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Original Article

Morphological characteristics and soil attributes of five species of Galium (Rubiaceae) from North Africa

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ABSTRACT
The morphological variations between selected five *Galium* species from Libya, and the role of soil factors that affect their distribution in their natural habitats were investigated. Thirty-seven macromorphological characters (11quantitative, 26 qualitative) representing vegetative parts were subjected to numerical-taxonomic analysis. Five branches and clusters were distinguished; each was linked to a specific species. Representatives of these groups were clustered together according to characters with high factor loading in the Principal Components Analysis (PCA). The results showed congruence between the UPGMA clustering and PCA in suggesting five species groups. Seventeen soil factors were used in this assessment of soil factors responsible for the distribution of the 5 species of *Galium*. Calcium and chloride ions content exhibited the most significant difference (p=0.05) among the five species groups, while the other examined soil variables showed no significant differences. The relationship between the examined soil variables and the studied populations of *Galium* species was assessed by Principal Components Analysis (PCA). Each of the studied species of *Galium* was affected by one or more of the examined soil parameters.

KEYWORDS: Vegetation, Libya, *Galium*, soil analysis, distribution.

INTRODUCTION

The Rubiaceae (Coffee family; Bedstraw) is the fourth family in number of species in the Angiosperms after Orchidaceae, Asteraceae and Leguminosae. Govaerts *et al.* (2006), in their world checklist, confirm reporting 611 genera and 13,100 species. *Galium* is by far the largest and most widespread genus within the tribe Rubieae (subfamily Rubioideae). It includes more than 400 species in 16 sections containing annual and perennial herb that are distributed in temperate and tropical regions of the world (Dempster and Delprete, 2004). *Galium* itself is problematic taxonomically, because taxa from different sections exhibit similar habit, many species are widely distributed and polymorphic, and species groups often are poorly differentiated both morphologically and geographically (Schischkin, 2000).

The flora of Libya is not rich in the number of species; however, the Al-Jabal Al-Akhdar Mountain landscape comprises the richest vegetation and the highest number of species known from Libya (Boulos, 1972, 1977, 1997). The floristic composition of plants in Libya is still comparatively unknown as far as in-depth ecological and botanical studies go (Pergent and Djellouli, 2002). There are 2103 species belonging to 856 genera and 155 families in Libya. The main component of the flora, 2088 species, 844 genera and 145 families, are angiosperms. Fifteen species of 12 genera and 10 families are Pteridophyta, but gymnosperms appear in mountains (Ali and

Jafri, 1976/77; Klopper et al., 2007). The geographical affinity of the flora is mainly East Mediterranean rather than neighboring regions of North Africa (Scholz, 1974; Le Houérou, 1997; El-Kady, 2000; El-Bana and Al-Mathnani, 2009). Much of the indigenous vegetation of Libya consists of surviving remnants of a more favourable climatic age (Keith, 1965).

The application of multivariate analyses approaches showed useful and significant results in taxonomic studies of several taxa. For instance, in *Plantago* from Turkey (Doğan et al., 1992), among populations of *Quercus petraea* from Italy (Bruschi et al., 2003), in representatives of *Potentilla* sect. *recta* (*Rosaceae*) from the Iberian islands (Rico et al., 2003), in olive cultivars from Italy (Rotondi *et al.*, 2003), in the genus *Vasconcellea* (Caricaceae) from the Ecuador (Kyndt et al., 2005), among rare endemic *Oncocyclus* irises (Iridaceae) of Lebanon (Saad and Mahy, 2009), in *Micromeria* (Lamiaceae) species from Turkey (Arabaci et al., 2010), in the genus *Serapias* from Croatia (Hršak et al., 2011), among the annual species of *Alyssum* (Brassicaceae) from Iran (Bolourian and Pakravan, 2011), in *Veronica* Sect. *Beccabunga* (*Plantaginaceae* s.l.) from Egypt (Abd El-Ghani et al., 2011b), in *Pancratium* from Egypt (El-Hadidy et al., 2012), in Leguminosae-*Papilionoideae* from Egypt (El-Gazzar *et al.*, 2013a), and in tribe Aveneae from Egypt (El-Gazzar et al., 2013b).

In the arid region, there are considerable studies focused on the effects of environmental factors on plant communities (for more literature, see Zahran and Willis, 2009). Parker (1988) examined the role of soil texture, elevation/nutrients, and xericness (based on slope aspect and angle) on the vegetation associations of columnar cacti in the northern Sonora desert, and the relationships between environmental factors and the distribution patterns of sedges were studied in the wetland important bird areas of Uganda (Ssegaw et al., 2004). However, detailed ecological and floristic accounts remain very scarce particularly for the arid and hyper-arid regions of North Africa. Significance of soil attributes to the distribution of some genera was profound. In some species of *Convolvulus* in Egypt (El-Khatib et al., 1998), in the endangered species of *Randonia africana* (Abd El-Ghani and Marei, 2006), in climbing plants (Abd El-Ghani et al., 2011a), in some succulent plants (Abd El-Ghani et al., 2014), in *Brassica nigra* (Gomaa et al., 2012).

Apart from the genus *Galium* published in the flora of Libya (Ali and Jafri, 1976/77), nothing has been published on this genus. Therefore, the present study aims to assess the morphological variation in the studied species of *Galium* based on a large number of characters through morphometric analyses of natural populations using numerical analyses approach, and to detect the role of soil factors that affect the distribution of the studied species of *Galium* in its natural habitats.

MATERIALS AND METHODS

The study area

Libya is a country in the Maghreb region of North Africa. It is bordered by the Mediterranean Sea, Egypt, Sudan, Chad, Niger, and Tunisia. It lies between 18°-33°N and 9°-25°E and has an area of 176,000 km^2 consisting mainly of desert and the Mediterranean coast. About 94 to 96% of the land is desert, and it is one of the driest countries in the world (Holdridge, 1974). Libya comprises three main provinces: Tripolitania, Cyrenaica and Fezzan. Tripolitania extends over the north western corner of the country and Fezzan south of Tripolitania. Cyrenaica, the largest geographic region, covers the entire eastern half of the country. Tripolitania holds the Nafusah plateau and Cyrenaica houses Al-Jabal Al-Akhdar which is the most vegetated part of the country. Fezzan is home to desert lands, including the Sahara (Johnson, 1973). The mountainous landscape confines about 50% of

the endemic species in Libya (Qaiser and El-Gadi, 1984). Southwards, arid climate prevails and xerophytic vegetation is dominant. More than 85% of the country's mean production of wood is used in charcoal production (Al-Idrissi et al., 1996), where the mountainous landscape is intercepted by heavily forested wadis.

Climatically, temperatures are very high with an annual average temperature of 27°C. Its desert climate is very hot in the summer, with extreme day/night temperature differences and the winters are mild. Rainfall in the northern part of the country varies between 100-500 mm/year; the southern section receives only as much as 10 mm/year and some parts are rainless (Wheida and Verhoeven, 2007). The harsh conditions and physical barriers limit human settlements and intensive agricultural activities (Alredaisy, 2011).

The plant material

One hundred and eighty specimens of the studied species of *Galium* were collected in their natural habitats from different locations of Libya. Field trips were made to the selected localities to collect flowering and fruiting specimens (Table 1). In the natural populations, individuals were sampled according to their availability and ease of access for observation and/or collection and, consequently, the number of samples varied from one population to another.

For nomenclature, several literature were consulted, e.g., Täckholm (1974), Feinbrun-Dothan (1978), Jafri (1979), Ehrendorfer and Schönbeck