58a	0.60b	0.30b	2.55a	1.71a	5.52ab
Atrazine + Glyphosate	5.70b	4.82a	49.93a	0.55a	0.60b	0.30b	2.53a	1.76a	5.35ab
Atrazine + Paraquat	5.06a	5.53a	8.09c	0.62a	0.53b	0.27b	2.48a	1.25a	4.85ab
Glphosate + Paraquat	4.96c	3.81b	33.29a	0.55a	0.67b	0.33b	2.56a	1.45a	5.85ab
Control	5.61b	3.98b	14.39b	0.48a	2.07a	1.03ª	2.48a	1.70a	7.64a

*Means followed by the same letter within each column are not significantly different (P=0.05) as indicated by Tukey's HSD Test

The long term changes in soil chemical properties of herbicide treated soils is a response to the disruption of biological and chemical equilibrium reaction in soil which in turn influence the activity of soil microorganisms. The fluctuating soil chemical properties level which was observed in this study could be ascribed to the adsorption of some herbicides on colloidal sites and organic matter which concealed the effects of these herbicides on soil microbial biomass, and subsequently led to increased hydrolysis of microbial cells (Jayamadhuri and Rangaswamy, 2005). It is obvious from this study that herbicides has various stimulatory effect on soil microbial activity which affects their biodegradation rates, and this mechanism is dependent on the type of herbicide, and application rates that can significantly alter the soil microbial pool quantitatively and qualitatively over an extended period (Anderson and Armstrong, 1981). Herbicides can also interfere with vital processes involving non-target microbial community; and these action includes respiratory activity, molecular composition, biosynthetic reactions, cell growth and division (De Lorenzo et al., 2001).

Effect of herbicides on soil organic carbon

Changes in soil organic carbon (SOC) values as observed in this study could have been induced by continuous herbicide application (Figure 1). Result indicate a significant (P=0.05) increase in SOC with atrazine + paraquat treated plots recording higher values of SOC (3.21%) while low values (0.61%) of SOC was observed in paraquat treated plots.

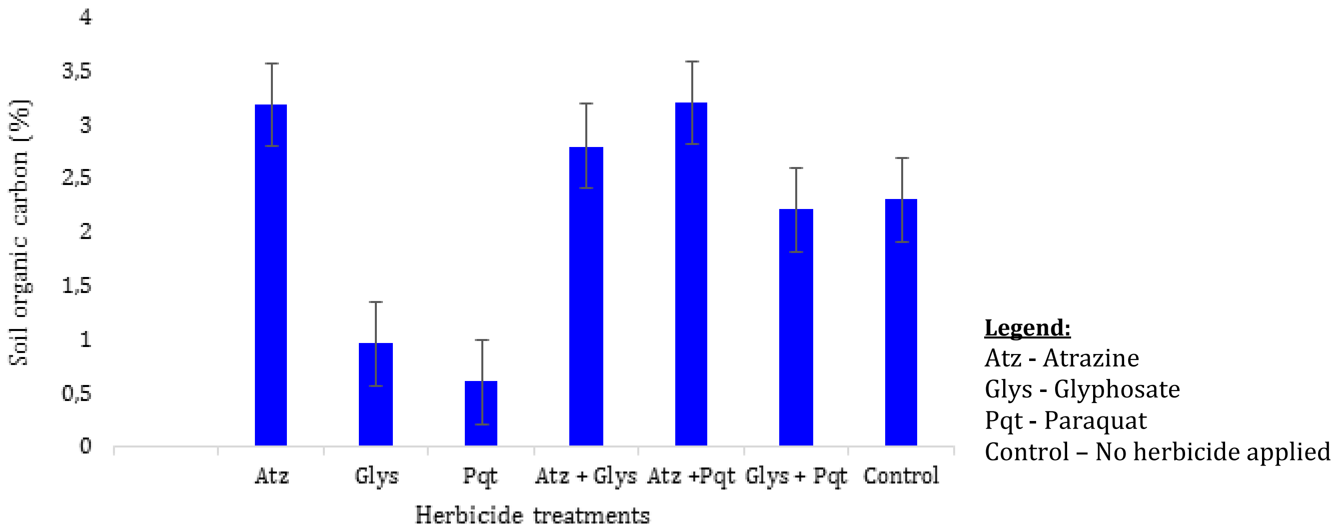

Figure 1. Effect of herbicide treatments on soil organic carbon

The increase of SOC in herbicide treated soil could be attributed to the faster putrefaction of herbicides by the dynamic microbial activity which further influence the movement and persistence of these herbicides due to increasing SOC levels which aids a faster biodegradation process (Ayansina and Oso, 2006). Result from this study was quite different from Sebiomo et al. (2011) who observed significant reduction in percentage organic matter after initial application of herbicides to soils in an incubatory study, although organic matter levels increased after continuous application from the second to the sixth week of treatment. Ayansina and Oso (2006) also reported that soil treatment with atrazine resulted in significant changes in soil organic matter levels. Considerable changes in soil organic matter were observed by Ayansina and Oso (2006) from herbicide treatment with atrazine, while in this study changes were only observed in glyphosate and paraquat treatments. Furthermore, Yaron et al. (1985) opined that soil containing high levels of organic matter would exhibit elevated microbial activity which would enable the soil to adsorb applied herbicide thereby decreasing its concentration in soil solution and reducing biodegradation and elongating its persistence in the soil. Suppression and poor root growth of weeds due to the actions of glyphosate, paraquat and their combined levels could also induce the decline in organic matter by interfering with the root-rhizosphere mechanism.

Effect of herbicides on microbial communities

Dominant fungal and bacteria communities isolated from the herbicide treated and untreated soil are presented in Tables 2, 3 and 4. Results indicate that beneficial bacteria's such as *Pseudomonas spp, Bacillus spp, Enterobacter spp* e.t.c were the dominant colonies across the herbicide treated fields. Under the atrazine treated plots, *Pseudomonas fluorescens* was the highest bacteria genera population (20 x 10^{-8} cfu/g) while the lowest population was *Chromobacterium lividium* and *Xanthomonas spp* (12 x 10^{-8} cfu/g) respectively. In the glyphosate treated plot, isolated bacteria genera; *Bacillus subtilis* and *Thiobacillus thiooxidans* were the dominant colonies at (20 x 10^{-8} cfu/g) respectively, while *Citrobacter freundii* was observed to have the lowest colony (8 x 10^{-8} cfu/g). Paraquat treated plots generally recorded low bacteria colonies, beneficial bacteria such as *Rhizobium, Bacillus subtilis,* were isolated under this plot, however, *Bacillus subtilis* recorded the highest colony count (5 x 10^{-8} cfu/g)

Table 2. Bacteria genera isolated from untreated and herbicides treated soil

Treatments	Isolated Bacteria	Colony count (10^{-8}cfu/g)
Atrazine	*Pseudomonas aeruginosa*	18
	Pseudomonas fluorescens	20
	Chromobacterium lividium	12
	Xanthomonas spp	12
	Enterobacter liquefacieus	15
	Flavobactererium spp	15
	Azotobacter spp	18
Total		110 x 10^{-8} (cfu/g)
Glyphosate	*Flavobacterium spp*	12
	Bacillus subtilis	20
	Proteus mirabilis	8
	Enterobacter spp	10
	Klebsiella aerogenes	10
	Yersinia spp	12
	Citrobacter freundii	8
	Thiobacillus thiooxidans	20
Total		100 x 10^{-8} (cfu/g)
Paraquat	*Proteus vulgaris*	4
	Bacillus subtilis	15
	Citrobacter freundii	3
	Enterococci spp	4
	Rhizobium spp	3
Total		29 x 10^{-8} (cfu/g)
Atrazine + Glyphosate	*Bacillus spp*	10
	Micrococus spp	6
	Pisteurella spp	5
	Leuconostoc spp	15
	Corynebacterium spp	10
	Kurthia spp	5
	Bacillus licheniformis	20
Total		71 x 10^{-8} (cfu/g)
Atrazine + Paraquat	*Chromobacterium lividium*	3
	Bacillus pumilus	6
	Klebsiella aerogenes	5
	E. coli	15
	Proteus mirabilis	20
Total		49 x 10^{-8} (cfu/g)
Glyphosate + Paraquat	*Citrobacter spp*	1
	Chromobacterium lividium	2
	Enterobacter spp	8
	Proteus mirabilis	7
	Acinetobacter anitratus	5
	Genella spp	1
	Branhamella catarrhalis	3
Total		27 x 10^{-8} (cfu/g)
Control (Untreated soil)	*Clostridium tetanomorphium*	20
	Brucella spp	3
	Bordetella spp	3
	Chromobacterium lividium	3
	Actinobacter antratus	5
	Acinetobacter spp	5
	Rhizobium spp	10
	Xanthomonas campestris	4
	Brevibacillus brevis	5
Total		58 x 10^{-8} (cfu/g)

Table 3. Fungi genera isolated from untreated and herbicides treated soil

Treatments	Fungi Isolated	Colony count (10^{-8} cfu/g)
Atrazine	*Aspergillus flavius*	15
	Doratomyces cordia	12
	Heterocerphalum spp	12
	Sclerothium spp	13
	Chaetomella spp	12
Total		64 x 10^{-8} (cfu/g)
Glyphosate	*Fusarium oxyporium*	8
	Geotrichum candidum	5
	Thallospora spp	6
Total		19 x 10^{-8} (cfu/g)
Paraquat	*Penicillium spp*	6
	Collectotrichum gleosporoides	3
	Geotricum candidum	2
	Fusarium spp	7
	Aspergillus nigericum	8
	Verticillium spp	5
Total		31 x 10^{-8} (cfu/g)
Atrazine + Glyphosate	*Fusarium spp*	1
	Trichoderma spp	2
Total		3 x 10^{-8} (cfu/g)
Atrazine + Paraquat	*Aspergillus niger*	3
	Coniothyrium spp	1
	Sphaeropsis spp	1
	Aspergillus fumigatus	3
	Trichothecium spp	2
	Botrytis cenerium	2
Total		12 x 10^{-8} (cfu/g)
Glyphosate + Paraquat	*Trichophyton spp*	3
		3 x 10^{-8} (cfu/g)
Total		
Control (Untreated soil)	*Aspergillus spp*	25
	Rhizoctonia solani	15
	Botryodiplodia spp	20
Total		60 x 10^{-8} (cfu/g)

Table 4. Bacterial and fungal population of herbicide treated and untreated soil

Treatments	Bacterial population (10^{-8} cfu/g)	Fungal population (10^{-8} cfu/g)
Atrazine	16^{a}	13^{b}
Glyphosate	13^{b}	6^{c}
Paraquat	6^{d}	5^{c}
Atrazine + glyphosate	10^{c}	2^{d}
Atrazine + paraquat	10^{c}	2^{d}
Glyphosate + paraquat	4^{d}	3^{d}
Control (Untreated)	7^{c}	20^{a}

Values assigned with different letters are statistically different at (P = 0.05)

Different bacteria genera's were isolated from the combined use of Atrazine and glyphosate treated fields. *Clostridium sporogenes,* and *Bacillus licheniformis* were the dominant isolated colonies (20 x 10^{-8} cfu/g) respectively. *Proteus mirabilis* (20 x 10^{-8} cfu/g) was observed to be the dominant bacteria colony in the atrazine and paraquat treated field. Different bacteria genera were isolated from the combined use of glyphosate and paraquat treated plot; however the colony counts were considerably lower when compared with herbicide treated and untreated (control) plots. Isolated bacteria genera *Enterobacter spp* was observed to have the highest colony count (8 x 10^{-8} cfu/g), while other less dominant bacteria colonies isolated include; *Chromobacterium lividium* (2 x 10^{-8} cfu/g), *Genella spp* (1 x 10^{-8} cfu/g) and *Citrobacter spp* (1 x 10^{-8} cfu/g).

Fungal colonies isolated from herbicide treated plots were generally low with respect to population count irrespective of the different herbicide types. However, *Aspergillus spp*, *Fusarium spp* and *Geotrichum spp* were the dominant colonies identified in all herbicide treated plots. *Aspergillus flavius* was the dominant colony in the atrazine treated plots (15 x 10^{-8} cfu/g). Glyphosate treated plots recorded the lowest fungal communities across all treatments, with population count as low as (5 x 10^{-8} cfu/g) observed for *Geotrichum candidum*. Paraquat treated plots also portrayed a linear decreasing trend in isolated fungal genera, *Aspergillus nigericum* (8 x 10^{-8} cfu/g) and *Fusarium spp* (7 x 10^{-8} cfu/g) were identified as the dominant fungal colonies under this plot. Combined atrazine and glyphosate treated plots remarkedly recorded the lowest fungal colonies amongst the herbicide treated plots with a total population count of (3 x 10^{-8} cfu/g). Despite the low population count, *Trichoderma spp* a beneficial fungi was observed to have the highest colony count (2 x 10^{-8} cfu/g). *Aspergillus spp* (6 x 10^{-8} cfu/g) were the dominant colonies isolated under the combined atrazine and paraquat herbicide treated plots. Although the fungal colonies were generally low, atrazine treated fields had the highest fungal colonies when compared with other herbicide treated plots (Table 3). Glyphosate and paraquat treated fields were also dominated by low fungal colonies. Fields under this herbicide treatment recorded a total fungal population count of (3 x 10^{-8} cfu/g) and was inhabited by the fungi genera *Trychophyton spp.* Diverse bacterial and fungal genera were identified and isolated from the untreated (control) fields. Bacteria colonies such as *Rhizobium spp* and *Clostridium spp* were the dominant colonies with respective population count of (10 x 10^{-8} cfu/g and 20 x 10^{-8} cfu/g). Fungal communities isolated from this field includes *Rhizoctonia spp*, *Aspergillus spp* and *Botryodiplodia spp* and their total population count (60 x 10^{-8} cfu/g) when compared with the herbicide treated fields was marginally higher except for Atrazine treated fields. Statistical analysis of total microbial population isolated and identified indicate a positive impact of herbicides on soil bacterial communities and a negative impact on fungal communities (Table 4). Fields treated with atrazine was significantly (P = 0.05) different from the untreated (control) soil and other herbicide treatments with respect to total bacterial population count as a high enumeration count was recorded (16 x 10^{-8} cfu/g). However, Atrazine + glyphosate and Atrazine + paraquat treated fields bacterial population count were not significantly (P=0.05) different from the untreated field except for glyphostate treated fields (Table 4). 128% increase in bacterial communities was observed in atrazine treated fields, 86% increase in bacterial communities isolated from glyphosate treated fields, 43% increase in Atrazine + glyphosate and Atrazine + paraquat treated fields respectively while over 42% decrease in bacteria population count was estimated from Glyphosate + paraquat treated fields relative to the untreated field.

Herbicide treatments exerted significant reduction in fungi colonies isolated and identified from treated fields. However, soil samples assayed from untreated fields was observed to have the highest fungi colonies amongst other treatments with a total fungal count of (20 x 10^{-8} cfu/g) (Table 4). Generally, fungal colonies enumeration count declined significantly across all herbicide treated plots when compared with the untreated field (control), with the lowest population count observed in atrazine + glyphosate, atrazine + paraquat and glyphosate + paraquat. A 35% reduction in fungal colonies was observed in atrazine treated plots, while over 10% decrease in fungal populations was observed in all herbicide treated fields irrespective of herbicide type and combinations when compared with the untreated field. Deleterious effect of herbicide application may lead to the reduction and negative stimulation of certain microbial communities with essential functions to perform in the soil environment and ecosystem. Herbicide treatments of glyphosate, paraquat, atrazine and their combined levels showed significant effects on isolated and identified fungal population within the soil environment. Fungal population increase and decrease observed due to the effects of herbicide treatments also varied among isolated fungal species and the types of herbicide treatments. The abundance of bacterial communities and fungal communities in atrazine treated fields suggest a stimulatory effect on select microbial growth and a high acclimatization to atrazine which could possibly create a colonies of microbes resistant to atrazine and can be used for degrading atrazine and bioaugmentation. This study also observed that the increase and decrease of some fungal species varied with differing herbicide types and this could be ascribed to the differing abilities of fungal mycelia to absorb herbicides for their utilization as some species are more active as herbicide degraders (Romero et al. 2009). Paraquat, glyphosate and their combined levels were found to be more inhibitory causing significant reductions in fungal populations. An earlier study had reported the Paraquat and glufosinate-ammonium were found to be inhibitory than glyphosate and metsulfuron-methyl, causing 80-100% growth inhibition to the fungal species. Paraquat was toxic *in vitro* to the radial growth of fungi as *Colletotrichum dermatium*, *Alternaria* sp., *Macrophomina phaseolina* and *Phomopsis* sp. isolated from soybean seeds at a concentration as low as 600 μg a.i. mL-1 (Cerkauskas and Sinclair, 1982;Zain et al., 2013). A sharp decline in fungal specie population in response to glufosinate-ammonium was also observed for *Trichoderma harzianum* and *T. longipilus* (Ahmad and Malloch, 1995) and *Magnaporthe grisea* and *Cochliobolus miyabeanus* (Ahn, 2008). Glyphosate and its combined levels with other herbicide types also inhibited fungal population in this study and this could be due to the blocking of EPSPS enzyme in the shikimic acid pathway that ultimately affected the amino acid synthesis in fungal species. Previous *in vitro* studies also reported the growth-inhibitory effects of glyphosate on soil fungi communities such as *Fusarium solani*, *Pythium ultimum* and *Trichoderma viridae* at 100 and 140 ppm (Meriles et al., 2006), *Sclerotium rolfsi* at commercial recommended rate of 3.6 g L^{-1} (Westerhuis et al., 2007). Several studies have also reported the short-term effects on soil microbial communities upon single application of herbicide exposure (Hance, 1980) and at combined herbicides levels higher than the recommended field rate were observed to cause transitory effects on microbial biomass (Das et al., 2006; Weaver et al., 2007). Several studies have also reported significant decline of a number of cellulytic and pathogenic soil fungi by paraquat (Smith and Mayfield, 1977) and glyphosate (Anderson and Kolmer, 2005) in soil. The decline in the population of fungi and bacteria in this study was further corroborated by previous study conducted by Novak et al. (1999) who made use of post emergence herbicides and observed a decrease in the microbial biomass carbon (MBC). Decrease in bacteria, fungi and Actinomycetes population has also been reported by Sebiomo et al. (2011) from the use of atrazine, primeextra, paraquat and glyphosate herbicide treatments in an incubatory study. The reduction in MBC could be ascribed to the decrease in organic matter due to the mortality of soil microbes by herbicide residues (Abbas et al., 2014). The decline in bacteria population and some specific beneficial bacteria e.g phosphate solubilizing bacteria (*Enterobacter spp.* etc.) by glyphosate, paraquat and their combined herbicide treatment in this study could be attributed to the soil textural characteristics. High clayey contents could increase the persistence of herbicides in the soil and prolong interaction between herbicides and soil bacteria which could cause significant drop in their population. This result was in agreement with the findings of Cupples et al. (2005) who also observed prolong persistence of herbicides in the soil due to high clay content. The significant increase in both fungal and bacterial communities observed in atrazine treated soils could be ascribed to their intrinsic ability to tentatively mineralize and use the herbicides as energy source (Kunc et al., 1985). The decline in microbial counts exhibited in other herbicide treatments may be due to the fact that these microbial communities were less tolerant of the applied herbicides and were susceptible to the soil-herbicide interactions, which could have possibly been inhibitory (Taiwo and Oso, 1997). However, microbial population could be increased as these microbial communities adapt to the stress induced by the herbicides over a period of time. This could be ascribed to the recovery ability of different microbial species and enzymatic activities after the initial inhibition phase due to the microbial adaptation to these herbicides or biodegrading abilities.

Conclusion

Soil microbial communities are a very important component of the soil ecosystem. A functioning agro-ecosystem is dependent on these microbial communities as their abundance, activities and biodiversity are good indicators of a sound agro-ecosystem. This study indicated that glyphosate, paraquat, atrazine and their combinations induced both detrimental and beneficial changes in bacterial and fungal communities isolated and identified after a period of five year application and this could be attributed to organic matter levels, clay contents and the elongated persistence of these herbicides in the soil. Therefore, farmers are implored to observe a cautious approach by strictly following manufacturers recommended rates when applying these herbicides. The idea of combining herbicides for increased weed eradication efficacy so as to combat herbicide resistant weeds should be discouraged as this poses deleterious effect on soil microbes and ecosystem functioning. Farmers could also combat weed infestation by mulching their farmlands with mulch materials with allelopathic abilities for weed suppression which also exert beneficial effects on crop growth, soil health and are non-ecologically destructive. Soil microbial communities with increased population in herbicides treated fields as observed from this study could be isolated and cultured for use in bioaugmentation programs to biodegrade persistent herbicides in contaminated soils in the study area.

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Effect of phosphorus solubilizing bacteria on some soil properties, wheat yield and nutrient contents

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Abstract

Application of chemical fertilizers besides economic concerns has been a reason of environmental and ecosystem degradation, so sustainable organic agriculture is becoming popular in researches and among farming communities. Phosphorus holds second position after nitrogen among macronutrients required for better plant growth and is needed in higher amounts. Meeting this high phosphorus input for better crop yields causes environmental problems like eutrophication, so phosphorus solubilizing bacteria (PSB) and plant growth promoting rhizobacteria (PGPR) are being emphasized to utilize phosphorus fixed in soil layers. This study was carried out to evaluate the effect of PSB on plant growth, soil biological properties including enzymes and soil respiration. Treatments including control, 50 mg kg^{-1} nitrogen, 50 mg kg^{-1} nitrogen and 12 mg kg^{-1} phosphorus applications reduced dosage of nitrogen 25 mg kg^{-1} with PGPR and 25 mg kg^{-1} nitrogen along with 0.12 g raw phosphate and PGPR. Results indicated that plant parameters like above and below ground plant biomasses (fresh and dry weight), plant nitrogen and phosphorus content were significantly enhanced in all the treatments when compared with the control. While soil pH in rhizosphere significantly increased with the treatments, bulk soil pH decreased with PGPR treatments when compared with all other treatments. EC values in rhizosphere and bulk soils were not significantly influenced with the treatments. Rhizospheric and bulk soil showed high amount of N, P and organic matter in PGPR treatments. Alkaline phosphatase and β-glucosidase activities were found significantly higher in the last treatment than the other treatments. Basal soil respiration was interestingly found higher in control soil but did not differ statistically from the other treatments. Concluding, application of PGPR with lower amounts of chemical fertilizers can reduce the use of chemical fertilizers and has also potential of improving soil health in long term aspects.

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Introduction

More food production is required for constantly increasing population but is limited by decreasing agricultural land due to urbanization pressure (Hamuda and Patkó, 2013) and other diverse factors. Meeting this required food without damaging the ecosystems is a major concern which has become more demanding these days. Use of chemical fertilizers is under continuous criticism because of their high losses and escape to environment due to different processes like denitrification in case of nitrogen and eutrophication in case of phosphatic fertilizers (Hamuda and Patkó, 2013). Along with ecosystem pollution, extensive use of mineral fertilizers is also leading to land degradation. These circumstances force us to adopt environment friendly and sustainable agriculture which is possible solely through use of organic and biofertilizers.

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Phosphorus being macronutrient stands second in plant requirements after nitrogen and is involved in various metabolism activities within the plants. Plant available phosphorus is usually deficient in the soil because it is fixed in soil layers. (Wang et al., 2009; Shenoy and Kalagudi, 2005; Khan et al., 2009; 2014). This insoluble or fixed phosphorus is in different forms fixed with calcium $(Ca_3PO_4)_2$, aluminum (Al_3PO_4) and iron (Fe_3PO_4) and can be turned to soluble forms by P-solubilizing organisms (Gupta et al., 2007; Song et al., 2008; Sharma et al., 2013) where P-solubilizing soil microbes have gained importance because of their ability to mineralize complex compounds (Bishop et al., 1994; Ponmurugan and Gopi, 2006; Toro 2007; Wani et al., 2007a). Release of different organic acids by these microorganisms lead to acidification of microenvironments (Maliha et al., 2004) and consequently replacement of P ions with cations which finally is termed as phosphorus solubilization (Goldstein, 1994; Mullen, 2005; Trivedi and Sa, 2008).

Phosphorus solubilizing bacteria (PSB) hold most important position in P-solubilizing soil microbes due to their multifunctional capabilities. Along with enhancing phosphorus availability for the plants, some PSB are also responsible for production of siderophore (Tank and Saraf, 2003; Hamadali et al., 2008; Wani et al., 2008), indole acetic acid and gibberellin (Sattar and Gaur, 1987; Souchie et al., 2007), antibiotics (Lipping et al., 2008; Taurian et al., 2010), secondary metabolites (Wani et al. 2007b) and 1-aminocyclopropane-1-carboxylate (ACC) deaminase enzyme (Glick et al., 2007). Use of PSB as biocontrol agents against soil borne pathogens have also been reported (Khan et al., 2002; Vassilev et al., 2006; Singh et al., 2010).

Rhizosphere is termed as the soil zone directly surrounding the roots (Walker et al., 2003) and also bio-influenced zone (Hinsinger et al., 2008). Inhabiting maximum of soil microbes, rhizosphere also supports plant growth through supplying plant nutrients (Gianfreda, 2015). Among the rhizosphere abundant bacteria; genera *Azotobacter, Bacillus, Beijerinckia, Burkholderia, Enterobacter, Erwinia, Flavobacterium, Microbacterium, Pseudomonas, Rhizobium* and *Serratia* are thought to be most promising PSB (Bhattacharyya and Jha, 2012). Several reports of increased plant yield and soil health through application of PGPR importantly PSB have been published. Application of *Pseudomonas* spp. significantly enhanced plant growth parameters in soybean (Son et al., 2006), maize (Mehrvarz et al., 2008), wheat (Afzal and Bano, 2008; Majeed et al., 2015), barley (Cakmakci et al., 2007), tomato (Calvo et al., 2010), green gram and alpine Carex trees (Bartholdy et al., 2001). Improvement in soil nutrient content regarding nitrogen and phosphorus was observed with application of PGPR in barley (Cakmakci et al., 2007).

Some soil biological properties such as enzyme activities and respiration activity are used as bio-indicators for soil quality and health for environmental soil monitoring (Kızılkaya et al., 2004; Akça and Namlı, 2015). Basal soil respiration of soil microflora provides useful information on the physiological condition of the pedoecosystem even though it is a matter some controversy. This respiratory activity takes into account the use of energy by microflora and expresses the efficiency of organic carbon by soil microorganisms (Ananyeva et al., 2016). In the prsent study, soil enzymes respentative of C and P cycle were selected. Glucosidases are widely distributed in nature and their hydrolysis products as low molecular weight sugars are important source of energy for soil microorganisms. β-glucosidase catalyzes the hydrolysis of β-D-glucopyranoside and is one of the three or more enzymes involved in the saccharification of cellulose (Kızılkaya and Bayraklı, 2005). Phosphatase is an enzyme of great agronomic value because it hydroles compounds of organic phosphorus and transforms them into different forms of inorganic phosphorus, which are assimilable by plants. Variations in phosphatase activity apart from indicating changes in the quantity and quality of a soil's phosphorated substrates, are also a good indicator of its biological state (Aşkın and Kızılkaya, 2006).

Soil biologic properties including enzyme activities indicate comprehensive results regarding bacterial inoculation to the soil (Caravaca et al., 2003). Soil enzyme activity is used in monitoring soil nutrient concentration changes as a result of inoculants' interaction with indigenous microbial population (Naseby et al., 1998) and these enzymes can alter the availability of different nutrients to the plants (Gianfreda, 2015). Soil enzymes also serve as soil quality indices and help us measure soil quality indirectly (Karaca et al., 2011). Enhancement of SEA through application of PGPR and PSB has been reported. Increase in phosphatases and dehydrogenases was observed in rhizospheric soil of lettuce through application of PGPR, arbuscular mychorhiza and PSB alone or in consortium (Kohler et al., 2007). Dehydrogenase activity was increased in soybean rhizosphere by application of Pseudomonas spp (Sharma et al., 2011).

The objectives of the present study were to evaluate the effects of PSB/PGPR applications on i) some chemical (such as; pH, EC, organic matter, N and P) and biological properties (such as; alkaline phosphatase activity, β-glucosidase activity and basal soil respiration) of rhizosphere and bulk soil, and ii) nutrient content and yield of wheat plant.

Material and Methods

Soil and PSB/PGPR

The soil used in this experiment had 30.8 % clay, 32.7% silt, 36.5% sand and classified as clay loam (CL). The pH in water was 7.9, the oxidizable organic matter content was 1.1%, the total N was 0.02%, $NaHCO_3$ extractable P was 6 mg kg^{-1}, the soil C:N ratio was 31.9. Bacillus sp. #189 tested in the present study was obtained from Konbiyo R&D Ltd. Co., Konya Technocity, Konya, Turkey, by Dr. M.Ogut. The bacteria used in this study were previously isolated from wheat rhizosphere, identified, and tested for their ability to solubilize P and to reduce pH in culture conditions and microcosms, and also identified and reported as phosphorus solubilizing bacteria (PSB) and plant growth promoting rhizobacteria (PGPR) by M.Ogut (Ogut et al., 2011; Ogut and Er, 2016).

Counting and culturing PSB/PGPR

The PSB were grown in liquid nutrient medium aerobically (200 rpm) at 35°C for 24 h. The bacterial cultures were mixed with sterile zeolite, and the mixtures were further incubated at 35°C for a week and dried in aseptic conditions. The numbers of the PSB were in the 10^8 CFU g^{-1} as determined by plate count. 1g dried PSB/PGPR added in sterile saline solution (0.85% NaCl) and the bacterial density of each suspension was adjusted to be 10^8 CFU ml^{-1}. Pure cultures of PSB-PGPR used for inoculation were grown in sterile saline solution (0.85% NaCl). Unsterilized soil samples in the pots were innoculated with 1 mL of 10^8 CFU mL^{-1} bacterial suspension 30 min before sowing under sterilized conditions.

Greenhouse experiment

Soil samples were air dried in a laboratory and sieved through a 2-mm screen. The soil sample (2 kg air-dried soil) was placed in 2,5 L cylindrical plastic pots. A pot experiment was carried out to investigate the effects of PSB/PGPR inoculation on some chemical and biological properties of rhizosphere and bulk soils, and nutrient content and yield of Spring Wheat (*Triticum aestivum*) plant in the greenhouse of Soil Science & Plant Nutrition Department in Ankara University. The experiment was conducted with 5 treatments and four replications, and the pots were distributed in completely randomized plot design. Soil without PSB-PGPR inoculation was used as a control treatment.

T1: control,
T2: 50 mg N kg^{-1},
T3: 50 mg N kg^{-1} + 12 mg P kg^{-1},
T4: 25 mg N kg^{-1} + 1 mL PSB/PGPR,
T5: 25 mg N kg^{-1} + 0.12 g raw P/2 kg soil + 1 mL PSB/PGPR,

Ten seeds were sown in each pot and thinned to five plants per pot after the full emergence of the first leaf. The pots were regularly irrigated to maintain a proper moisture level. Plants in pots were harvested 80 days after sowing. The soils in pots were than moistened up to 70% of field capacity and maintained at this moisture throughout the experiment. At the end of the experiments, plant samples were collected from the pots. Studied plant parameters were shoot biomass, root biomass, N and P concentration in plants. All parameters were measured according to Ryan (2001).

Soil analyses

At the end of the experiment, soil samples were taken from each pot. Selected some chemical properties of rhizosphere and bulk soils were determined by the folowing methods; pH in 1: 2.5 (w:v) in soil:water suspension by pH-meter, electrical conductivity (EC) at the same suspension by EC-meter, soil organic matter by the wet oxidation method (Walkley-Black) with $K_2Cr_2O_7$, total N by Kjeldahl method and available P by $NaHCO_3$ extraction method (Rowell, 1996). Basal soil respiration (BSR), alkaline phosphatase activity (APA) and β-Glucosidase activity (GA) were determined according to the methods by Naseby and Lynch (1997).

Statistical analysis

One-way analysis of variance (ANOVA) was used to determine significant treatment effects at 5% significance level. Least significant difference (LSD) test was used to determine significant ($P < 0.05$) differences. All statistical analyses were performed by statistix (Version 8.1).

Results

Chemical properties of rhizosphere and bulk soils

The effects of different treatments on chemical properties of rhizosphere and bulk soils are illustrated in Figure 1. Rhizosphere soil pH was significantly affected by the treatments ($P<0.05$) which ranged from 7.71 to 7.89 where the lowest pH was recorded in the control and the highest was found in T3 which did not statistically differed from all other treatments, except the control.

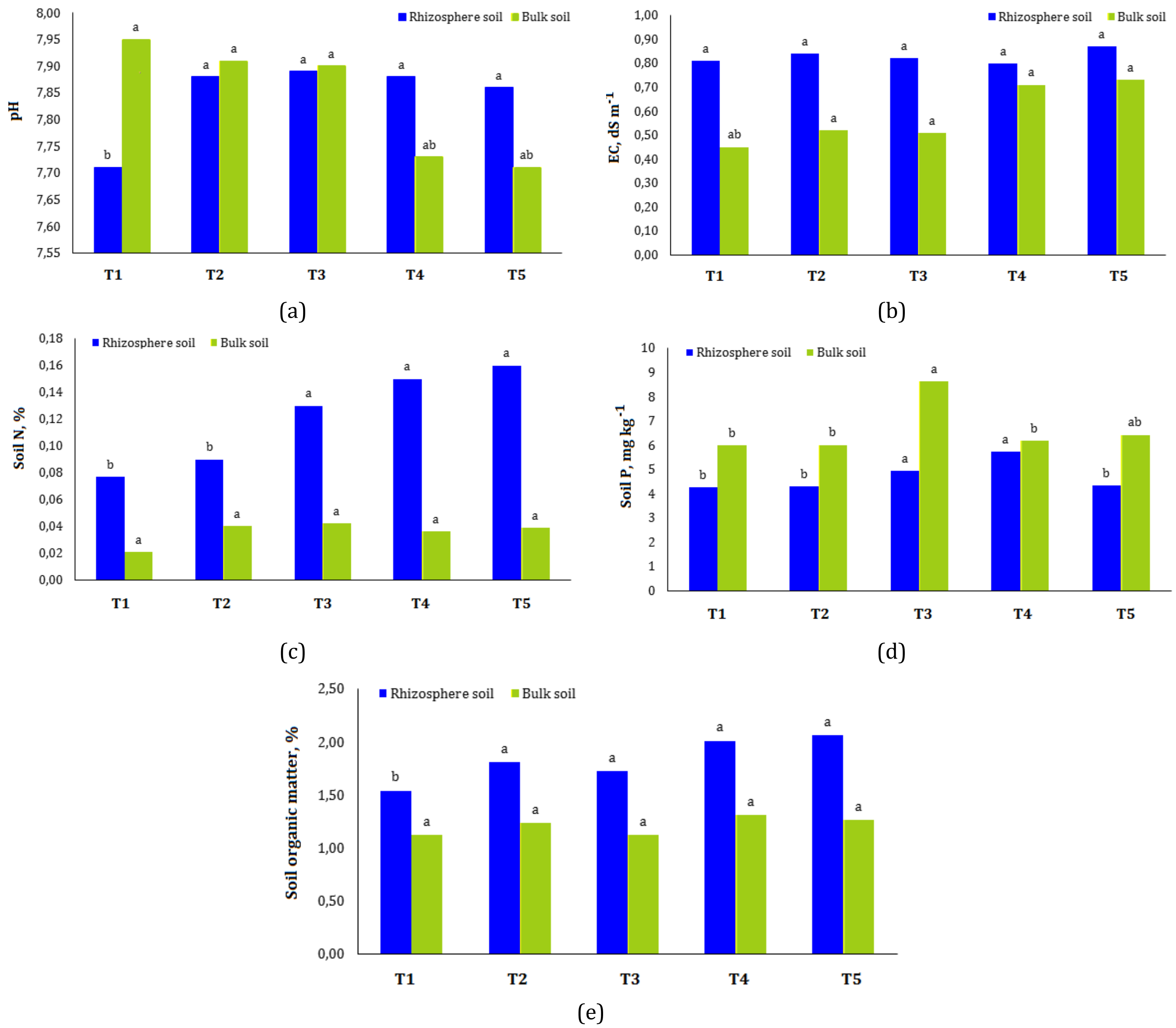

Figure 1. The effect of different treatments on chemical properties of rhizosphere and bulk soils

Bulk soil pH also significantly differed ($P<0.05$) within the treatments and ranged from 7.71 to 7.95. The lowest pH was found in T5 which was not different from pH measured in T4 whereas the highest pH was found in the control which was similar with T2 and T3 (Figure 1a). Rhizosphere and bulk soil EC values were not significantly affected by the treatments (Figure 1b).

Rhizosphere soil N content was significantly affected by the treatments ($P<0.05$) and ranged from 0.077% to 0.16% where the lowest N content was found in the control and the highest one was found in T5 which was not significantly different from the values with T3 and T4 statistically. Bulk soil N content was not significantly affected by the treatments ($P>0.05$) and ranged from 0.021% to 0.042% where the lowest N was determined in the control which was statistically same with that in all other treatments (Figure 1c).

Rhizosphere and bulk soil P content was significantly affected by the treatments applied (P<0.05). The rhizosphere P content ranged from 4.26 to 5.73 mg kg^{-1} where the lowest amount was observed in the control which was not different statistically from T2 and T5 treatments. The highest P content was calculated in T4 which was statistically the same with T3 treatment. Phosphorus content in bulk soil varied between 5.98 and 8.63 mg kg^{-1}. The lowest P content was found in the control which was not significantly different from the values determined in T2, T4 and T5, while the highest P content was obtained in T3 treatment (Figure 1d).

Rhizosphere and bulk soil organic matter (SOM) contents followed similar trend. The treatments only affected the rhizosphere SOM content significantly (P<0.05) while the bulk SOM was not significantly affected (P>0.05). The rhizosphere SOM ranged from 1.54% to 2.06% among the treatments where SOM was increased significantly over control but did not differ significantly among the treatments of T2, T3, T4 and T5 (Figure 1e).

Nutrient content and yield of wheat plant

The effects of different treatments on nutrient content and yield of wheat plant are illustrated in Figure 2. Plant N content was significantly affected with the applications of different fertilizer sources (P<0.05) when compared with the control. Plant N content varied from 0.94% in the control to 1.52% in T2 treatment (Figure 2a). Plant P content was significantly affected by the treatments (P) and ranged from 0.10% to 0.21%. The lowest P content was found in the control which was statistically the same with T2 and T4, and the highest P content was measured in T3 which was the same with T5 tatistically (Figure 2b).

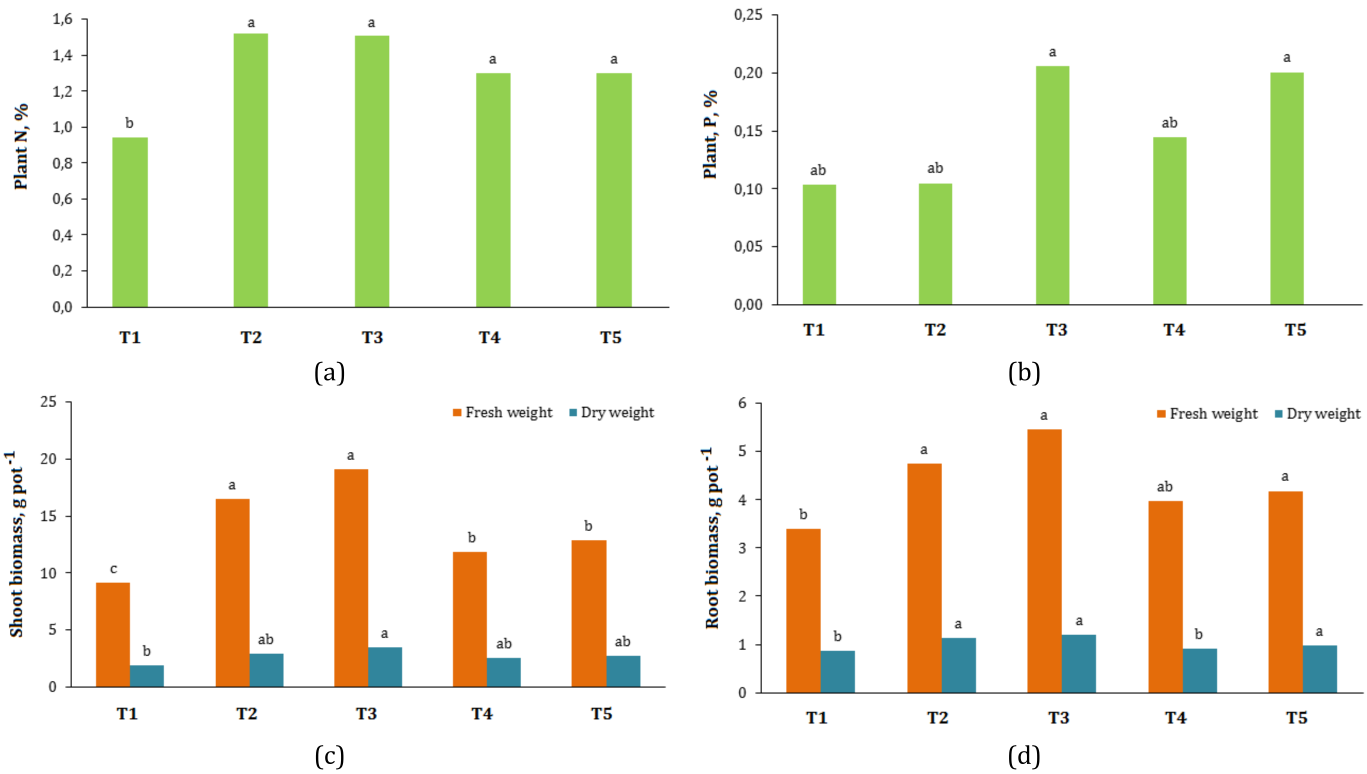

Figure 2. The effect of different treatments on nutrient content and yield of wheat plant

Fresh and dry weights of shoot and root biomass were significantly affected by the different treatments. Fresh weights of wheat plant ranged from 9.1 g in control treatment to 19.09 g in T3. Dry weight of the plants was the lowest in control plant with a value of 1.87 g whereas all other treatments had higher dry weight values than control statistically (Figure 2c).

Root biomass was significantly affected by the treatments (P<0.05) and followed a similar trend with shoot biomass. Fresh weight of root biomass ranged from 3.39 g to 5.45 g. The highest fresh root weight was determined in T3 which was significantly higher than control treatment, but it was not significantly diferent from the other treatments. Dry weights of the roots varied from 0.86 g in control to 1.19 g in T4 (Figure 2d). There was not significant differences for dry root weight among the treatments, except the control.

Biological properties of rhizosphere and bulk soil

The effects of different treatments on biological properties of rhizosphere and bulk soils are illustrated in Figure 3. Alkaline phosphatase activity (APA) measured in the treatments varied between 20.1 to 22.76 µg pNP g^{-1} in rhizosphere soil and not significantly differed each other. However treatments significantly affected the APA in bulk soil. The highest APA in bulk soil was detrmined in T5 which was statistically the same with T1, T2 and T4 but different from T3.

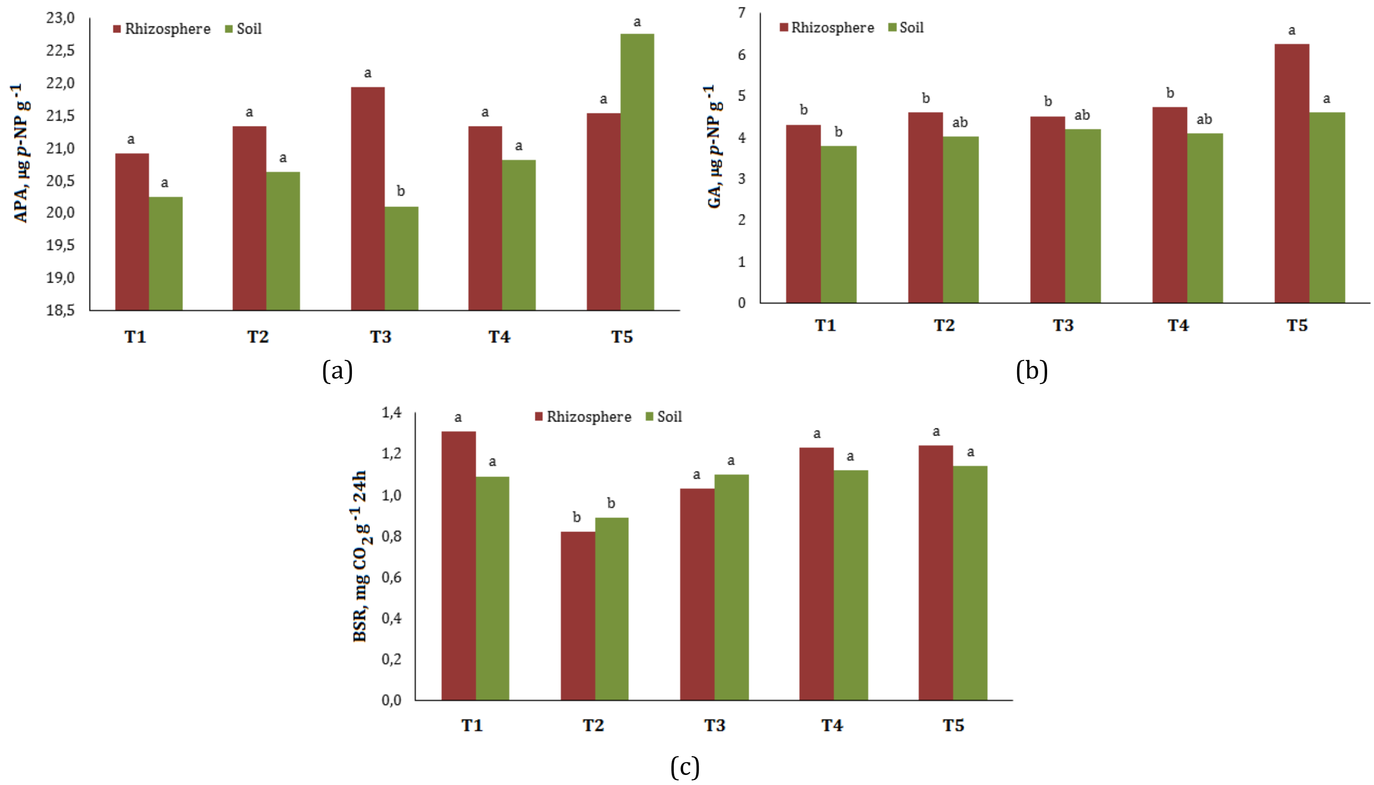

Figure 3. The effect of different treatments on biological properties of rhizosphere and bulk soils

β-glucosidase (GA) activity measured in the treatments varried between 3.79 and 6.25 mg pNP g^{-1}. The highest GA in rhizospere and bulk soils was observed with T5 treatment. In bulk soil, GA increased with T2, T3 and T4 treatments, but they were not different from the control treatment statistically (Figure 3b).

BSR varied between 0.82 and 1.31 mg CO_2 g^{-1} $24h^{-1}$. Similar trend was observed in both rhizosphere and bulk soils (Figure 3c). Basal soil respiration (BSR) values in the control treatments were generally higher than that in the other treatments. The lowest BSR, obtained from T2 treatments in rhizospeher and bulk soils, were significantly different from the other treatments. BSR increased with T4 and T5 treatments.

Discussion

Application of PSB-PGPR has been long reported to enhance soil properties like; nitrogen fixation in soil (Zahid et al., 2015), phosphorus solubility (Khan et al., 2009) and plant growth like agro-morphological parameters (Egamberdieva, 2010), enhanced uptake of nutrients in above ground parts of the plant (Cakmakci et al., 2007; Sarker et al., 2014; Zahid et al., 2015) and physiological parameters (Turan et al., 2014).

A decrease in bulk soil pH was observed in the study by Chen et al. (2006). They reported that application of PSB reduces pH of the soil in certain conditions due to release of organic acids to solubilize the insoluble phosphorus fixed in soil layers. Other microbial metabolites are also responsible for decreasing the soil pH (Abd-Alla, 1994), however decrease in bulk or rhizosphere is not always evident. The electrical conductivity of soil also increases with increase in metabolite concentration as observed in this study. Increase in rhizosphere soil nitrogen content with application of PGPR was also observed by Cakmakci et al. (2007). Nitrogen fixation is the ultimate reason behind increased nitrogen concentration in rhizosphere (Saharan and Nehra, 2011). Several free living and symbiotic bacteria enhance nitrogen content of soil in different crop conditions. Hussain et al. (2013) observed that phosphate solubilizing bacteria increased phosphatase and organic phosphate mineralization activity in this study, rhizosphere and soil phosphorus content increased with phospahatase activity. Phosphatase activity is considered as main contributor towards increased phosphorus and its availability in the soil (Xu and Johnson, 1995). Certain soil microbes also release organic acids which dissolve the rock phosphate along with chelating the calcium ions which consequently release phosphorus into the soil solution (Saharan and Nehra, 2011).

Bulk soil organic matter almost remained the same in all treatments while SOM content of rhizosphere generally increased by the treatments over the control. The results for rhizosphere SOM were similar to findings of the study by Ul Hassan and Bano (2015). They observed that SOM significantly increased with PGPR bacteria application. Prevention of organic matter due to application of biofertilizers may be the reason for increased SOM in treated pots (Hamuda and Patkó, 2013).

Nitrogen and phosphorus contents in the above ground parts of wheat plants increased significantly in all the treatments, and they were similar to findings of previous studies by Majeed et al. (2015) and Ul Hassan and Bano (2015). Multidimensional roles of PGPR also contribute to enhanced nutrient uptake by plants thus leading to more nitrogen and phosphorus content in above ground plant parts (Kuan et al., 2016). Root growth in response to PGPR bacteria inoculation also leads to better nutrient uptake by the plants (Bhattacharyya and Jha, 2012). Vikram (2007) also reported auxin assisted improvement in roots thus leading to more nitrogen and phosphorus content in above ground plant parts.

Increases in plant root and shoot biomass were observed in different crops in response to PGPR bacteria as witnessed in this study (Yildirim et al., 2008, 2011a, 2011b; Turan et al., 2014). Different plant growth promoting characteristics of PGPR bacteria and PSB are linked to enhanced plant growth in wheat plants (Salantur et al., 2006). Gianfreda (2015) reported that soil enzyme activities and respiration give an idea about microbial population and functioning in the soil where enzyme measurement in the soil also defines health of the plants. Gianfreda (2015) determined that enzyme activity is higher in rhizosphere soil when compared to bulk soil which is similar to the results of this study. Hamuda and Patkó (2013) found similar results and observed that when soil was augmented with microbes along with NPK had higher enzyme activities compared with sterile soils. Roca et al. (2013) indicated that the logic behind higher alkaline phosphatase enzyme activity is presence of insoluble phosphate for which PSB can produce enzymes and organic acids to solubilize this unavailable phosphorus. Dinesh et al. (2013) determined that application of PGPR bacteria and chemical fertilizers activates soil enzymes on different levels. They concluded that soil biological activities are activated and increased due to application of chemical and biological fertilizers.

Conclusion

Treatments had no or minimum effects on agro-morphological parameters, but had more prominent effects on soil biological properties. Application of raw phosphate enhanced phosphate solubilizing bacterial activity and showed more enzyme activities along with soil respiration. Similarly, application of PSB-PGPR like in T4 treatment also showed increase in soil biological properties, but absence of substrate like raw phosphate did not enhance biological properties when compared with other treatments. Differences between rhizosphere and bulk soil were observed in few soil parameters. The effects of treatments on soil properties were minimum in bulk soil and more in rhizosphere soil.

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Depositional environments signatures, maturity and source weathering of Niger Delta sediments from an oil well in southeastern Delta State, Nigeria

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Abstract

Attempts have been made to classify the sediment on their degree of maturity. Compositional maturity is a reflection of intensity of weathering and a function of labile grains, unstable/stable rock fragments and stable quartz arenites. The main aim of this study is to investigate maturity and area of deposition and attempt to shed light on source area paleo-weathering conditions. Twenty one samples of shales and sandstones units were collected from a depth precisely between 1160 to 11,480m at a well in western Niger Delta, grinded, pulverized and sieved with less than 75µm. About 10g was packed and sent to Acme analytical Laboratory LTD., Vancouver, Canada. From the results, various plots and indexes inferring maturity and area of deposition were utilized. Using the A-K-F ternary plots of Englund and Jørgensen (1973), the depositional environment is transition zone. The silicate weathering indexes CIA, CIW and PIA values ranges from 45-65, on average indicates low to moderate weathering in the source area with extreme weathering of some sand fraction. Various calculated values of the weathering indices: Chemical Index of Alteration (CIA), Plagioclase Index of Alteration (PIA), Chemical Index of Weathering (CIW) and scatter plots of formulated ratios of Al/Na, K/Na, and Rb/K vs chemical index of alteration (CIA) were plotted. The moderate values below average suggest low to moderate weathering conditions in the source area or during transportation. This also inferred their recycling processes are insignificant. The clay content is low and feldspars are averagely high implying immaturity. The calculated ZTR index for the sand ranges from 36.4-75.0 from with an average mode of 55.5% implying almost all contain mineralogically immature sediments. The calculated Zircon- Tourmaline-Rutile (ZTR) index shows that majority of the sample depths have >43% ZTR index but below 75% which corresponds to generally immature sediments.

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Introduction

The use of geochemical composition of siliciclastic sedimentary rocks is a vital tool to know weathering and erosion dynamics (Nesbitt and Young, 1982) and post-depositional changes (Fedo et al., 1995, 1996). The extent and duration of weathering in siliciclastic sediments can be estimated by examining the relationships among alkali and alkaline earth elements (Nesbitt and Young, 1982). The use geochemical data from sediments to understand such sedimentary processes is increasing in use due to the specificity of some key trace elements in identifying minor components that are not easily recognized petrographically (Abd El-

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Rahman et al., 2009). The upper continental crust is established to be dominated by the presence of feldspars and volcanic glass (Nesbitt and Young, 1982, 1984). As a result, the dominant process during chemical weathering, transportation, deposition and soil formation is the degradation of labile feldspars from source rocks to its corresponding clay minerals. The intensity of degradation can infer its maturity, distance travelled from provenance. These and other such chemical processes are preserved and ultimately transferred to sedimentary records which gives a tenable signature for evaluating the original composition maturity and following weathering conditions (Akarish and Dessandier, 2011).

The Niger delta is situated at the intersection of an early cretaceous Atlantic and gulf of guinea triple junction. The southern and northern arms developed into Atlantic and gulf of guinea respectively as they grow into spreading events which cause separation of South-America from Africa (Ofoegbu, 1984). The third arm which is the Benue trough failed during late cretaceous to become an aulacogen. The development of cretaceous pro-deltas was terminated in the Paleocene by a major transgression. Niger Delta which stretches from Eocene to recent in age. The offshore boundary of the province is defined by the Cameroon volcanic line to the east, the eastern boundary of the Dahomey to the west, a two-kilometer sediment thickness to the south and sediment thickness greater than two kilometers to the southwest.

The Niger Delta province covers 300,000 km^2 and includes the geologic extent of the Tertiary Niger Delta (Akata-Agbada) Petroleum System (Kulke, 1995). First, shale diapirs formed from loading of poorly compacted, over-pressured, prodelta and delta-slope clays (Akata Fm.) by the higher density delta-front sands (Agbada Fm.). Second, slope instability occurred due to a lack of lateral, basin ward, support for the under-compacted delta-slope clays (Akata Fm). For any given depobelt, gravity tectonics were completed before deposition of the Benin Formation and are expressed in complex structures, including shale diapirs, roll-over anticlines, collapsed growth fault crests, back-to-back features, and steeply dipping, closely spaced flank faults (Evamy et al., 1978; Xiao and Suppe, 1992). The Tertiary section of the Niger Delta is divided majorly into three formations, representing prograding depositional facies that are distinguished mostly on the basis of sand-shale ratios. The three formation type are described in Short and Stäublee (1965) and Doust and Omatsola (1990). The Akata beginning in the Paleocene and through the recent was formed during lowstands when terrestrial organic matter and clays were transported to deep water areas characterized by low energy conditions and oxygen deficiency (Stacher, 1995). Little of the formation has been drilled so its estimated depth is about 7,000 meters thick (Doust and Omatsola, 1990). The formation is estimated to underlie the entire delta and it is as expected over pressurized. The deposition of the overlying reservoir; Agbada Formation which is the major petroleum- bearing unit began in the Eocene and continues into the Recent. The formation consists of parallic siliciclastics over 3700 meters thick and represents the actual deltaic portion of the sequence (Doust and Omatsola, 1990). The Agbada Formation is overlain by the third formation, the Benin Formation, a continental latest Eocene to Recent deposit of alluvial and upper coastal plain sands that are up to 2000 m thick (Avbovbo, 1978).

Weathering processes: The compositional maturity basically reflects the weathering process in source area and degree or extent of reworking/recycling and transportation. Typical compositionally immature sediments are located close to their source area or they have been rapidly transported and deposited with little reworking from a source area of limited physical and chemical weathering. The classification of sandstones and shales in this study is based on widely used but simple classification system which is a function of feldspar content, rock fragments, quartz grains and matrix after Pettijohn et al. (1972). Two percentages are used to subdivide terrigenous sandstone which is percentage of matrix (any clastic material finer than 30 microns or coarse silt). The other is its Composition of framework grains which is basically sand, quartz, feldspar, and rock fragments (lithics). These two major groups based on texture can be viewed as also being divided into whether the sandstones are composed of grains only, the arenites, or contain more than 15% matrix forming the wackes. For the arenite, quartz arenite is applied to those with 95% or more quartz grains, Arkosic arenites is a term for the arenite with more than 25% feldspar which exceeds the rock fragment content and litharenite is applied where the rock fragment exceeds 25% and is greater than feldspar. Two rock types transitional with quartz arenite are sub-arkose (5-25% feldspar and > rock fragments) and sub-litharenite (5-25% rock fragments and > feldspar). Climate in the source area can also play a major role in producing quartz arenites: tropical climate will breakdown and evacuate many of the unstable feldspar grains. A matures river channel, without steep slopes and generally undulating relief will enhance slow sedimentation rates and as such, quartz will dominate the detritus. Arkoses are known to be derived from feldspar especially orthoclase K-bearing feldspar in granites and granitoid gneiss. Climate and source area relief may also influence the production of arkoses apart from its provenance geology. Under humid conditions, feldspars are weathered to clay minerals, so that semi-arid and glacial climates favor arkose formation. A very rapid erosion in a very high-relief environment will also favor arkose formation.

Various works has been done on the Niger delta clastic sediments but the precise area of deposition, their maturity and sediment transport has been very limited. The main aim of this study is to investigate maturity and area of deposition and attempt to shed light on source area paleo-weathering conditions using different perspective.

Material and Methods

The sampled were collected from a well in an oil field in Niger Delta. The exact coordinates is not detailed due to propriety and ownership rights of the oil well. The pictorial location of the well on regional Nigerian map is shown in figure 1.

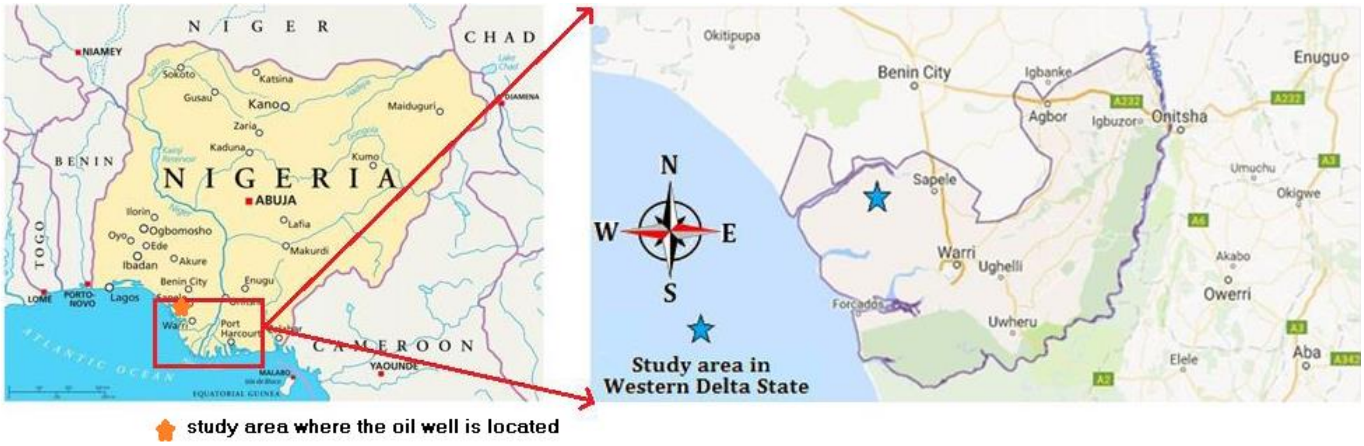

Figure 1. Pictorial location of the study area in western Niger Delta, Nigeria

The Niger delta extends from about longitudes 3°E and 9°E and latitudes 4°30′N to 5°21′N. It is situated at the intersection of an early cretaceous Atlantic and gulf of guinea triple junction. The low to intermediate weathering of the studied sediments may infer climate change towards arid and cold conditions that are unfavorable to weathering from situ. In this study, twenty-one core samples were collected and used for analysis. The analysis used in this project the major oxides, trace elements and heavy mineral analysis. The samples are first oven dried, washed with de-ionized water and dried again.

Major Oxides and Trace Elements

The samples were grinded and pulverized for both the major oxides and trace elements. The samples were reduced by sieving and crushing through a < 75μm. About 10g of the pulverized sample was packed in a suitable bag and sent to Acme analytical Laboratory LTD., Vancouver, Canada. The analyses were carried out by both Induced Coupled Plasma-Mass Spectrometry (ICP-MS) for trace elements and Induced Coupled Plasma-Emission Spectrometry (ICP-ES). The results of the major oxides are as illustrated in table 1 while trace elements in table 2.

Heavy Mineral Separation: Pretreatment, Setup and Procedure

Heavy mineral analysis of the sandstone sequence entails preparation of samples which includes size reduction, disaggregation and cleaning. The eight sand samples were subjected to pre-treatment by dis-aggregating about 50g 0f each of the coherent sediment samples to liberate individual mineral grains. It is followed by acid digestions which include boiling in dilute HCl for 15 minutes to remove dolomites. It was later soaked in dilute HCl for 24 hours so as to remove the carbonates and calcareous particles from the unconsolidated sediment. It also rid the grains of iron oxide coating. The samples are washed thoroughly with distilled water to decant the removed carbonate and any clay fraction present. The sample is spread on filter paper, air dried and further oven dried.

The gravity settling techniques was used for the separation of the heavy minerals. This was accomplished by the use of bromoform of specific gravity 2.80. The light mineral grains either floated or were trapped as middling within the bromoform while the heavy mineral settled into the stem of the separating funnel. 8 grams of each pre-treated sample is poured into the separating funnel (containing bromoform) and stirred thoroughly until the heavy mineral could no longer be observed to settle into the stem of these separating funnel.

Table 1. Eleven major element oxides in percentages after Oni et al. (2014)

Samples Depth (in meters)	Lithology	FeO	Fe_2O_3	CaO	P_2O_5	MgO	TiO_2	Al_2O_3	Na_2O	K_2O	MnO	SiO_2	Total
		%	%	%	%	%	%	%	%	%	%	%	%
1160-1180	Sand	0.5	0.5	1.1	bdl	0.1	0.1	1.7	0.2	0.2	bdl	95.5	100.0
1560-1580	Shale	0.4	0.5	0.6	bdl	0.1	0.1	1.5	0.4	0.3	bdl	96.0	100.0
1960-1980	Sand	1.0	1.1	0.8	bdl	0.1	0.1	1.7	0.6	0.6	bdl	93.8	100.0
2960-2980	Shale	2.2	2.4	0.4	bdl	0.2	0.3	5.1	1.8	1.7	bdl	85.8	100.0
3960-3980	Shale	4.3	4.7	0.4	bdl	0.4	0.7	11.1	2.6	3.0	bdl	72.7	100.0
4560-4580	Shale	3.9	4.4	0.4	bdl	0.4	0.7	13.5	3.3	3.1	bdl	70.1	100.0
5460-5480	Shale	3.6	4.0	0.2	bdl	0.1	0.3	3.8	4.6	2.9	bdl	80.4	100.0
5760-5780	Shale	4.7	5.2	0.2	0.1	0.1	0.8	6.5	4.5	2.9	bdl	74.9	100.0
6160-6180	Shale	3.4	3.8	0.7	0.1	0.2	0.6	11.0	3.7	2.8	bdl	73.8	100.0
7060-7080	Sand	2.9	3.2	0.7	0.2	0.1	0.3	3.1	6.0	4.3	bdl	79.2	100.0
7260-7280	Sand	2.8	3.2	0.7	0.1	0.1	0.3	4.4	6.8	4.7	bdl	76.9	100.0
7560-7580	Sand	3.2	3.6	0.6	0.1	0.2	0.3	5.4	6.0	4.5	bdl	76.0	100.0
7760-7780	Shale	4.3	4.8	0.8	0.1	0.6	0.6	9.0	1.9	3.1	bdl	74.7	100.0
7960-7980	Shale	2.8	3.1	0.8	0.1	0.2	0.3	5.4	4.7	3.9	bdl	78.6	100.0
8060-8080	Shale	2.9	3.2	1.4	bdl	0.6	0.2	3.5	1.0	1.7	bdl	85.5	100.0
8160-8180	Sand	3.0	3.4	3.1	0.1	1.6	0.3	4.8	0.7	1.7	bdl	81.2	100.0
8560-8580	Sand	3.8	4.2	1.7	0.1	0.4	0.2	3.9	1.0	1.6	bdl	82.9	100.0
8960-8980	Shale	4.7	5.2	0.9	0.1	0.4	0.8	11.7	0.9	2.5	bdl	72.7	100.0
10360-10380	Shale	2.7	3.0	0.5	0.1	0.3	0.4	6.0	1.3	2.8	bdl	82.8	100.0
11060-11080	Shale	7.7	8.6	0.7	0.2	0.9	0.8	13.5	1.2	2.7	0.1	63.6	100.0
11460-11480	Shale	6.1	6.8	0.5	0.1	0.5	0.9	14.6	1.1	2.1	0.1	67.2	100.0

*bdl values are values below detection limit at approximately 0.0.

Table 2. Some trace element in ppm as reported from the analysis with their corresponding MDL values

Samples Depth In meters	Lithology	Mo	U	Th	Sr	Cd	Cr	W	Zr	Sn
1160-1180	Sand	1.42	0.7	3.4	59	0.05	10	3.0	43.9	1.0
1560-1580	Shale	0.63	0.6	2.6	69	0.03	7	3.1	32.4	0.6
1960-1980	Sand	1.09	0.8	3.2	68	0.07	12	5.7	44.0	0.9
2960-2980	Shale	3.50	1.6	5.5	64	0.16	36	8.2	90.4	1.7
3960-3980	Shale	3.38	2.8	9.9	123	0.19	70	13.7	163.0	2.3
4560-4580	Shale	3.31	2.8	10.4	97	0.19	80	8.6	148.0	2.8
5460-5480	Shale	6.29	0.8	3.8	34	0.19	63	17.1	93.3	1.9
5760-5780	Shale	6.70	1.2	5.8	46	0.26	82	44.8	176.5	2.6
6160-6180	Shale	3.50	1.7	6.8	252	0.20	67	6.1	154.8	2.5
7060-7080	Sand	2.85	0.5	2.6	447	0.32	61	9.4	77.8	1.8
7260-7280	Sand	3.67	1.1	1.7	247	0.33	94	9.6	100.7	2.0
7560-7580	Sand	2.90	0.8	2.2	236	0.50	125	10.4	83.6	1.8
7760-7780	Shale	2.35	1.6	6.6	225	0.14	57	8.4	115.6	2.0
7960-7980	Shale	2.94	0.7	2.2	284	0.54	166	17.4	76.3	2.1
8060-8080	Shale	3.58	1.1	3.4	183	0.17	41	26.8	69.0	1.2
8160-8180	Sand	6.37	1.5	6.4	168	0.48	51	36.5	105.2	1.5
8560-8580	Sand	2.97	0.8	3.8	162	0.53	197	58.0	53.8	1.6
8960-8980	Shale	3.34	2.3	10	193	0.45	94	11.4	156.0	2.4
10360-10380	Shale	1.57	1.1	6.6	164	0.26	46	2.8	97.6	1.1
11060-11080	Shale	2.15	1.9	10.1	168	0.21	93	2.2	182.3	3.1
11460-11480	Shale	2.67	1.9	9.0	235	0.22	96	2.8	190.8	3.1
	MDL	0.05	0.1	0.1	1	0.02	1	0.1	0.2	0.1

The mixture was then allowed to settle for about 20 minutes. The accumulated heavy minerals in the stem were allowed to pass with the bromoform into the filtering funnel by carefully opening the stop-lock. The heavy minerals were held back on the filter paper in the funnel while the bromoform drained away into the 500ml conical flask as filtrate. The re-cycled bromoform is used again. The filter paper was then removed from the measuring funnel and placed on a clean flat surface where it was mildly washed with acetone, and allowed to dry. The acetone helps to neutralize the effect of the bromoform. Separated heavy minerals were spread on the mountant which consist of a glass slide with Canada balsam. The preparation includes the splitting of the heavy mineral fractions. It also includes grain mounts in which the grains were sprinkled evenly unto the surface of a glass slide with Canada balsam. The advantage of the Canada balsam is that it has mollass-consistency which is excellent for crystal rolling. This enables the examination of grains in different orientations and thus assists greatly in the identification of difficult minerals.

The microscopic identification and grain counting which involves the use of ribbon grain counting method was employed. Five strips of masking tapes were used to cover the section of the slide on which the heavy minerals were mounted mimicking the ribbon technique after Galehouse (1971). The sample slides were thoroughly previewed in systematic transverse starting from the base. Following standards used for identification, a tally of 300-500 grains in several transverses across the slides were used to get a reasonable percentage of each mineral present. This was achieved by removing a strip of masking tape at a time and then counting from base to top of the exposed section of the slide the total number of mineral grains. After that the non-opaque minerals were identified, counted and tabulated.

Results and Discussion

Depositional Environments Signatures

Ternary plots of Englund and Jørgensen (1973) proposed that certain classification of soil samples may be employed to ascertain the depositional environment of the sediments of the basin. This employs the chemical classification on the basis of AKF [Al_2O_3-(K_2O+Na_2O+CaO)-(Fe_2O_3+MgO)] contents. The samples were plotted on the ternary diagram of the AKF plots which reveals whether the sediments are deposited in continental, transition and/or marine zone. The results show a gradual transition of the sediments of the basin from continental to marine environment, majorly falling within the transition zone as indicated in figure 2.

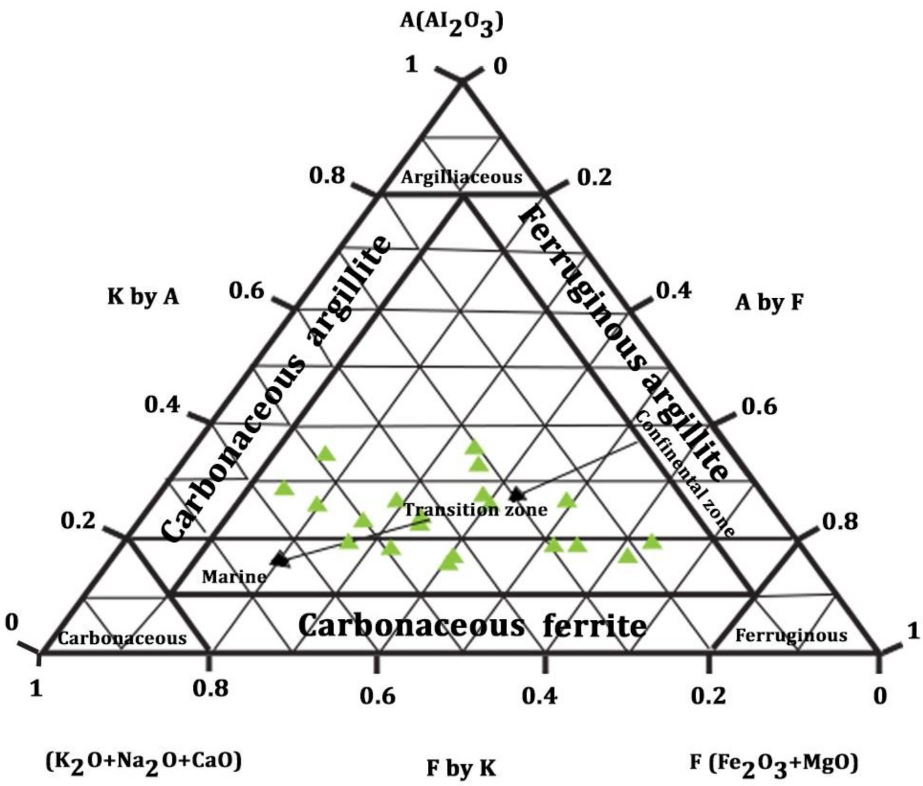

Figure 2. A-K-F plots for sediments from well "Y" after Ternary plots of Englund and Jørgensen (1973).

Maturity of Sediments

Major oxide geochemical analysis can be used to determine sediment classification and maturity conditions. The clastic sediments are considered as sodic and non-calcareous continental sands. A Low CaO oxide suggests a chemical destruction under oxidizing conditions during weathering. Sediments with little or no tertiary material have little or no MnO, P_2O_5 and relatively high Al_2O_3 values

Ratio of Quartz to Feldspar and Rock Fragments

This ratio is an index of compositional maturity, reflecting the difference between super mature quartz arenites typically white to light gray consisting almost entirely of sand-sized monocrystaline quartz grains which contains heavy minerals such as tourmaline, zircon, and rutile as well as resistant grains of chert, metaquartzite (95% - 97% SiO_2, 0.5% - 1.0% Al_2O_3), and dirty sand with lots of soft, unstable, decomposable rock fragments and feldspar. It is a ratio that zones the samples into arenites that are texturally clean, matrix free/matrix poor arenacious material or into argillaceous matrix rich wackes/dirty sandstones. Continuous weathering and recycling should ultimately generate sand residues composed of only the most resistant materials which are the quartz arenites. In these ratio plots, the quartz usually SiO_2 is used to correlate with feldspars. In terms of mineralogical maturity, a rock is immature when it contains a mix of stable (quartz arenites) and unstable (feldspar and clay) minerals, and is mature when it contains only stable (quartz) minerals. By describing sedimentary rocks in terms of their percentage of quart, feldspar, and lithic fragments (and ignoring accessory minerals), it is possible to use ratios as index markers. This implies that the ratio of feldspar to lithic fragments can serve as an index of sands provenance. If a rock has more lithic fragments relative to feldspars, then the source rock is probably resistant supracrustal rocks (like volcanics). If a rock has more feldspars to lithic fragments, then the source rock is probably less resistant subcrustal rocks (like plutons).

Commonly employed geochemical criteria for estimate of sedimentary maturity are the SiO_2 content as well as SiO_2/Al_2O_3 ratio (Pettijohn et al., 1972). This reflects the relative presence of clays, feldspar and quartz content. There is a more efficient method to determine chemical maturity by determining the alkali content (Na_2O + K_2O), which is a function of the feldspar content. Using an index of chemical maturity and the Na_2O/K_2O ratio, Pettijohn et al. (1972) proposed a classification of terrigenous sands based upon a plot of log (Na_2O/K_2O) vs log (SiO_2/Al_2O_3). The plot for the analyzed samples is as shown in figure 3. Herron (1988) modified the diagram of Pettijohn et al. (1972) using log (Fe_2O_3/K_2O along the Y-axis instead of log (Na_2O/K_2O). The SiO_2 content and SiO_2/Al_2O_3 ratio are the most commonly used geochemical criteria to determine the abundance of quartz, feldspar and clay contents and for differentiating mature and immature sediments (Potter, 1978). The ratio Fe_2O_3 (total)/K_2O allows arkoses to be more successfully classified and its mineral stability may be easily accessed. As confirmed for ferromagnesians, they tend to be less stable during weathering and this is illustrated as Fe-Shale transition to Fe-sand and finally disappearing with intense weathering in the more resilient quartz arenites. The plot is shown in figure 4. The ratio Fe_2O_3 (total)/K2O allows arkoses to be clearly illustrated.

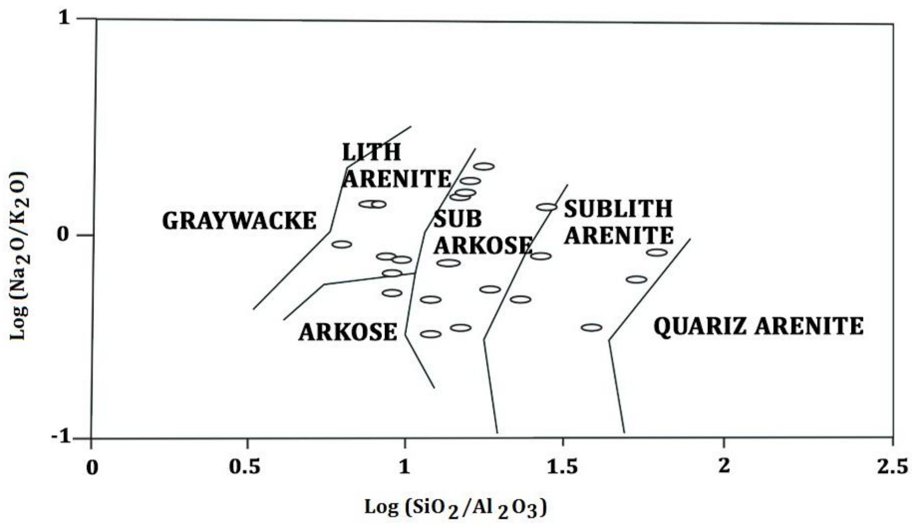

Figure 3. The classification of terrigenous sandstones using log (Na_2O/K_2O) vs log (SiO_2/Al_2O_3) from Pettijohn et al. (1972) with boundaries redrawn by Herron (1988).

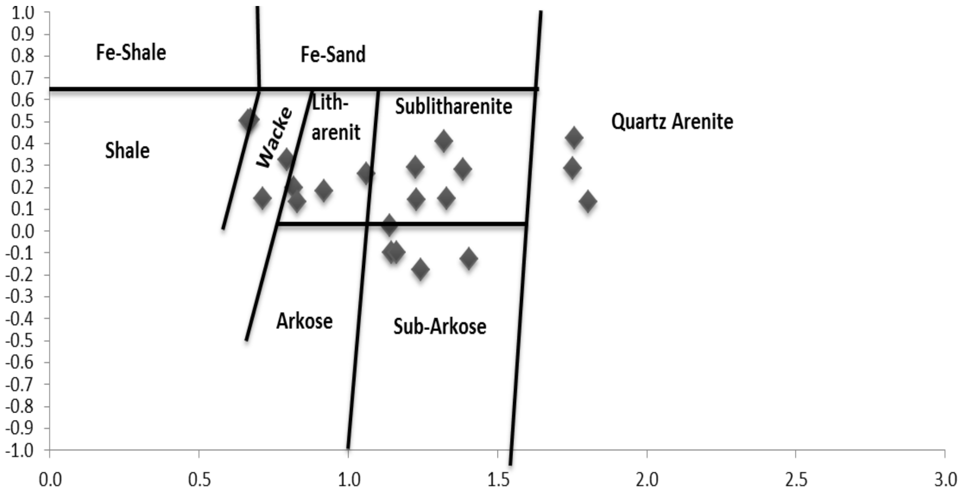

Figure 4. The classification of terrigenous sandstones and shales using log (Fe_2O_3/K_2O) vs log SiO_2/Al_2O_3) after Herron (1988).

The plot of figure 3 and figure 4 based on the basic oxides can be used to deduce the percentage of quartz, feldspars and lithic fragments. As observed from figure 3 and 4, most of the samples plotted as litharenites, sublitharenites and subarkose. Majority of the samples in litharenites implies the rock fragment exceeds 25% and is greater than feldspar. Litharenites usually contain 30-80% quartz and 25-50% lithic fragments. The compositional maturity may vary broadly depending on the nature of the lithic fragments but generally litharenite sediments are termed immature according to Folk classification system that prescribed sediments containing more than 5% clay and sand grains as poorly sorted, angular as immature. The sublitharenites implies 5-25% rock fragments and > feldspar while sub-arkose is 5-25% feldspar and > rock fragments. According to folk classification of textural maturity, a sedimentary rock is texturally immature when it contains greater than 5% clays/silt or a larger portion of unstable minerals such as feldspar, and lithic fragments. The litharenites, sublitharenites and subarkoses points to textural immaturity of the sediments

The ratio of quartz to other components (feldspar and lithic fragments combined) is an index of compositional maturity. Sediment with low proportion of quartz and a higher proportion of soft-unstable elements is an immature rock. If the sample contains a high proportion of resistant, stable quartz compared to the unstable elements it is a more mature rock. In terms of textural maturity, a rock is immature when it contains angular, poorly sorted grains, and it is mature when it contains rounded, well-sorted grains. Very few samples plotted in quartz arenites in figure 4 and none in figure 3 confirms majority of the sediments are angular and poorly sorted, probably produced from short transportation and limited reworkings. This emphasized the feldspars, lithic and angular fragments have not been broken down by mechanical weathering, wearing and abrasion to remain very stable and resilient quartz arenites.

Source Area Weathering Provenance

The concentrations of major elements can be affected by diagenesis and metamorphism, so that prior to any interpretation, their weathering from the source before mobility must be studied (McLennan, 2001). The weathering history of ancient sedimentary rocks can be evaluated in parts by examining the relationship among the alkali and alkaline earth elements (Al_2O_3, CaO, Na_2O and K_2O) (Nesbit and Young, 1989). Generally the alteration of igneous rock during weathering results in the depletion of alkali and alkaline earth elements and preferential enrichment of Al_2O_3 in the sediments. Al_2O_3 and K_2O content may relate to the presence of potassium feldspars (orthoclase and microcline), illite and mica. The source of Na_2O is principally related to plagioclase feldspar since its (Ca-Na) feldspar. MgO content is related mostly to the presence of dolomitic materials as fragments or cement. Calcite cement and rock fragments are the main source for CaO. The weathering effects are evaluated in terms of the molecular percentage of the oxide components as well as estimated mineralogical composition. A good measure of the degree of chemical weathering can be obtained by the calculation of Silicate weathering indexes such as

Chemical index of alteration CIA (Nesbit and Young, 1989).

$$\text{The CIA} = 100 * [(Al_2O_3 / (Al_2O_3+CaO^*+Na_2O+K_2O)]$$

CaO* is the calcite bound in silicates. The CIA can be shown graphically with the A (Al_2O_3) – CN (CaO+Na_2O) – K (K_2O) composite plots generally termed A-CN-K ternary diagram, with the corresponding CIA values to the left as shown in figure 5. This values obtained from the ternary plots sharply equates the calculated values from the CIA formula given above. High CIA values reflect removal of labile cations (Ca^{2+}, Na^+, K^+) relative to stable residual constituents (Al^{3+}, Ti^{4+}) during weathering implying intensive weathering activities probably with long distance, slow sedimentation rates and humid tropical conditions. Conversely, low CIA values indicate the near absence of chemical alteration and might reflect cool and arid conditions (Fedo et al., 1995).

Table 3. Table showing the sample depth with corresponding silicate weathering indices: Ruxton ratio, CIW, PIA and CIA values

Samples Depth In meters	Lithology	RUXTON RATIO	CIW VALUES	PIA VALUES	CIA VALUES
1160-1180	Sand	57	55	52	51
1560-1580	Shale	64	59	53	52
1960-1980	Sand	56	54	43	45
2960-2980	Shale	17	70	61	56
3960-3980	Shale	7	79	73	65
4560-4580	Shale	5	79	74	67
5460-5480	Shale	21	44	16	33
5760-5780	Shale	12	58	44	46
6160-6180	Shale	7	72	65	60
7060-7080	Sand	21	32	-20	22
7260-7280	Sand	18	37	-5	26
7560-7580	Sand	14	45	12	33
7760-7780	Shale	8	77	68	60
7960-7980	Shale	15	50	21	37
8060-8080	Shale	8	60	44	47
8160-8180	Sand	17	56	45	47
8560-8580	Sand	21	60	46	48
8960-8980	Shale	6	87	84	73
10360-10380	Shale	14	76	63	56
11060-11080	Shale	5	87	85	75
11460-11480	Shale	5	90	89	80
AVERAGE		18.95	63.19	48.2	51.4

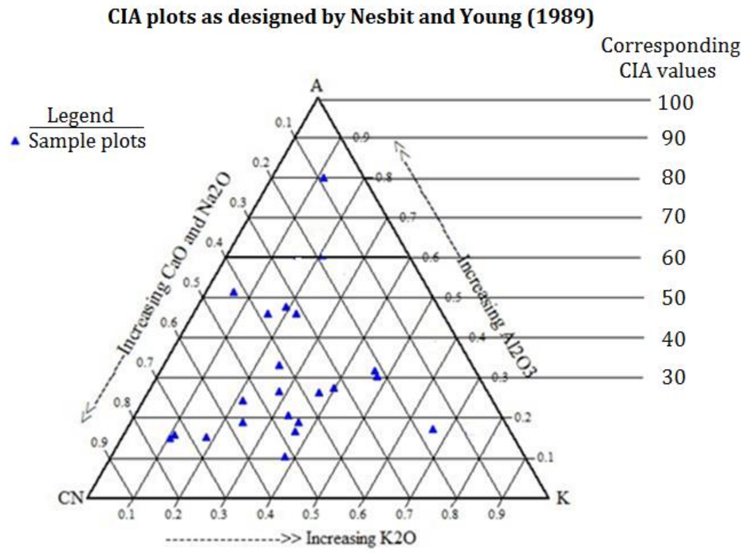

Figure 5. CIA value from the samples on plot of A-CN-K denoting (Al_2O_3-CaO&Na_2O-K_2O) as designed by Nesbit and Young (1989).

The calculation of chemical index of alteration is a useful means to describe the degree of weathering. This is because the CIA provides vital information on the relative abundance of the unweathered materials especially the feldspars which contains the labile cations (Ca^{2+}, Na^{+} and K^{+}) (Osahe et al., 2006). The higher the CIA values, the closer the CIA value is to 100, the more weathered the rock. A CIA value of 100 suggests complete alteration of feldspar and labile to fine argillaceous aluminum rich clay weathering products, which are basically kaolinite and chlorite (with no feldspar).

Weathering indices of sedimentary rocks can provide useful information of tectonic activity and climatic conditions in the source area. According to Jacobson et al. (2003), the increase in chemical weathering intensity has been attributed to decrease in tectonic activity and/or the change of climate towards warm and humid conditions which are more favorable to chemical weathering in the source region. The low to intermediate weathering of the observed sediments may thus be linked with increase in tectonic activity and/or climate change towards arid and cold conditions that are unfavorable to weathering from situ.

A derived CIA value between 90 and 100 (near 100) implies fine argillaceous smectite or illite sediments with very minute/minimal feldspars. The rate of chemical weathering and soil formation is largely controlled by climate. Weathering intensity and chemical maturity is a function of climate and rates of tectonic uplift (Wronkiewicz and Condie, 1989). Kaolin minerals tend to develop preferentially within the tropics while chlorite and smectite tends to be preserved in artic regions. Average shale CIA value ranges around of 75. CIA and PIA (Plagioclase index of alteration PIA) between 60 and 75 indicates moderately/average intensive weathering in the source area. This two indices also helps to prove that although there was intensive weathering in the source area, the sediments as well did not travel far before been deposited. We can thus conclude a high CIA and PIA values (75-100) indicates intensive weathering in source area with little or no feldspar present whereas low values that are lesser than 50 indicates low weathering in the source area. Unweathered granite has a CIA of 50 which also the reference CIA for upper continental crust. At 50, it corresponds to upper continental crust and anything below 40 indicates that the site has consistently received sediments (with feldspars) that have undergone very little or no chemical weathering (Nesbitt and Young, 1989).

CIA is also much affected by grain size. It increases as grain size decreases. Also noteworthy is to mention that with increase in CIA, the porosity and permeability decreases. CIA values needed to be interpreted with caution. If the samples plot mostly in the upper part of the Illite /Kaolinite, it is an indication of a high degree of alteration as shown in figure 5. It will be seen most of the unweathered samples plotted near the calcium-sodium (CN) end indicate that the feldspars present are in form of plagioclase feldspars The dominance of unaltered feldspars as inferred from geochemical data suggests that the immature nature of sediments. The Niger Delta sediments probably formed from low residence times in the source region waters or river basin and quick removal of materials without continuous recycling or formed from rapid moving waters/ steep mountainous rivers (physical weathering dominates). Furthermore, deviations from ideal weathering trends can give information on the type of alteration, as for example K-metamorphism which discloses the alteration of plagioclase/K-feldspar to aluminum rich illite clay minerals to (Fedo et al., 1995)

Plagioclase index of alteration PIA (Fedo et al.,1995)

$$\text{The PIA} = 100 * [(Al_2O_3-K_2O) / (Al_2O_3+CaO+Na_2O-K_2O)]$$

The PIA values can also be shown pictorially with the ternary plot of (Al_2O-K_2O)-CaO-Na_2O diagram. And just like CIA, the values given by the ternary plot is the same as the values calculated from the formula above. The combination of CIA and PIA avoids misleading interpretation of too low CIA values and reveals a more realistic degree of chemical weathering. The PIA was determined following Fedo et al. (1995). The PIA generally reports higher values than CIA in K-metasomatized rocks. From the studied samples results, it is shown in table 6 that PIA is lesser (48.2) than CIA in table 5 (51.4). It is very correct to infer that the provenance of Niger Delta clastic sediments were more of plagioclaise (NaO, CaO) than orthoclase (K-metasomatized) rocks. Integrating the CIA and PIA values of both sand and shales, it seems that the Niger Delta sediments were derived from the different zones of weathering, and likely from rapid erosion of fast rising recycled orogens. The values obtained from the PIA are very similar to the CIA and their inference or interpretations are the same.

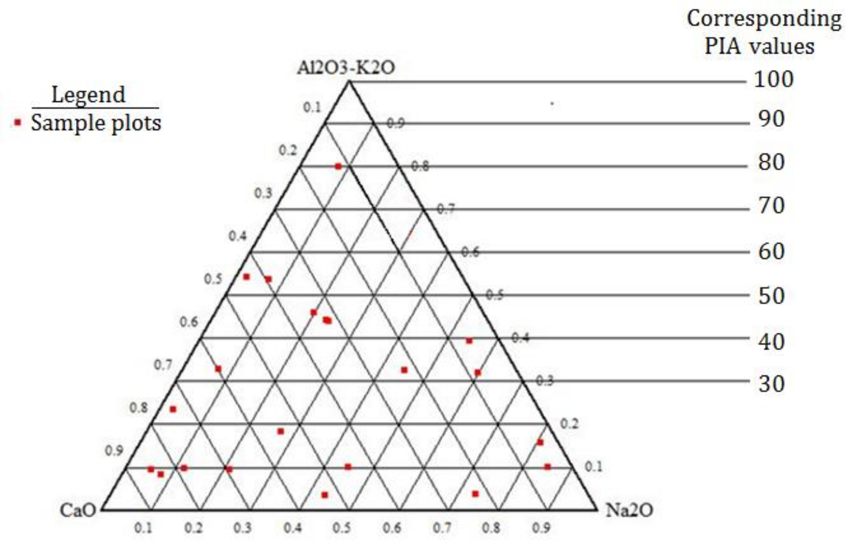

Figure 6. The values of the (Al_2O_3-K_2O)-C_aO-Na_2O and the corresponding PIA values after Fedo et al. (1995).

Chemical index of weathering CIW (Harnois, 1988)

The CIW = 100 * [(Al_2O_3) / (Al_2O_3+CaO+Na_2O)]

The role of chemical and mineralogical weathering indices is essentially to quantify the degree of depletion of mobile components relative to immobile components during weathering (Harnois, 1988). According to Harnois (1988), a CIW between 0-40 is classified as low weathered, 40-65 as intermediate or moderately weathered and greater than 70 as highly weathered sediments. From table 2, the average CIW is derived as 63.19, which made the sediments to be interpreted as moderately weathered.

Ruxton ratio: The ruxton ratio RR is given by RR = (SIO_2/AL_2O_3)

Ruxton (1968) prescribed the Ruxtion ratio of a digit (0-9) as intense chemical weathering, 10-30 as intermediate or moderate weathering while greater than 30 is interpreted as very weak chemical weathering. RR value greater than 30 suggests weak chemical weathering while those with one digit value (0-9) indicate high level of chemical weathering. From the RR results obtained, it is observed that majority of the samples have two digit value >10 implying low to medium level of weathering. However the instability or the alternation between high and low RR values is a clear indication of the highly weathered shale and less weathered (poorly sorted, immature) sand sediments.

Maturity in terms of feldspar/quartz and argillaceous sediments

Colin et al. (1994) identified that sediments that evolved from increased physical and chemical weathering as characterized by sediments deposited during the cretaceous is observed to have a decrease smectite (illite-chlorite) ratio. It implies that there is more of chlorite than illite with increasing intensity of weathering. As long as feldspars persist, the sediments will remain compositionally immature (Nesbitt and Young, 1996). In line with Medaris et al. (2003), absence of feldspar in sediments and sedimentary rocks is a characteristic feature of super mature sedimentary rocks. This agrees with Fedo et al. (1996) that if the source rocks experienced intense chemical weathering, feldspars present in the source rock would be altered totally as aluminous clay. Repeated cycles of weathering and abrasion during transport eventually result in destruction of feldspars and formation of clay minerals (Nesbitt and Young, 1996). As such decrease in smectite (illite-chlorite) ratio or increase in magnitude of samples plotting near the chlorite/kaolinite end which is at the top of the triangle is an indication of supermature, feldspar free sediment/sedimentary rock. However the presence of feldspars in the samples of this study as inferred from CIA and PIA plot suggest their derivation by physical and/or moderate chemical weathering.

Large-ion lithophile elements (Rb, Sr, K, and Na) in relation to CIA values and chemical weathering.

It is established that in chemical weathering intensity rapidly leaches Sr compared to Rb (Nesbitt and Young, 1982); implying that Rb/Sr ratio increases with increasing weathering and consequently increasing CIA values (Ma et al., 2000). In the same manner, K will shows rapid depletion against Rb also implying

Rb/K values varies directly with weathering intensity and CIA as well (Wronkiewicz and Condie, 1989). Rb has been studied and considered to be primarily fixed in weathering residues and less reactive than Ca, Na, and Sr (Woyski and Harris, 1963). Rb/Sr ratios in the illite minerals are usually greater than 1 (Chaudhuri and Brookings, 1979). None up to 1 in the studied samples as shown in table 11. It has a maximum value of 0.46 implying that the sediments had not suffered intense chemical weathering and is low in alumina/clay. This is confirmed by the low CIA value averaging 51.4 as well as the samples plotting below the Illite level (90). The Rb/Sr ratios of sediments and sedimentary rocks can thus be used to monitor the degree of source rock weathering (McLennan et al., 1993). The Rb/Sr, Rb/K ratios of studied sediment samples are as shown in table 4.

Table 4. Some analysed elemental ratios of trace elements in the samples.

Samples Depth	Lithology	AL/NA	K/NA	Rb/Sr	Rb/K
1160-1180	Sand	5.06	0.98	0.23	80.00
1560-1580	Shale	2.48	0.87	0.20	50.00
1960-1980	Sand	1.88	1.04	0.30	41.63
2960-2980	Shale	2.01	1.08	0.46	20.41
3960-3980	Shale	3.08	1.32	0.39	18.92
4560-4580	Shale	2.94	1.06	0.40	15.06
5460-5480	Shale	0.58	0.69	0.45	6.50
5760-5780	Shale	1.04	0.72	0.32	6.22
6160-6180	Shale	2.12	0.85	0.14	14.79
7060-7080	Sand	0.37	0.79	0.06	7.20
7260-7280	Sand	0.46	0.78	0.09	5.41
7560-7580	Sand	0.64	0.83	0.12	7.86
7760-7780	Shale	3.31	1.80	0.20	17.42
7960-7980	Shale	0.83	0.94	0.09	7.94
8060-8080	Shale	2.59	1.93	0.17	22.16
8160-8180	Sand	5.09	2.85	0.20	23.99
8560-8580	Sand	2.83	1.84	0.15	17.50
8960-8980	Shale	9.16	3.05	0.24	22.23
10360-10380	Shale	3.25	2.40	0.30	20.72
11060-11080	Shale	7.82	2.41	0.27	20.36
11460-11480	Shale	9.52	2.18	0.18	11.81

Scatter plot of Al/Na ratio versus chemical index of alteration (CIA): This is another way to show the silicate weathering extent at glance is to plot the Al/Na against CIA values as shown in fig 8. From the scatter plot of Al/Na ratio versus chemical index of alteration (CIA) it is observed that there is a very strong correlation between both indexes, which reflects the silicate weathering intensity. The Al/Na ratio expresses the abundance and increase of intensely weathered Alumina and or clay particles (Al_2O_3) over unweathered arenacious arenites and wackes; especially feldspars. As the Al/Na ratio increases, the Alumina, weathering intensity and CIA values increases and the feldspar decreases.

Heavy Mineral Analysis

Apart from major, trace and rare earth elements, heavy mineral studies of the sedimentary rocks are a powerful tool in identifying provenance (Morton, 1985). The term heavy mineral applies to minor assessory mineral constituents of rocks having ordinarily specific gravities higher than 2.89g/dm^3. This high density accessory mineral constituents of siliciclastic sediments are separated using liquids with densities of 2.89 (bromoform) or 2.96 (tetrabromoethane) in which they sink hence the name heavy minerals. Heavy minerals in their parent rock they are present either as essential rock forming minerals (e.g. amphiboles, pyroxenes, micas) or as accessory components such as zircon, apatite, tourmaline, occurring in a variety of rock types. Having a good knowledge of the mineral characteristics in surface and diagenetic environments can be used to deduce sedimentary processes and factors operating in depositional environments. This is because crystallization of minerals begins during the petrogenesis of the rock when the properties of the crystal such as structure, morphology, color, twinning and many others are formed. The effects of transport, climate, alluvial storage, sedimentary reworking and redeposition, burial diagenesis and recycling are mostly preserved within the structural properties and surface textures of a particular heavy mineral grain. The recognizing of these chemical and physical signatures enables the critical assessment and interpretation of the prevalent conditions and the paleo-environment under which the sediments are deposited. The chemical stability of a particular heavy mineral can be determined from the pH of the geochemical environment. A heavy mineral assemblage seems to respond differently to extremes of acid environments (such as typical lateritic or humid–tropical weathering conditions) and alkaline environments (as typical in desert soils or saline brines associated with hydrocarbon reservoirs).

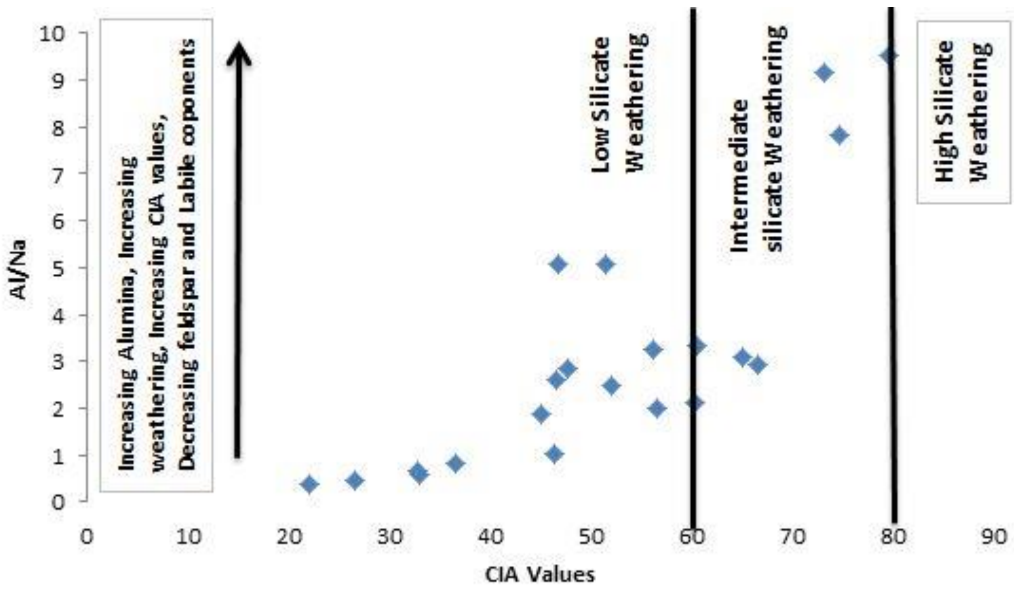

Figure 7. Scatter plot of Al/Na ratio versus chemical index of alteration (CIA)

Table 5. Table showing the stable and ultrastable elements as observed by grain count under the microscope.

Sample Depth in meters	Zr	Ru	To	Ga	Ap	Ep	Mu	St	Total Opaque	Total Non Opaque	Z+T+R	Z%	R%	T%	ZTR INDEX
1160-1180	11	10	6	5	8	5	5	2	64	36.0	27.0	40.7	37.0	22.2	75.0
1960-1980	13	8	8	12	4	3	2	2	93	46.0	29.0	44.8	27.6	27.6	63.0
7060-7080	15	11	13	9	2	-	4	5	55	58.0	39.0	38.5	28.2	33.3	67.2
7260-7280	9	5	6	7	2	2	7	4	69	55.0	20.0	45.0	25.0	30.0	36.4
7560-7580	11	6	5	11	3	2	4	1	80	51.0	22.0	50.0	27.3	22.7	43.1
8160-8180	10	7	6	9	4	1	2	1	72	52.0	23.0	43.5	30.4	26.1	44.2
8560-8580	9	10	3	13	3	2	6	1	43	37.0	22.0	40.9	45.5	13.6	59.5
Average	11.1	8.1	6.7	9.4	3.7	2.1	4.3	1.0	68	47.9	26.0	43.3	31.6	25.1	55.5

Key

Zr	Zircon	Ru	Rutile
To	Tourmaline	Ga	Garnet
Ap	Apatite	Ep	Epidote
Mu	Monazite	St	Staurolite
ZTR	Zircon Tourmaline Rutile Index		

ZTR Index

Heavy mineral suites may also be used as an index of maturity using the ZTR index. The ZTR index is a method of determining how weathered, both chemically and mechanically a sediment (or a corresponding sedimentary rock) is. The letters in ZTR stand for 3 common minerals found in ultra-weathered sediments: zircon, tourmaline, and rutile. Other minerals that can be used along the ZTR index are garnet, magnetite, sphene, and other minerals from local provenance sources. The ZTR index is commonly high in beach or littoral zone depositional environments due to the long transport distances from the source and the high energy of the environment. These minerals are found in abundance due to their high specific gravity and resistance to weathering. The ZTR index can also be used as a scale for the estimation of the degree of modification or maturity of the entire heavy mineral assemblage.

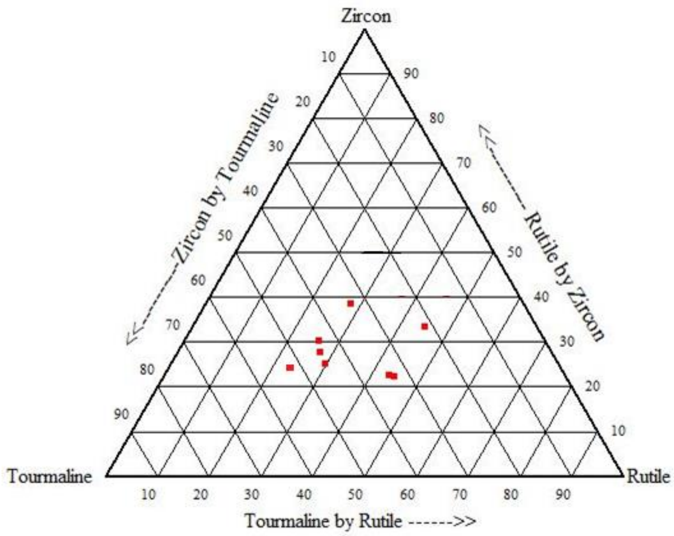

Figure 8. The ZTR ternary diagram of the eight sand samples

Statistics indicates that zircon of most sandstone are represented predominantly by rounded grains or angular fragments. Zircon is common heavy mineral, derived from granitic, volcanic, and metamorphic recycled sources. Kaolin deposits display a mature to super-mature assemblage of heavy minerals, represented dominantly by zircon. The lower zircon content (most samples plotted relatively far from Zircon end) for the plotted samples is an indication of immaturity, absence of Kaolin and presence of feldspars.

Rutile grains were yellowish to reddish brown, showed adamantine luster in reflected light and occurred mostly in small prismatic crystals. Rutile is an ultrastable mineral and is one of the three index species (ZTR), which are used to characterize the mineralogical maturity of a heavy mineral suite. Rutile is widespread accessory metamorphic mineral and mostly from high grade metamorphic rocks, particularly in schist, gneisses and amphibolite. The relatively higher rutile proportion indicates recycled metamorphic origin (Hubert, 1962).

Tourmalines are widespread in all types of detrital sediments and are ultrastable both mechanically and chemically. Tourmaline has proven particularly useful as a provenance mineral due to its presence in many rock types, chemical responsiveness to environment of formation, complex and variable chemical and mechanical weathering, and stability through digenesis and metamorphism. Tourmalines hardness and chemical stability make it extremely durable in sedimentary cycle. The heavy mineral assemblage (relatively higher concentrations) of tourmaline of rutile and zircon may indicate both igneous and metamorphic origin.

The opaque mineral species exceeded the non-opaque minerals (table 5). The large amount of heavy opaque minerals suggests oxic (oxygen-rich) environments of deposition (Odumoso et al. 2013). The heavy mineral garnets indicate a high grade metamorphic source while tourmaline, rutile and zircon indicate both igneous and metamorphic origin (Feo-Codecido, 1956).

The calculated ZTR index for the sand samples range from 36.4-75.0

ZTR Index = [(Z + T + R)/ Total non-opaque] * 100

Z = Zircon, T = Tourmaline, R = Rutile, ZTR Index = 55.5

The detrital epidote-group minerals, garnet and titanite in some of the samples indicate a major contribution from metamorphic sources. It is high (like 10%) in some samples while it is like 1-1.5% in some other samples. This variation probably is the result of influences from different sources for the different sediments. The variation could be produced by fluvial systems which change tracks through time, or that the sediments were fed by partly different fluvial systems. Further, it might mirror different tectonic activity in the source regions of the sediments.

According to Hubert (1962), the non-opaque or transparent non-micaceous heavy mineral assemblage of the quartz are predominantly zircon, tourmaline and rutile and these grains are ultimately concentrated in sands by prolonged abrasion. According to modification of heavy mineral association and provenance by Feo-Codecido (1956) the presence of zircon, rutile and tourmaline indicates an acid (felsic) igneous rock source of the sediments. The possibility of the source rock being basic igneous rock is very low because augite, diopside, hypersthene or olivine are largely absent from the heavy mineral assemblage. Instead Staurolite, Rutile and garnet occur in relatively fairly large quantities with respect to Epidote and Sillimanite, which are indicative of dynamo thermal metamorphic rock source.

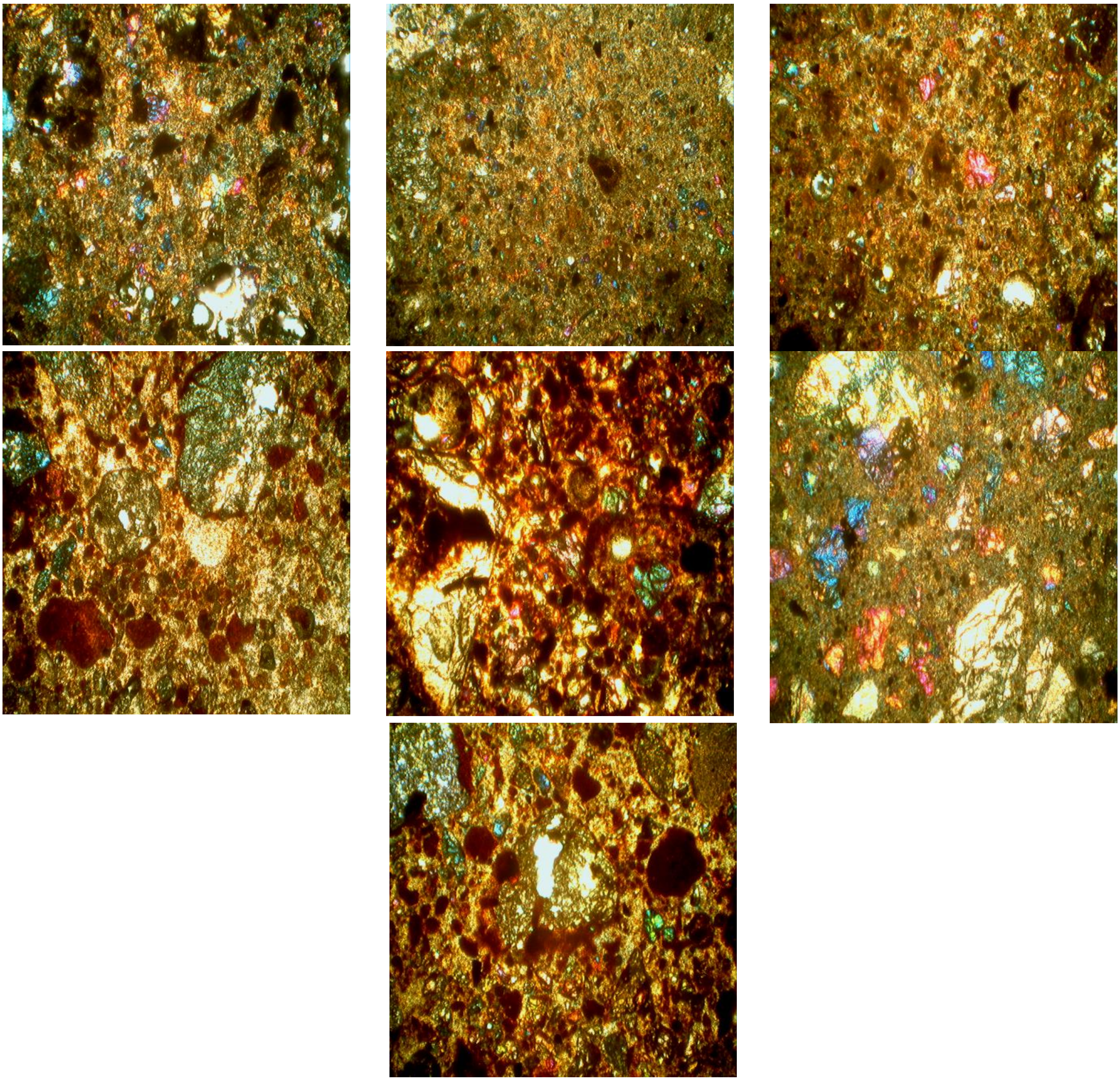

Figure 9. The photomicrograph of some of the samples

Conclusion

It is very obvious that the sediments are not matured. From the classification of terrigenous sandstone and shale based on Herron (1988) and Pettijohn et al. (1972), most of the samples plotted in litharenite, sublitharenite to sub-arkose and arkose. There is presence of matrix, while the grain shape sub-angular to sub rounded and only very few plotted in Quartz Arenite portion in Herron's diagram of figure 4. All this infer moderately mature sediment. From the CIA, PIA CIW and RR value obtained, it can be concluded that the trough has consistently received sediments (with feldspars) that have undergone very little or no chemical weathering and minimized sediment reworkings. The dominance of unaltered feldspars as inferred from geochemical data emphasize the immature nature of sediments. The less than 50 CIA and PIA average values, coupled with poor sorting, alternating values of Ruxtion ratio and angular fragments is an indication of the immaturity of the sediment. Repeated cycles of weathering and abrasion during transport will eventually result in destruction of feldspars and formation of clay minerals and since feldspars persist, the sediments are compositionally immature.

From the A-K-F ternary plot, it is very clear that the depositional environment is a transition zone between continental and marine zones. The AKF plots show a gradual transition of the sediments of the basin from continental to marine environment.

The heavy mineral analysis prescribes a granitic-metamorphic origin of these sediments. Comparisons of specific indicator heavy minerals, such as garnet and tourmaline also strongly support this conclusion. Tourmalines indicate granitic and low-grade metamorphic provenances. Garnets are indicator of granitic and metamorphic, reflecting the fact that probably the granite suffered from regional metamorphism on a large scale. A metamorphic origin for the angular and euhedral pink to green tourmaline can also be inferred by its association with garnet and staurolite. The concentration of garnet in sediments can be due to increase erosion of metamorphic rocks. The angular to sub-angular shape of the sandstone is an indication of a short distance of transportation or closeness to the source area. The appreciable concentration of feldspar, the dominance of sub-angular to angular shape and the poorly sorted nature of the sandstones of the study area infer textural and mineralogical immaturity. Z.T.R. averaging 55.5% equally corresponds to immature sediments. The opaque mineral more than non-opaque is indicative of oxic paleoenvironment. The occurrences of zircon, tourmaline, apatite, and sphene suggest a felsic igneous source (Oni et al., 2014). Thus the provenance or source of the sediments is acidic or felsic igneous rock. The results of the geochemical analysis revealed that the Niger Delta sandstones consist of relatively high SiO_2, Al_2O_3, Na_2O, K_2O, and CaO and these suggest a quartz and feldspar-rich source area. From all the analysis above, we can conclude that the sediments are immature to moderately matured and of felsic/acidic igneous (probably granitic) and/or metamorphic provenance.

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Land suitability evaluation for irrigating wheat by Geopedological approach and Geographic Information System: A case study of Qazvin plain, Iran

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Abstract

Land evaluation, using a scientific method, is essential to recognize the potential and limitation of a given land for specific use in terms of its suitability, and certifies its sustainable use. The soil is such a source that its renewal takes a long time, so effective use of soil and land resources requires a thorough understanding of the effective morphological processes of soil forming in different regions. The current study identified available soil in the area in terms of interpretation of aerial photographs and Geopedological approach. After mapping the geoform area, 61 profiles of the designated area were drilled and sampling was done for all diagnostic horizons. Then, the samples were transported to the laboratory for Physico-chemical analysis. By the end of the profile classification process, which was based on the Soil Survey Staff (2014), the soil map, was prepared by integration of the soil data and the geoform map in ArcGIS software. There are several limiting factors for wheat in Qazvin plain, namely; electric conductivity (EC), gypsum, coarse fragment, soil depth, soil organic carbon (SOC), texture, calcium carbonate and climate. The map of the land units was prepared, and land requirements for the type of utility were calculated. Land suitability evaluation was performed according to FAO. The results showed that land unit's number 17 and 18 were unsuitable (N1) for irrigating wheat with limiting factors such as; high levels of EC and gypsum in the studied profiles. Moreover, the land unit's number 10, 20, and 23 are suitable (S_1) for the wheat production and have the highest rate of predicted yield.

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Introduction

The term land suitability is the suitability of a given type of land to support a defined land use, either in its current state or after improvements (Gong et al., 2012). The explosion of the population and rising of living standards has led to greater demand for food. It is impossible to separate the subject of crop production from the environmental issues, so human attention has always been focused on increasing the production of the horticultural crops and more proper use of land. In their land evaluation, (Ayoubi and Jalalian, 2010). Rahsamavati (2012) used land suitability evaluation to determine the potential performance of the products and to monitor the compatibility of land for specific uses (FAO, 1976). In other words, addressing the prediction potential of land for a variety of applications, land evaluation concerns land requirements and

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general characteristics of the potential of the soil in various uses (Senol et al., 1996). In many cases, especially in semi-arid areas, accessing to usable agricultural land is an important limitation. It increases pressure on available land and in turn, it leads to land erosion and degradation (Elaalem et al., 2011). Therefore, it seems necessary to perform an accurate and reliable assessment of the land in order to take a decision on the processes involved in the development of land use policies. Today, land suitability evaluation in developing countries is considered as the main factor for selecting crop adapted to soil and climatic conditions of each region (Baqer Zadeh et al., 2012). There are several methods for assessing the land which is now increasingly used. Due to soil separation ability, which is more homogenous than the traditional soil mapping, the Geopedological method has been used in several studies (Gholizadeh et al., 2001) to evaluate land suitability assessment. This approach uses a hierarchical structure that leads to the purity of soil map units, especially in areas where there is a close relationship between geomorphology and area soils (Zinck, 1989). Remote sensing techniques (RS) and Geographic Information System (GIS) are used as analyses and prediction methods for making variety of planning and test results obtained from various decision makings (Rossiter, 2000). Although the GIS spatial analysis is not a novel concept, it constitutes a significant portion of land suitability analysis and mapping (Feizizadeh and Blaschke, 2013). A study conducted in Turkey evaluated the qualitative land suitability using Geographic Information System (GIS). The researchers reported that 40.1% of the land was allocated for wheat cultivation, and 54.1 and 65.8% of the land were used for cultivating crops such as citrus, tomato, and cotton with the suitable class of S1, S2 and S3, respectively (Ozcan, 2006). Qualitative assessment of land suitability in the Nishabur plain used GIS for cultivating wheat, cotton and corn. Soil physical properties were reported as the main limiting factors for planting wheat in the area, whereas the production of maize and cotton was mainly limited by climatic conditions (Baqer Zadeh et al., 2012). Some studies were conducted in different parts of Iran to evaluate suitability of land for a given utilization (Mohammed, 2004; Teka and Haftu, 2012; Maharia and Alebachewa, 2013; Teshome et al., 2013; Gizachew, 2014) and to find an optimum use for each land unit (Jafarzadeh et al., 2005, 2008; Navidi and Sarmadian, 2011; Safari et al., 2013; Kamkar et al., 2014; Tati and Sarmadian, 2014; Hashemvand Khiabani and Sarmadian, 2014). According to the above- mentioned items, the main purpose of this study is to evaluate strategic land evaluation parametrically for irrigating wheat crop using the geopedological approach and GIS techniques on some parts of the agricultural plain located in Abeik, Qazvin province.

Material and Methods

Study site

The study area is located between 36° 1' and 36° 9' N, and 50° 21' and 50° 14' E, approximately 16630 ha, in the Abyek area, Qazvin Province, Iran (Figure 1). The mean annual precipitation and temperature at the site are 284 mm and 14°C and the coldest and hottest months are December and July, respectively (IMO, 2015) (Table 1). Soil moisture and temperature regimes are dry xeric, weak aridic, and thermic, respectively, according to Van Wambeke (2000).

Table 1. Climatic characteristics of the synoptic meteorological station of Qazvin (IMO, 2015)

Characteristics	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Mean min temp. (°C)	-2.3	-2.8	1.5	6.4	10.4	14.5	17.6	17.0	12.9	7.9	2.8	-4.3
Mean max temp. (°C)	5.8	8.2	13.8	20.2	25.8	32.2	35.4	34.9	30.9	23.4	15.2	8.4
Mean temperature (°C)	0.8	2.7	7.6	13.3	18.1	23.4	26.5	26.0	21.9	15.7	9.0	3.4
Rainfall (mm)	35.9	30.7	50.1	40.5	30.3	3.9	2.1	1.7	0.8	18.3	30.8	38.9
Relative humidity (%)	70.0	65.0	56.0	51.0	38.0	38.0	37.0	38.0	46.0	57.0	68.0	51.0
Sunshine (hours)	159.8	167.3	195.2	226.9	281.2	349.2	354.8	345.8	306.3	343.3	182.6	142.9
Calculated ETp (mm)	23.6	37.5	72.5	106.5	142.6	181.9	191.8	179.8	128.5	78.1	40.2	23.5
Mean wind speed (m/s)	1.27	1.78	2.39	2.24	2.55	2.44	2.29	2.09	1.68	1.47	1.27	1.98

The study area belongs to the quaternary period and had been developed by gravel and sand sediments and alluvial fans (Navidi and Sarmadian, 2011). The dominant landscapes of the study area are hillands, Peneplains, Piedmonts and plains that have classified the area into four different geomorphic units (Zink, 2013). The pasture, irrigated and fallowed cultivation field, rainfed lands, Rajai power plant and residential section are the major land uses in this area.

Preparation of geoform map

Firstly, an interpretation of aerial photos (1:40,000 scale from Iran Surveying Organization) was performed based on the expert opinion, the systematical structure of geopedology (Zinck, 1989) and the geology as well as the topography maps of the study area. According to the geopedological method, the geomorphologic units were classified into four levels; landscape, relief, lithology, and landform. To determine the lithology layer, a geological map of the area with a scale of 1: 100,000 was used. Then, the accuracy of boundaries was studied and finally the geoform map of the area (1: 40,000 scale) was prepared. This map was used as the base map for geological field studies and preparation of the soil map.

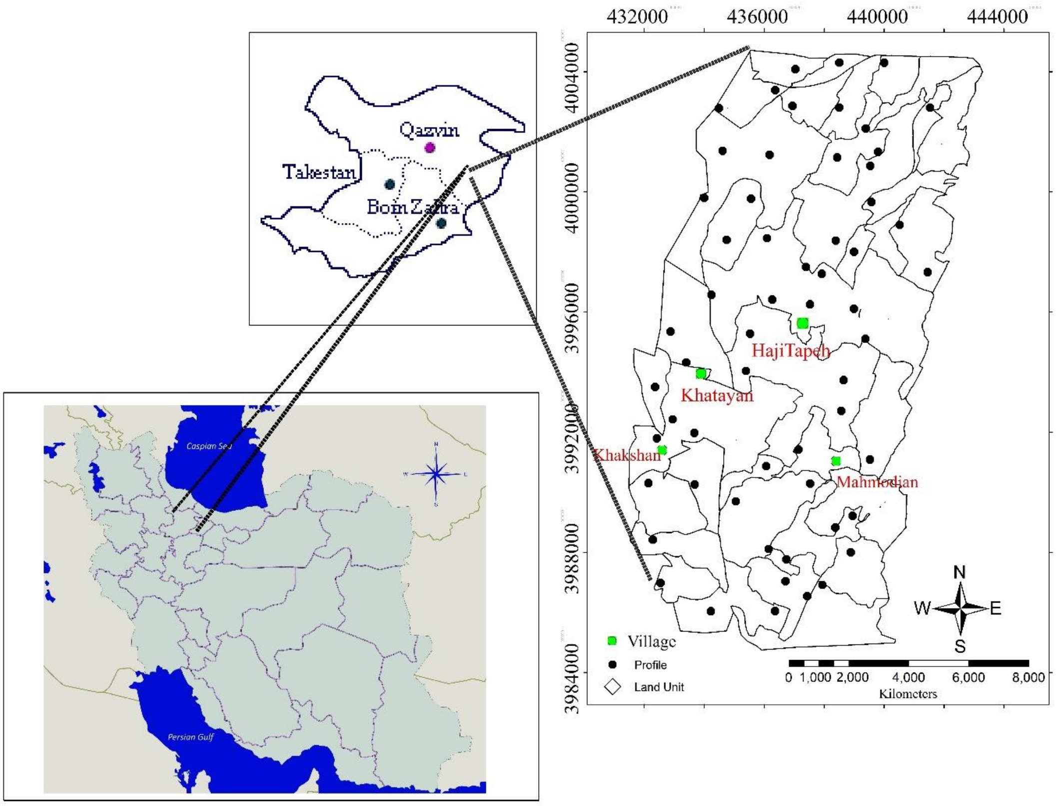

Figure 1. The sampling point location in studied area

Field work and laboratory analysis

To obtain basic data, 61 soil profiles were described in different parts of the study area. Physico-chemical variables were performed on fine fractions (<2 mm) after drying and grinding the samples. The specifications of the surface and sub-surface horizons were measured as follows; color (Munsell Color, 1994), structure, soil texture by hydrometer method (Gee and Boudry, 1986), organic carbon by Walkley-Black method (Walkley and Black, 1934), lime (calcium carbonate) content by Scheibler calcimeter, gypsum by the Aston method, coarse fragment percentage in terms of volume, electrical conductivity by electrical conductivity meter, soil reaction by pH meter (Black, 1965), cation exchange capacity by Bover method (Bover et al., 1952), available phosphorus by Olsen method (Olsen et al., 1954), and total soil nitrogen according to Black (1965). The classification of sampling points was performed to family level based on the Soil Survey Staff (2014).

Preparation of Soil map

Providing the soil map based on the relationship between soil and geomorphology is one of the main objectives of this study. To achieve this goal, the soil map with 24 units was prepared using incorporating soil data and geoform map in GIS software environment.

Land suitability evaluation using FAO method

Required features for this assessment are climatic information (rainfall, temperature, radiation, relative humidity) and the characteristics of the profile of the land, and soil (slope, flooding, soil texture and structure, coarser sand particles, the amount of calcium carbonate, gypsum percentage, cation exchange capacity, soil pH, soil organic carbon (SOC), electrical conductivity (EC) and exchangeable sodium percentage (ESP)), the correction factor applied to the weighted average for efficiency types on the basis of standard tables (Sys et al., 1991b; Nahusenay and Kibebew, 2015). Matching and classification of land suitability for wheat were also done based on the parametric methods (stories and square root). The water production potential was used to determine the production potential of wheat in the region. This model evaluates the vivid, pure products and its yields for the best varieties in terms of favorite conditions of weather, nutrition's, and controlling pests and diseases. Equation 1 was used in order to calculate the net biomass of all living plants.

$$Bn = 0.36\,bgm \times KLAI / [(1/L) + 0.25Ct] \tag{1}$$

Where, Bn is net biomass of the living plant, bgm is maximum gross biomass production rate (kg CH_2O /ha.hr), LAI is leaf area index, K is correction factor which for LAI less than 5 m^2/m^2.

Equals 0.95. L is the length of the growing season (days) and Ct is respiration quotient that is obtained from following formula (Eq. 2).

$$Ct = C_{30}(0.444 + 0.0019 \times t + 0.001t^2) \tag{2}$$

Where, t is average daily temperature during the growing season (in Celsius), C30 is the ratio of non-legume, 0.0108, and 0.0283 for legumes.

Equation 3 was used for calculating the production of water potential (Sys et al., 1991b).

$$Y = Bn \times Hi \tag{3}$$

In this equation, Y is yield of a crop (kg/ha), Bn is net biomass production (kg CH_2O/ha), and Hi is harvest index as a part of net biomass that is economically usable (e.g. grain in cereals, sugar in sugar beet and sugar cane) (Sys et al., 1991b).

Parametric index in land evaluation

The main core of these methods by calculated of indices obtained from combining numerical grades of several factors. In terms of their impact on the target, land, properties are graded between 0 and 100. Given that it has a relative scale (e.g. comparing with lands with a slope of 40°, those with 80° slope is much more proper for cultivation, both the Storie and the square root equations were used respectively in the (Eq. 4 and 5), in order to determine different degrees of land (Sys et al., 1991b). In this study, eight characters such as; EC, gypsum, coarse fragment, depth, SOC, soil texture, calcium carbonate and climate were used for determining the final class of land suitability for wheat. Theses parameters had the most significant role in determining the suitability classes (Table 6). Storie method (Storie, 1978) is described as below:

$$I = A \times \frac{B}{100} \times \frac{C}{100} \times \cdots \tag{4}$$

In this equation, I is the land index and A, B, C,... are the characteristic of various properties. The Square Root Method (Khiddir, 1986):

$$I = R_{min} \times \sqrt{\frac{A}{100} \times \frac{B}{100} \times \cdots} \tag{5}$$

Where, I is square root index, R_{min} is minimum rating between different characteristics, A and B are other rating beside the minimum. After calculating the index value of each land unit, the rate of the suitability class was determined for each unit (Table 2).

Table 2. Determine classes of land suitability for FAO methods (Sys et al., 1991a)

Land suitability class	land index	Symbol
Very suitable	75- 100	S1
Moderate suitable	50-75	S2
Marginally suitable	25 - 50	S3
Temporary unsuitable	25 – 12.5	N1
Permanently unsuitable	12.5 - 0	N2

Calculation of Predicted yield or potential yield

This product is the factor which is predictable according to the specifications of each product per land unit. It is achieved by multiplying the land index per land by the estimated potential product using FAO model in Eq. 6, (Bagheri, 2010).

$$PY = LI \times LPY \tag{6}$$

Where, PY is the predicted yield (kg/ ha), LI is land index, LPY is land potential yield.

Results and Discussion

Geoform and Soil map

The geoform map was prepared based on the Systematic approach of Geopodologic. The studied area was divided into four units on landscape level, seven units in terms of relief, and 13 units in terms of landform level, and the finally, the area geoform map was prepared with 32 units. The landscape units included hilland, peneplain, piedmont, and plain and each unit included 189 ha (1.14%), 1533 ha (9.22%), 7255 ha (43.63%), and 7571 ha (45.53%), respectively (Table 3). Thus, the plain and Piedmont units had the largest parts in the study area. Figure 2 indicates the distribution map of geoform units in the study area.

Table 3. Geoform units as identified in the area

Area				Area			
%	Hectare	Geoform unit	Land scape	%	Hectare	Geoform unit	Land scape
1.2	200	Pe444	Piedmont	189	1.13	Hi111	Hilland
2.75	458	PL111		377	2.26	Pe111	
1.31	218	PL112		243	1.46	Pe122	
3.94	656	PL113		232	1.39	Pe214	Peneplain
3.95	657	PL123		254	1.5	Pe223	
3.92	653	PL124		123	0.73	Pe224	
5.36	893	PL131	Plain	114	0.68	Pe333	
2.11	351	PL223		1034	6.21	Pi111	
0.75	125	PL232		371	2.23	Pi112	Piedmont
7.14	1189	PL233		813	4.88	Pi121	
3.48	580	PL234		1289	7.75	Pi123	
1.25	208	PL235		120	0.72	Pi124	
4.01	667	PL242		205	1.23	Pi125	
3.2	533	PL243		2006	12.06	Pi134	
1.64	274	PL254		1372	8.25	Pi143	
0.26	44	PL326		259	1.55	Pi145	

On the Geopedological approach, it is possible to study a wide geographic area quickly, especially if the relation between geomorphology and the soils is well defined (Rossiter, 2000). The soil map of area (1:40000) was prepared based on the relationship between geoform and soil layers. For this purpose, the layer of soil data, including classification of the sampling points and the map of the geoform units were integrated, and the soil map with 24 map units were prepared through applying the geopedological method (Zink, 2013), (Figure 3). The results revealed that the properties of soil vary from place to place. These diversities incur that natural soil bodies are the result of climate and living organisms acting on parent material with topography or local relief exerting a modifying influence and with time required for soil-forming processes to act (Zaremehrjardiri, 2011). Results of soil classification (Soil Survey Staff, 2014) in the subgroup, in each landscape unit are presented in Table 4. According to the table 4, distribution of soil class in each landscape are 50% in plain, 35% in piedmont, 7/5% in peneplain and 7/5% in hilland, respectively. The results showed that the highest soil variation was observed in the plain landscape.

Table 4. Soils in the study area (Sub group) (Soil Survey Staff, 2014)

Landscape	Soils has the most frequency	Other soils	% soils in each landscape	Label of soil map unit
Hilland	Lithic xerorthents	-	7.5	6
Peneplain	Typic calcixerepts	-	7.5	2-4-5-NR
Piedmont	Fluventic haploxerepts	Typic calcixerepts, Typic haploxerepts, Typic xerorthents	35	3-7-8-9-10-20-22-24
Plain	Sodic Xeric Haplocalcids	Sodic Xeric Calcigypsids, Xeric Calcigypsids, Gypsic Aquasalids, Gypsic haplosalids, Xeric haplocalcids, Xerofluventic haplocambids	50	11-12-13-14-15-16-17-18-19-21-23-1

Land suitability evaluation

To determine a map of the land units for each type of desired efficiency, the requirement tables of climate, soil and land were examined for water irrigated wheat (Sys et al., 1991b). Since the desired products are irrigated with water, rainfall limitations have no effect on climate class of the area, because they were irrigated at every stage of the plant water requirement (Sys et al., 1991b). Considering the history of wheat cultivation and weather station data available in the area (IMO, 2015), climatic suitability classes of wheat were separately calculated based on the square root and the Story parametric methods (Sys et al., 1991b). The results showed that the studied area had the perfect climate for wheat (S1) and there was no limitation to its growth during the growing season.

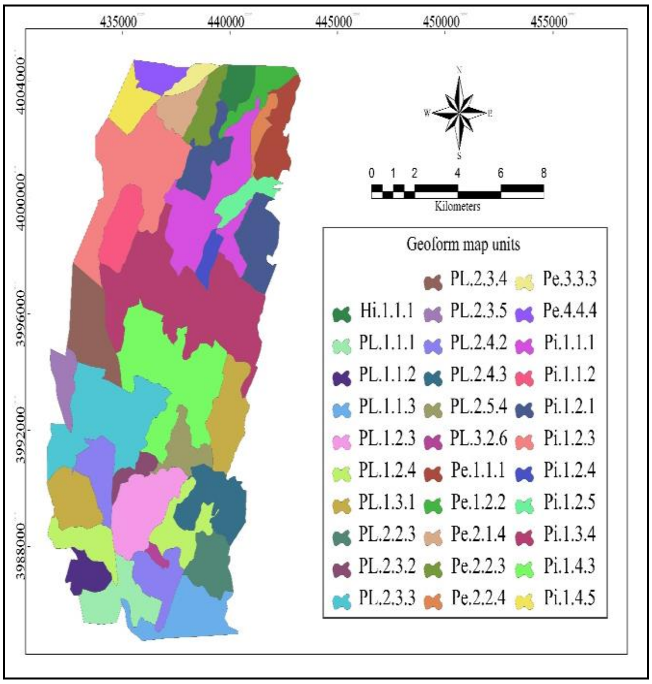

Figure 2. Geoform map units in the study area

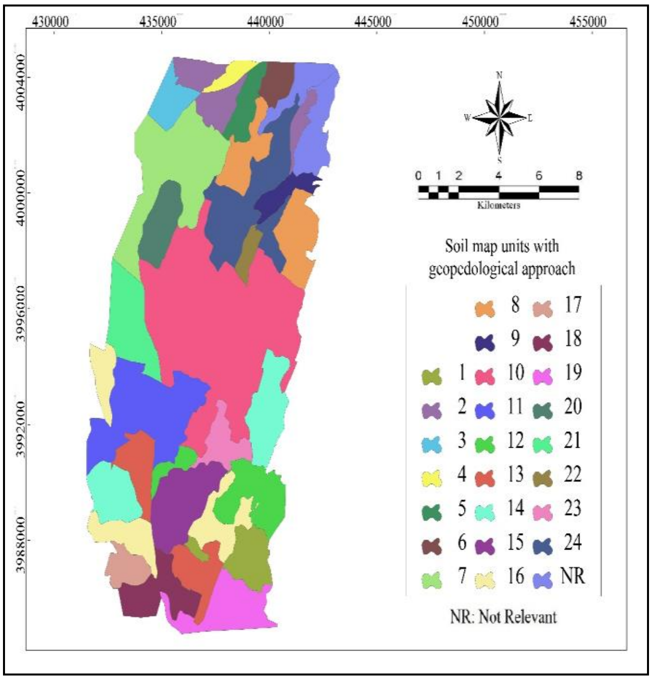

Figure 3. Geopedological soil map of study area

The required values of climate, soil and land for irrigated wheat in the region are provided in Tables 5 and 6, respectively. According to FAO method, the potential production of wheat was 6666 kg/ ha. Navidi and Sarmadian (2011) and Tati and Sarmadian (2014) in separate researches calculated the potential production of wheat in Qazvin plain with the similar results. After calculating the land requirements for wheat and in accordance with the FAO method, each of the eight characteristics of climate, soil and land received a rate between 0 and 100 (Sys et al., 1991b). Next, the final values of the land index were separately calculated based on the square root and the parametric Storie methods. Table 7 shows the rates of land values under the suitable class and the predicted yield for each land unit. Classification of changes in land suitability classes for wheat is shown in both Figures 4 and 5. The results of Table 8 also illustrate that based on Storie method, 24.1% of the area had a high suitability class S1, 36% of the area had a moderate suitability class S2, 30.85% of the area had a low suitability class S3, and 5.33% of the land had unsuitable class N1. However, the results of the land classes in the square root method showed that 34.9% of the area had a high suitability class S1, 42.86% of the area had the moderate suitable class S2, 14.4% of the area had a low suitability class S3, and 4.18% of the area had unsuitable class N1. The results obtained by the parametric square root method are probably more realistic when compared with other reports (Sarvari and Mahmoudi, 2001; Jafarzadeh et al., 2005, 2008; Taati and Sarmadian, 2014) that were applied by different methods in different parts of the country.

Table 5. Rating of climatic factors for wheat crop in Qazvin plain (IMO, 2015)

Suitability class	Rating scale	Temperature degree (°C)	Climate features
S2	89	11.44	Mean temperature of the growing cycle (°C)
S1	99	8.31	Mean temperature of vegetative stage (°C)
S1	99.85	14.12	Mean temperature of the Flowering stage (°C)
S1	96.89	19.11	Mean temperature of the ripening stage (°C)
		0.77	Average daily minimum temperature. coldest month combine with
S1	100	10	Average daily maximum t° coldest month

Figures 4 and 5 represent changes in land suitability for wheat based on the Storie parametric method and the square root methods respectively. To better understand the changes in distributions of land suitability classes, a change is shown in Table 8. As shown in Table 7, the land unit numbers (LUN's) 17 and 18 have the lowest yield prediction, for these two units are located in the southern part of the region and in the landscape of the plane lands. In fact, high levels of salt and gypsum would limit these two LUNs.

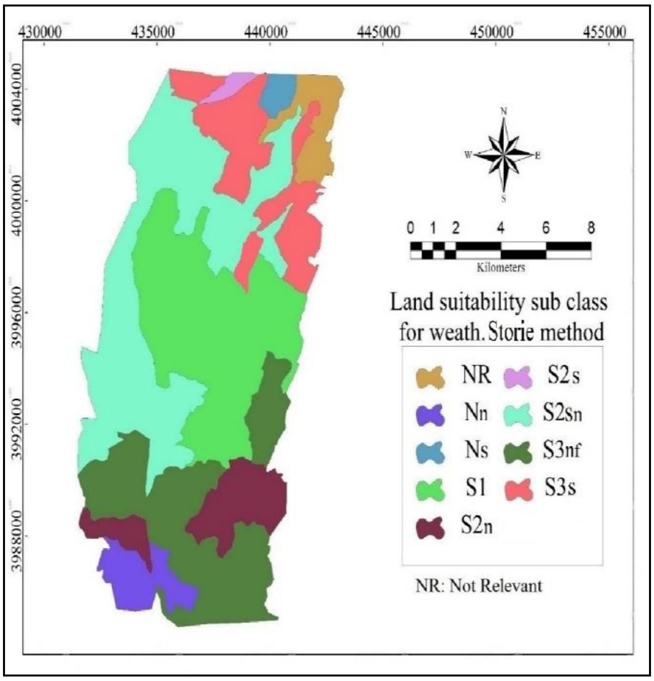

Figure 4. Land suitability sub class for wheat with Storie method

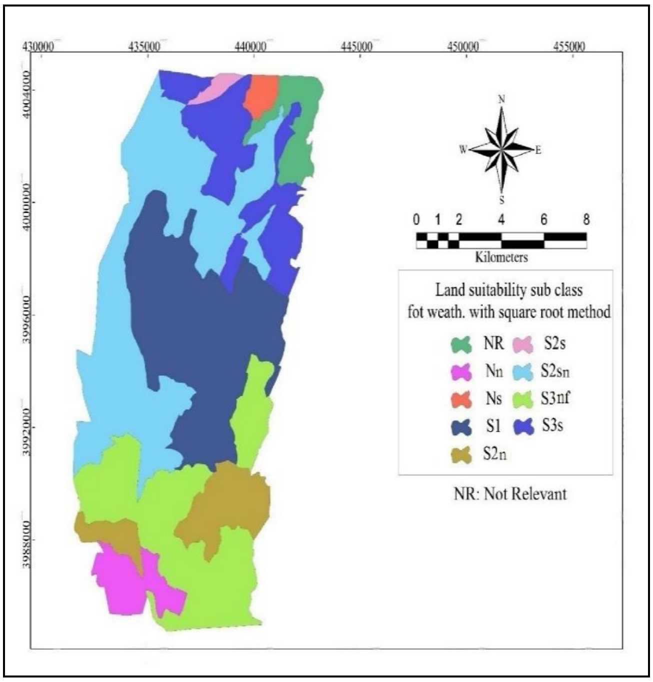

Figure 5. Land suitability sub class for wheat with Square root method

Table 6. Some soil characteristics and land requirements in the study area in terms of wheat cultivation

Coarse Fragment,%	Organic Carbon,%	TNV, %	Gypsum, %	Electrical Conductivity (dS/ m)	Texture*	Depth (cm)	LUN
0	0.41	18	15	3.5	C	110	1
31	0.42	25	0	0.62	SL, L	85	2
6.31	0.36	19.5	0	1.07	CL	105	3
23	0.44	20.73	0	0.54	L	90	4
15.3	0.48	4	0	0.48	SL	75	5
35	0.51	4	0	0.59	SL	20	6
5.2	0.28	6.7	0	0.7	L, SL	102	7
40	0.4	9.14	0	0.9	SCL,CL	40	8
20	1	8.85	0	0.77	SL	85	9
3.5	0.58	7.5	0	1.23	CL,SiCL	140	10
0	0.55	21.9	0	5.5	C	140	11
0	0.58	14.53	0	4.59	C	100	12
0	0.55	17.33	8.75	9	C	145	13
0	0.53	16	1.1	7	C	140	14
0	0.55	18.5	3.5	3.5	C	115	15
0	0.61	17.15	0.56	2.1	CL, C	120	16
0	0.56	15.14	2.2	38	CL	150	17
0	0.45	10	9.5	36	C	140	18
0	0.65	17.81	0	6	C	150	19
9	0.86	10	0	0.86	CL, L	120	20
0	0.56	11	0	0.91	CL, SiCL	130	21
13	0.39	2.6	0	0.65	SL	50	22
0	0.57	9.23	0	1.09	C	120	23
15	0.40	7.7	1.1	1.01	SL،L	17	24

*Note: C,SL, CL; L, SCL, SiCL, respectively, Clay, Sandy Loam, Clay loam, Sandy clay loam, Silty Clay loam

High rate of salinity more than 4 dS/m has limited effect on wheat production (Sys et al., 1991a). Moreover, it was observed that the LUN 6 was placed in the not suitable class N1, which according to its location, on the face of the land limited the properties of soil depth, large amounts of gravel with the slope factor for wheat cultivation. The LUN 23 had the highest level of the predicted performance in the area. This unit is located on the landscape of the plain, and all characteristics of this unit have a high proportion for irrigated wheat cultivation. Moreover, after this unit, the LUN's 10 and 20 had a high proportion for irrigated wheat cultivation. Of course, the results of the conformity class in the square root method confirmed these results and showed that LUN's 17 and 18 were placed in the unsuitable and the LUN's 23, 20, 16, 12, 10 and 3 had a high proportion for irrigated wheat cultivation. Zeinodini (2003) evaluated land suitability for wheat in Bardsir, Kerman. The results of suitability classification of land for the separated land units showed that the classes are various according to the parametric method from S1 to S3. The most significant limiting factors in mass wheat production in the region could be gypsum, texture, and soil structure. In their study on Qazvin plain, (Navidi and Sarmadian, 2011) also reported that the most important factors limiting wheat, barley and hay crops in most units were soil texture, lime content, and drainage condition.

Table 7. the rate of the land units, sub class, and the predicted performance for wheat based on the Storie and Square root methods

LUN	Storie method	Predicted Yield (Kg/ha)	Sub class	Square root method	Sub class	Predicted Yield (Kg/ha)
1	28.22	1881.14	S3ns	41.24	S3ns*	2749.05
2	49.83	3321.66	S3s	57.66	S2s	3841.61
3	72	4792.52	S2s	85.5	S1	5841.41
4	68	4532.88	S2s	67.7	S2s	4512.88
5	39.88	2658.40	S3s	44.3	S3s	2953.03
6	24.43	1628.50	N1s	38.2	S3s	2546.41
7	65.33	4354.89	S2s	74	S2s	4932.84
8	38.19	2545.74	S3fs	55.61	S2s	3706.96
9	37.68	2511.74	S3s	41.71	S3s	2780.38
10	80.54	5368.79	S1	86.47	S1	5644.10
11	53.2	3546.31	S2nf	62.84	S2nf	4188.91
12	67.5	4499.55	S2nf	77.5	S1	5166.15
13	44.6	2973.03	S3ns	47.7	S3ns	3179.68
14	43.52	2901.04	S3ns	51.2	S2ns	3412.99
15	49.3	3286.33	S3ns	59.4	S2ns	3959.06
16	69.75	4643.53	S2nf	80.85	S1	5389.46
17	17.47	1161.88	N1n	21.3	N1n	1419.85
18	13.3	886.57	N1n	18.43	N1n	1228.54
19	39.77	2651.06	S3ns	40.62	S3ns	2707.72
20	79	5266.14	S1	81.2	S1	5412.79
21	64	4266.24	S2s	65.85	S2s	4389.56
22	39.2	2613.07	S3s	43.58	S3s	2905.04
23	91.2	6079.39	S1	92.3	S1	6152.71
24	65.22	4347.56	S2s	67.7	S2s	4512.88

Note: *n, s and f, represent the salinity and alkalinity limitations, physical properties of soil and fertility.

According to the results mentioned in this study, the limitation caused by soil texture was observed in LUN's 2 and 8, so their results were consistent with the results observed in the wheat crop. Moreover, Lime limitation was observed in LUN 2 for the production of wheat in all calculations used for determining the degree of land, and the square root of the results of the parametric method presented a more logical result of the Storie method. Bagher Zadeh et al. (2012) reported that due to reverse multiplication of successive characteristics of soil and climate by each other, the Storie method's results were very strict and far from reality, whereas the second square root represented more balanced results for determination of the suitability of these classes and products.

Table 8. Related area to different suitability classes for weath base on Storie and square root method in the study area

Suitability	Weath (Storie method)		Weath (Square method)	
	Area (ha)	% Out of total	Area (ha)	% Out of total
Suitable	4007.83	24.10	5803.87	34.90
Moderate suitable	5986.80	36.00	7127.61	42.86
Marginally suitable	5130.35	30.85	2394.72	14.40
Unsuitable	1505.20	9.51	1303.80	7.84
Total area	16630.00	100.00	16630.00	100.00

Conclusion

Since a soil map is the basis of land evaluation studies, higher accuracy and purity of the map would improve the accuracy of the assessment of land suitability. Because it is based on a hierarchical structure, the geopedological approach would be able to identify the geomorphic units and justify the relationship between geoform units and the soil created on it. Based on the soil map prepared, the higher frequencies of the region's soils were observed in the profile of the plain. The results showed that these three LUN's 6, 18, and 17 had the lowest suitability class N1 for wheat. In addition, the three land unit's No. 23, 20, and 10 had the highest suitability S1 for wheat. The other results of this study differentiated in the discrepancy between the two parametric methods, the Story and the square root, which lead to a difference between the suitability classes in each land unit for the desired products. In addition, the presence of such difference between the correct equations of land index will result in a greater amount of land index in the second square method rather than the Storie method. It would finally improve the suitability class of the lands and the yield amount observed in the calculated land units will improve in the second root parametric method. Among these 8 features used for determining the characteristics of the final class of land suitability for each of the products, the sequence, soil salinity, gypsum, coarse fragment, soil depth, soil organic carbon, soil texture, lime content and climate, had the most significant role in determining the conformity classes of the products.

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Assessment of heavy metals contamination in the Nile River water and adjacent sediments: A case study from Khartoum City and Nile River State, Sudan

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Abstract

The current study aims to investigate the heavy metals concentration and the degree of pollution in the water and adjacent sediment of the Nile River and its main tributaries at Khartoum City and River Nile State, Sudan. For this purpose, thirty-three water and sediment samples were collected from River Nile, Blue Nile, and White Nile. Water chemical properties and sediment physico-chemical properties were measure. Concentrations of heavy metals (As, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Ti, Zn) were determined for both sediment and water samples using inductively coupled plasma (ICP-OES). Enrichment factor (EF) and geo-accumulation index (I_{geo}) were applied to quantify heavy metals pollution levels in sediment samples. The revealed that only Fe metal detected in the water samples and its concentrations within the permissible maximum limit. This indicated that water is highly suitable for irrigation. Depending on calculated enrichment factor (EF) and geo-accumulation index (Igeo), sediment samples were found to be enriched and polluted with Mn and Mo particularly at Berber site which may as consequence of gold mining activities in this area. The study revealed relatively strong to strong correlation between heavy metals of Co, Cr, Cu, Fe, Mn, Ni, Pb, Ti, Zn (r^2=0.84 to 0.99) and significant negative correlation with Mo (r^2=0.58 to 0.73). This study recommends regular monitoring of heavy metals in the Nile River and its main tributaries for conservation and protection from pollution.

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Introduction

From various water resources in the Sudan, the Nile River and its tributaries are considered to be the primarily source of water for human, agriculture, livestock, and wildlife. Despite this importance, the Nile River water and its suspended sediments are subjected to possibility of contamination by various hydro-chemical pollutants; especially heavy metals from various reasons mainly sanitation problems.

Anthropogenic activities not only lead to increasing heavy metals concentrations in the environment, but also it can cause an unnatural enrichment, leading to metal pollution of the surface soils. The soil enriched

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with heavy metals can significantly cause an adverse impact on the population via inhalation, ingestion and dermal contact. The soil-accumulated heavy metals can also pose potential long-term hazards to plants and animals as well as humans that consume these plants (Singh and Kumar, 2006).

Nile River sediments considered as a group of metals that could be released to the overlying water from natural and anthropogenic processes such as dredging and bioturbation, may lead up to potential adverse health effects (Kim et al., 2010). On the other hand the presence of heavy metals in the Nile River sediments is influenced by the particle size of the sediments, this actually attributed toco-precipitation, sorption, and complexing of metals on particle surfaces and coatings (Sakai et al., 1986; Krishna and Govil, 2008).

In Sudan, research in the Nile River water and its adjacent sediment contamination with heavy metals derived as a result of anthropogenic activities and its impacts on environment is not yet clearly understood. Thus, there is a need for re-assessment of heavy metals in the Nile River water and its adjacent sediment to ensure environmental sustainability. The study was carried selected certain location at Nile River and its tributaries in Khartoum city and Northern state, Sudan. The main objectives of this study were: (i) To determine the chemical composition and concentration of heavy metals in the Nile River water (ii) To assess the degree of the heavy metals contamination in the sediment of the Nile River particularly, Khartoum city and Nile River state, Sudan with reference to international standard.

Material and Methods

Study area

The study was conducted in five different locations along the Nile River and its main tributaries at Khartoum State and Northern State (Table 1 and Figure 1). The sample from Northern State was selected to be handled as control since the area is remote area and expected to be free from pollution.

Table 1. Location of samples using geographical coordinate system (longitude/latitude)

Site	Longitude	Latitude	River	State
Blue Nile	15º30'58"N	32º38'33"E	Blue Nile	Khartoum
White Nile	15º32'02"N	32º28'53"E	White Nile	Khartoum
Shambat	15º39'34"N	32º30'49"E	Nile River	Khartoum
Wawasi	16º02'36"N	32º34'01"E	Nile River	Khartoum
Berber	17º49'22" N	33º59'59" E	Nile River	Northern State

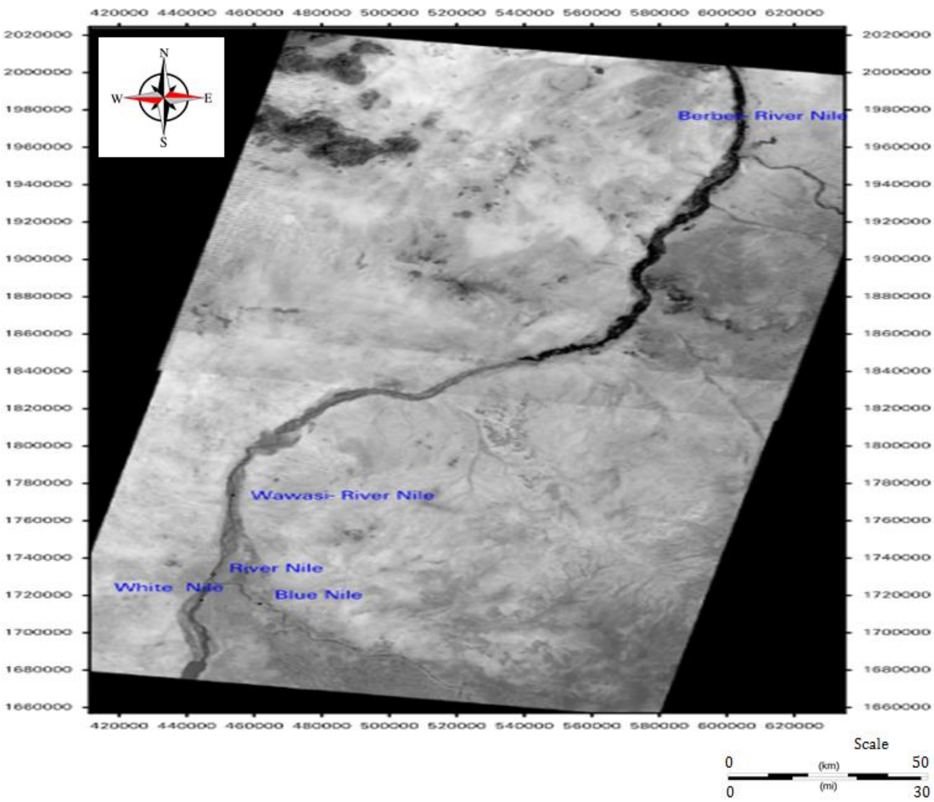

Figure 1. Distribution of sampling sites

Sampling and physico-chemical analyses

For water sampling, at each location, the polyethylene bottles were rinsed at least three times before sampling. Three surface water samples from 10 cm depth (about 0.25 L for each one) were taken at each sampling site and placed into a 500 ml polyethylene bottle, well closed in order to avoid contamination. A counterparts of sediment samples were collected from 0-10 cm using grab sampler, immediately transferred to the laboratory for analysis in order to avoid changing of redox potential, pH, and pore water. In the laboratory, sub-sediment samples were air-dried (23±1 °C) and passed through 2mm sieve to obtain the fine fraction. The particles-size distribution of these samples was determined using particle size analyzer model (Mastersizer 2000, Malvern) and the textural class was obtained by using the USDA textural triangle according to Soil Survey Staff (2014). Fresh sediments and water chemical properties were determined according to the procedure described by Binning and Baird (2001). Soil pH was measured in 1:5 sediment suspensions using a digital pH meter model (3510, Jenway). The electrical conductivity (EC) was determined in 1:5 sediment extract using a conductivity meter model (4510, Jenway). Percentage of calcium carbonate (%$CaCO_3$) was determined by Calcimeter. The samples were treated with 0.1N HCL; the volume of CO_2 from pure calcium carbonate and samples were recorded. Percentage calcium carbonate was then calculated according to Horvath et al. (2005). Soluble cations (Na^+, K^+, Ca^{2+} and Mg^{2+}), and anions (Cl^-, HCO_3^-, SO_4^{2-} and PO_4^{3-}) were determined in the extracted solutions using ion chromatography model (Dionex ™ IC 5000).

Determination of heavy metals in the sediment and water samples

Microwave digestion oven model (CEM Mars 5) was used to digest the sediment samples. 0.5 gram of air-dried sample was used after a well-milled, and then placed into a microwave oven pipes, 10 ml of nitric acid (HNO_3) was added to each pipe containing sample and well closed, then introduced into the microwave oven and digested using EPA-3051A according to the method described by Link et al. (1997). For extraction, digested samples were transferred quantitatively into 50 ml volumetric flask and the volume was completed by using distilled water. All digested sediment samples and water were filtered using Whatman No. 42. Concentrations of heavy metals (As, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Ti, Zn) were analyzed for sediment and water samples using inductively coupled plasma (ICP-OES) model (Optima 4300 DV, PerkinElmer Inc.)

Assessment of heavy metal pollution levels in the sediment samples

In order to verify the quantity of heavy metal pollution levels in the sediment samples; two indices have been applied including the following: Enrichment Factor (EF), and the geo-accumulation index (I_{geo}). Enrichment factor was calculated by using the equation described by Sutherland (2000), as follows:

$$\mathrm{EF}m = \frac{\mathrm{Cm(sediment)/CFe\ (sediment)}}{\mathrm{Cm\ (earth\ crust)/CFe(earth\ crust)}}$$

Where: C_m (sediment) is the metal concentration in the sediment sample; C_{Fe} (sediment) is the concentration of the reference metal (Fe) in the sediment sample; C_m (earth crust) is the metal concentration in the earth crust; and C_{Fe} (earth crust) is the concentration of the referenced metal (Fe) in the earth crust.

The EF values are classified into five categories: deficiency to minimal (EF<2), moderate (2<EF<5), significant (5<EF<20), very high (20<EF<40), and extremely high enrichment (EF>40).

Whereas, the geo-accumulation index (I_{geo}) was calculated by using the following equation:

$$Igeo = Log2(Cn/1.5Bn)$$

Where: Cn is measured concentration of heavy metal in the sediment samples, Bn is geochemical background value in average shale (Turekian and Wedepohl, 1961) of element, and 1.5 is the background matrix correction factor due to lithogenic effects.

The index of geo-accumulation consists of six categories: <1 (unpolluted), 1–2 (moderately to unpolluted), 2–3 (moderately), 3–4 (moderately to highly polluted), 4–5(highly polluted), and >5 (very highly polluted).

Statistical analysis

The values of maximum, minimum, and averages were calculated, and Tukey significant difference was tested for means separation (P< 0.05). All statistical analyses were performed by using statistical package for social science software SPSS Statistics version 16.0 (IBM Corp., 2012).

Results and Discussion

Chemical properties of the River Nile water and its main tributaries

The pH values of water samples for the River Nile and its main tributaries are alkaline (7.4-7.9), this is may be attributed to domination of $Ca^{2+}$and Mg^{2+} cations as well as HCO_3^- anion among various measured soluble ions (Table 2). EC values were ranged from 0.09 to 0.14 dS m^{-1} in all water samples. These findings were in line with those of previous studies (Osman and Kloas, 2010; Ali et al., 2017). Based on the average concentration values, the water soluble cations were found in the following order: Ca^{+2}> Mg^{+2}> Na^+> K^+, where, the soluble in the in following order: HCO_3^- > PO_4^{3-} > SO_4^{2-} > Cl^-. This result of soluble cations and anions orders agreed with those obtained by Ali et al. (2017).

Table 2. Summary statistics for the water chemical analysis of the River Nile and its tributaries

Location		pH	EC (dS m^{-1})	Soluble cations (meq L^{-1})				Soluble anions (meq L^{-1})			
				Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-	HCO_3^-	SO_4^{2-}	PO_4^{3-}
Blue Nile	Min	7.30	0.10	0.62	0.07	1.6	1.47	0.03	3.7	0.03	0.07
	Max	7.60	0.16	0.63	0.07	1.85	1.54	0.05	3.9	0.04	0.08
	Av.	7.47	0.13	0.63	0.07	1.70	1.52	0.04	3.79	0.03	0.07
White Nile	Min	7.40	0.09	0.78	0.09	1.13	1.69	0.11	3.49	0.03	0.07
	Max	7.50	0.10	0.82	0.09	1.16	1.73	0.15	3.54	0.04	0.08
	Av.	7.43	0.09	0.8	0.09	1.15	1.72	0.13	3.51	0.03	0.08
River Nile (Wawasi)	Min	7.30	0.09	0.26	0.02	1.88	1.22	0.01	3.26	0.07	0.07
	Max	8.60	0.10	0.30	0.03	1.92	1.31	0.01	3.32	0.09	0.08
	Av.	7.87	0.09	0.28	0.03	1.90	1.25	0.01	3.28	0.08	0.08
River Nile (Shambat)	Min	7.10	0.08	0.39	0.04	1.70	1.26	0.07	3.13	0.11	0.06
	Max	7.80	0.09	0.40	0.04	1.87	1.48	0.08	3.55	0.14	0.07
	Av.	7.40	0.09	0.40	0.04	1.80	1.37	0.08	3.34	0.13	0.07
River Nile (Berber)	Min	7.30	0.14	0.68	0.07	1.34	1.51	0.02	3.44	0.03	0.08
	Max	7.70	0.15	0.69	0.08	1.40	1.59	0.02	3.61	0.04	0.09
	Av.	7.47	0.15	0.69	0.07	1.38	1.54	0.02	3.52	0.04	0.08

Physico-chemical properties of the River Nile sediment

The textural class of sediment samples from Berber site (control site), Wawasi 1, and White Nile was Sandy; the sand fraction was dominant with an average value amounted to 97.52 %, 95.29 %, and 93.18 %, for the three sites respectively. Contrary to that, the textural class of sediment samples from Wawasi 2, Bule Nile, and Shmabat is silt loam and dominated by silt fraction that amounted to 71.22 %, 54.52 %, and 51.18 %, respectively. Clay fraction was higher in the sediment samples from Shambat and Waswasi 2 sites as compared to other studied sites.

The pH values of sediment samples were alkaline ranged from 7.7 to 7.79 for Blue Nile site, 8.52 to 8.53 for White Nile site, 8.18 to 8.19 for Wawasi 1 site, 7.73 to 7.75 for Wawasi 2 site, 8.01 to 8.03 for Shambat site, and 8.17 to 8.24 for Berber site, with an average value of 7.78, 8.53, 8.19, 7.74, 8.02, and 8.21, respectively. The higher values of EC recorded at Wawasi 1 site, with an average value of 1.2 dS m^{-1}. Meanwhile, this site showed higher values of soluble Na^+, Mg^{+2}, Cl^-, and SO_4^{-2}, with an average value of 3.76 meq L^{-1}, 11.80 meq L^{-1}, 6.66 meq L^{-1}, and 6.39 meq L^{-1}, respectively. Based on the average values, the sediment soluble cations were obtained in the following decreasing order: Mg^{+2}> Ca^{+2}> Na^+> K^+. In contrast, the average values of the soluble anions were found in the following order: HCO_3^- > Cl^-> SO_4^{-2}> PO_4^{-3}. The order of Na^+, K^+, and HCO_3^- remains same as observed in the water samples (details are shown in Table 3).

Heavy metals concentration in the River Nile water and its adjacent sediment

The concentrations of the heavy metals in the Nile River water and its main tributaries are shown in (Tables 4). The results showed that, in the River Nile water samples and its main tributaries, only Fe metal was detected (0.42 to 2.46 mg L^{-1}), with an average value ranged between 0.63 to 2.18 mg L^{-1}at the different sites. The results indicated that the average values of Fe metal concentrations were lower than maximum acceptable concentrations limit for irrigation water (Ayers and Westcot, 1994). Contrary to Fe metal, the other heavy metals (As, Cd, Co, Cr, Cu, Mn, Mo, Ni, Pb, Ti, and Zn) were not detected in all sites (Table 4).

Table 3. Summary statistics for the sediment physico-chemical analysis of the River Nile and its tributaries

Location		Particle size distribution (%)			Textural class (USDA)	pH	EC	Soluble cations (meq L^{-1})				Soluble anions (meq L^{-1})			
		Sand	Silt	Clay			dS m^{-1}	Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-	HCO_3^-	SO_4^{2-}	PO_4^{3-}
Blue Nile	Min	37.42	52.8	6.35	Silt loam	7.76	0.33	1.15	0.42	2.92	1.82	1.88	2.97	1.26	0.08
	Max	40.48	56.23	6.48	Silt loam	7.79	0.37	1.19	0.47	2.97	1.85	1.93	3.02	1.32	0.09
	Av.	38.95	54.52	6.42	Silt loam	7.78	0.35	1.17	0.45	2.95	1.84	1.91	3.00	1.29	0.08
White Nile	Min	92.26	5.90	0.50	Sand	8.52	0.90	2.64	2.11	3.32	7.32	4.22	5.40	4.88	ND
	Max	94.10	7.74	0.90	Sand	8.53	1.01	2.69	2.13	3.35	7.35	4.27	5.70	4.91	ND
	Av.	93.18	6.82	0.70	Sand	8.53	0.96	2.67	2.12	3.34	7.34	4.25	5.55	4.90	ND
River Nile (Wawasi 1)	Min	94.77	4.20	1.03	Sand	8.18	1.18	3.74	3.49	3.79	11.7	6.59	8.18	6.35	ND
	Max	95.80	5.23	1.50	Sand	8.19	1.21	3.78	3.52	3.82	11.9	6.72	8.24	6.42	ND
	Av.	95.29	4.72	1.27	Sand	8.19	1.20	3.76	3.51	3.81	11.80	6.66	8.21	6.39	ND
River Nile (Wawasi 2)	Min	12.92	70.62	13.09	Silt loam	7.73	0.46	2.35	2.03	2.94	6.14	2.88	7.60	2.25	ND
	Max	15.09	71.82	15.87	Silt loam	7.75	0.48	2.38	2.06	2.98	6.18	2.94	7.80	2.32	ND
	Av.	14.00	71.22	14.48	Silt loam	7.74	0.47	2.37	2.05	2.96	6.16	2.91	7.80	2.29	ND
River Nile (Shambat)	Min	36.58	50.61	11.66	Silt loam	8.01	0.48	2.39	3.71	2.59	3.35	3.38	5.64	2.60	ND
	Max	37.65	51.74	12.48	Silt loam	8.03	0.53	2.42	3.75	2.61	3.39	3.42	5.75	2.80	ND
	Av.	37.12	51.18	12.07	Silt loam	8.02	0.55	2.45	3.73	2.60	3.37	3.40	5.69	2.70	ND
River Nile (Berber)	Min	97.02	0.80	2.50	Sand	8.17	0.55	2.59	2.04	4.50	7.97	2.29	11.99	2.32	ND
	Max	98.01	0.94	1.05	Sand	8.24	0.58	2.63	2.07	4.70	8.20	2.33	12.15	2.43	ND
	Av.	97.52	0.87	1.78	Sand	8.21	0.57	2.61	2.06	4.60	8.09	2.31	12.07	2.38	ND

ND = not detectable

Table 4. Heavy metals concentrations in the water samples of the River Nile and its main Tributaries

Location		Heavy metals concentrations (mg L^{-1})											
		As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn	Ti	Zn
Blue Nile	Min	ND	ND	ND	ND	ND	0.96	ND	ND	ND	ND	ND	ND
	Max	-	-	-	-	-	1.16	-	-	-	-	-	-
	Av.	-	-	-	-	-	1.06	-	-	-	-	-	-
White Nile	Min						0.42						
	Max	ND	ND	ND	ND	ND	0.83	ND	ND	ND	ND	ND	ND
	Av.	-	-	-	-	-	0.63	-	-	-	-	-	-
Wawasi	Min	-	-	-	-	-	0.53	-	-	-	-	-	-
	Max	ND	ND	ND	ND	ND	0.97	ND	ND	ND	ND	ND	ND
	Av.	-	-	-	-	-	0.75	-	-	-	-	-	-
Shambat	Min	-	-	-	-	-	0.68	-	-	-	-	-	-
	Max	ND	ND	ND	ND	ND	0.90	ND	ND	ND	ND	ND	ND
	Av.						0.79						
Berber	Min	ND	ND	ND	ND	ND	1.90	ND	ND	ND	ND	ND	ND
	Max	-	-	-	-	-	2.46	-	-	-	-	-	-
	Av.	-	-	-	-	-	2.18	-	-	-	-	-	-

ND = not detectable

Consequently, their concentrations in the water samples indicating high suitable for irrigation according to the previous authors (Ayers and Westcot, 1994). For sediment samples, As and Cd metals were remains same as observed in the water samples, and not detected (Table 5). These findings indicated that their values concentrations were lower than those in the common range of soil (Lindsay, 1979). The concentrations of Co, Cr, and Cu ranged from 4.2 to 25.7 mg kg^{-1}, 3.2 to 54.3 mg kg^{-1}, and 4.7 to 42.3 mg kg^{-1}, respectively. This result indicated that the three metals concentrations were within the common range of soil according to Murthy (2008) although lower than their target value according to Dutch standards. Similarly, the concentrations of Fe, Mn, and Mo were in common range of soil according to the previous reference and their concentrations ranged between 3438 to 31140 mg Fe kg^{-1}, 123.7 to 999.5 mg Mn kg^{-1}, and zero to 2.6 mg Mo kg^{-1}. The concentrations of Ni, Pb, and Zn were in range of 0.97 to 44.3 mg kg^{-1}, zero to 9.5 mg kg^{-1}, and 5.2 to 71.9mg kg^{-1}, respectively, which in common range of soil (Lindsay, 1979). The concentrations of Mo, Ni, Pb, and Zn were lower than target value of Dutch standard. Furthermore, the concentration of Ti was found in range of 102.8 to 1612 mg kg^{-1}, with an average values ranged between 106.9 to 1604.5 mg kg^{-1} at the different sites.

Table 5. Heavy metals concentrations in the sediment samples of the studied sites.

Location		Heavy metals concentrations (mg kg^{-1})											
		As	Cd	Co	Cr	Cu	Fe	Mn	Mo	Ni	Pb	Ti	Zn
Blue Nile	Min	ND	ND	15.70	23.80	28.30	26355.0	598.20	0.50	28.30	5.80	1597.0	53.70
	Max	ND	ND	20.60	37.90	30.50	26430.0	612.10	0.80	31.40	8.10	1612.0	58.20
	Av.	ND	ND	18.15	30.85	29.40	26392.5	605.15	0.65	29.85	6.95	1604.5	55.94
White Nile	Min	ND	ND	7.80	9.30	10.50	11548.0	156.70	ND	18.00	ND	723.8	22.80
	Max	ND	ND	9.20	11.20	12.30	11570.0	159.00	ND	20.00	ND	728.8	25.50
	Av.	ND	ND	8.50	10.25	11.40	11559.0	157.85	ND	19.00	ND	726.3	24.15
Wawasi 1	Min	ND	ND	4.20	7.80	4.70	9147.0	123.70	1.90	6.70	ND	562.7	15.30
	Max	ND	ND	5.30	9.00	6.10	9152.0	128.80	2.60	7.30	ND	566.6	19.40
	Av.	ND	ND	4.75	8.40	5.40	9149.5	126.25	2.25	7.00	ND	564.7	17.35
Wawasi 2	Min	ND	ND	22.30	48.20	37.50	31098.0	982.70	ND	37.80	6.80	1413.0	64.80
	Max	ND	ND	25.70	54.30	42.30	31140.0	999.50	ND	44.30	9.50	1427.0	71.90
	Av.	ND	ND	24.00	51.25	39.90	31119.0	991.10	ND	41.05	8.15	1420.0	68.35
Shambat	Min	ND	ND	13.50	37.40	22.50	25187.0	587.80	ND	25.80	5.80	982.6	47.30
	Max	ND	ND	16.90	42.00	27.50	25210.0	602.50	ND	30.70	8.90	993.2	53.10
	Av.	ND	ND	15.20	39.70	25.00	25198.5	595.15	ND	28.25	7.35	987.9	50.20
Berber	Min	ND	ND	ND	3.20	4.70	3438.0	187.20	0.88	0.97	ND	102.8	5.20
	Max	ND	ND	ND	5.70	7.90	3452.0	193.30	1.40	1.60	ND	111.0	7.70
	Av.	ND	ND	ND	4.45	6.30	3445.0	190.25	1.14	1.29	ND	106.9	6.45

ND = not detectable

In order to describe the pollution levels of heavy metals in the study area, two indices were applied including enrichment factor (EF) and geo-accumulation index (I_{geo}). Recently, it has been reported that the EF is appropriate measure of geochemical trends and can be applied for contemplating on lithogenic or anthropogenic origin of heavy metals (Ye et al., 2011). Depending on the category and the obtained values of EF, all investigated metals were found to be in their minimum limits with EF<2, except for Mn and Mo at the Berber area were obtained to be moderate and significant with an EF values of 2.42 and 11.09, respectively (Figure 2). On the other hand, EF values for heavy metals more than 2 considered as major concern contaminant as suggested by some researchers (e.g. Yongming et al., 2006; Ye et al., 2011). In this context the EF values for Mn and Mo at the Berber area were slightly contaminated. Furthermore, Hernandez et al. (2003) suggesting that the value of EF≤2 indicates that the heavy metals may be as a result of crustal materials or natural weathering processes. Whilst, EF values higher than 2 indicate that the heavy metals are mainly due to anthropogenic inputs. Thus, the heavy metals of Mn and Mo having EF value higher than 2, indicating that these heavy metals might be enriched as a result of anthropogenic inputs might be probably from mining activities. Previously, it has been demonstrated that the geo-accumulation index (I_{geo}) can be used effectively in explaining soil quality. Based on its category and the obtained values of I_{geo}, for Cr, Cu, Ni, Pb, and Zn, all sediment samples at all sites were unpolluted (Figure 3).

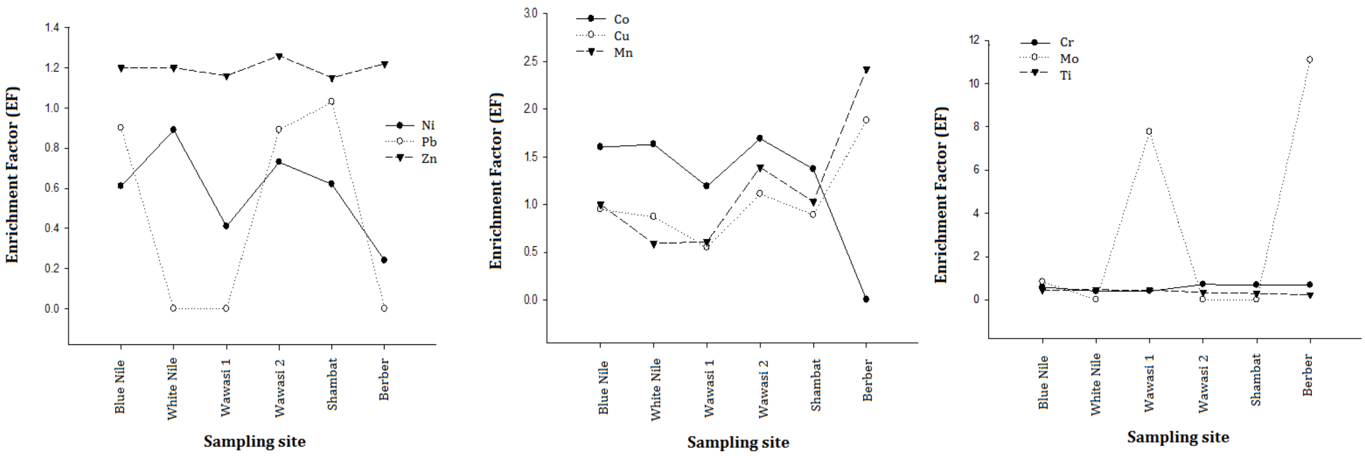

Figure 2. Enrichment factor (EF) values for heavy metals at different sites

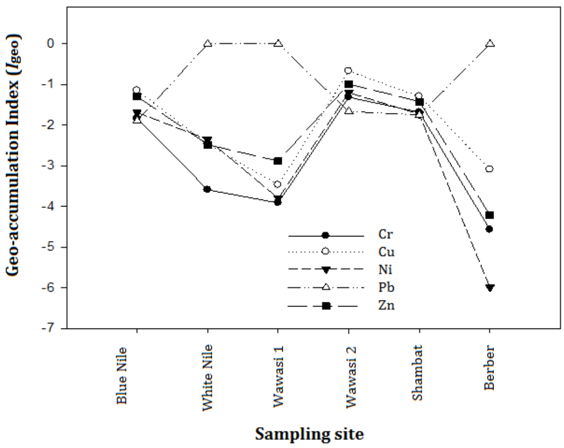

Figure 3. Geo-accumulation index (Igeo) values for Cr, Cu, Ni, Pb, and Zn at different sites

Correlation between the sediment solution composition and heavy metals

Table 6 showed the Pearson's correlation analysis between the different sediment solution composition and heavy metals. According to correlation's coefficient, clay content showed relatively strong positive correlation with silt (r^2=0.92), Co (r=0.86), Cr (r^2=0.96), Cu (r^2=0.92), Fe (r^2=0.90), Mn (r^2=0.95), Ni (r^2=0.88), Pb (r^2=0.92), and Zn (r^2=0.90).

Table 6. Correlation coefficients matrix (*r*) among physico-chemical properties and heavy metals of the sediment samples

	Clay	Silt	pH	EC	Ca	Mg	Na	K	Cl	HCO_3	SO_4	Co	Cr	Cu	Fe	Mn	Mo	Ni	Pb	Ti	Zn
Clay	1.00																				
Silt	0.92**	1.00																			
pH	-0.77*	0.87*	1.00																		
EC	-0.62*	-0.72	0.72*	1.00																	
Ca	-0.36	-0.63	0.55*	0.85*	1.00																
Mg	0.07	-0.25	0.32	0.51	0.77	1.00															
Na	-0.72*	-0.79	0.48	0.33	0.44	-0.01	1.00														
K	-0.54*	-0.73	0.54*	0.83*	0.92**	0.48	0.67	1.00													
Cl	-0.35	-0.48	0.54*	0.93**	0.87*	0.67	0.12	0.75	1.00												
HCO_3	-0.27	-0.52	0.29	0.19	0.56	0.32	0.83*	0.66	0.10	1.00											
SO_4	-0.52*	-0.65	0.66*	0.99**	0.88*	0.60	0.24	0.81*	0.97**	0.17	1.00										
Co	0.86*	0.97**	-0.78*	-0.58	-0.59	-0.29	-0.86	-0.68	-0.38	-0.64	-0.52	1.00									
Cr	0.96**	0.99**	-0.84*	-0.69	-0.56	-0.16	-0.80	-0.69	-0.44	-0.48	-0.61	0.96**	1.00								
Cu	0.92**	0.99**	-0.83*	-0.73	-0.63	-0.31	-0.75	-0.70	-0.53	-0.46	-0.66	0.97**	0.98**	1.00							
Fe	0.90*	0.99**	-0.81*	-0.62	-0.60	-0.22	-0.88	-0.72	-0.39	-0.63	-0.55	0.99**	0.98**	0.97**	1.00						
Mn	0.95**	0.97**	-0.86*	-0.73	-0.55	-0.24	-0.66	-0.61	-0.51	-0.32	-0.66	0.93**	0.98**	0.99**	0.93**	1.00					
Mo	-0.59*	-0.58*	0.15	0.52	0.55	0.20	0.64	0.68	0.55	0.41	0.50	-0.62*	-0.61*	-0.66*	-0.61*	-0.58*	1.00				
Ni	0.88*	0.94**	-0.69*	-0.56	-0.56	-0.24	-0.87	-0.67	-0.38	-0.61	-0.50	0.99**	0.95**	0.96**	0.97**	0.92*	-0.73	1.00			
Pb	0.92**	0.98**	-0.86*	-0.76	-0.64	-0.18	-0.80	-0.78	-0.51	-0.52	-0.68	0.92**	0.98**	0.95**	0.96**	0.94**	-0.58	0.90*	1.00		
Ti	0.69	0.90*	-0.76*	-0.53	-0.68	-0.25	-0.83	-0.71	-0.35	-0.79	-0.49	0.95**	0.85*	0.87*	0.93**	0.80*	-0.49	0.90*	0.84*	1.00	
Zn	0.90*	0.99**	-0.82*	-0.63	-0.59	-0.45	-0.85	-0.69	-0.40	-0.60	-0.55	0.99**	0.98**	0.98**	0.99**	0.95**	-0.61	0.98**	0.95**	0.93**	1.00

*P< 0.05, ** P<0.01

Contrary to that, significant negative correlation were found with pH (r^2=0.77), EC (r^2=0.62), Na (r^2=0.72), K (r^2=0.54), SO_4 (r^2=0.52), and Mo (r^2=0.59).Whilst, silt content showed relatively strong positive correlation with all studied heavy metals, except with Mo significant negative correlation were found between them (r^2=0.58). In addition, soil pH revealed significant correlation with EC (r^2=0.72), Ca (r^2=0.55), K (r^2=0.54), Cl (r^2=0.54), and SO_4 (r^2=0.66). Also, relatively strong positive correlation were found for EC against Cl (r^2=0.93) and SO_4 (r^2=0.99). On the other hand, relatively strong positive correlation were also obtained for Cl versus SO_4 (r^2=0.97). Moreover, heavy metals of (Co, Cr, Cu, Fe, Mn, Ni, Pb, Ti, Zn) showed relatively strong to strong positive significant correlation (r^2=0.84 to 0.99) and significant negative correlation were found against Mo (r^2=0.58 to 73).

Conclusion

The findings of this study showed lower values of salinity in both water and sediment samples, and domination of sand fraction in the sediment samples in most studied sites. The heavy metals in this study were obtained in the following decreasing order: Fe> Ti> Mn > Zn> Ni> Cr > Cu > Co> Pb > Mo. Among the different studied heavy metals, the Nile River sediment was polluted with Mn and Mo particularly at the Berber site. The results of the Pearson's correlation for heavy metals in our study pointed out relatively strong to strong positive significant correlation between them (r^2=0.84 to 0.99) and significant negative correlation were found versus Mo (r^2=0.58 to 0.73).

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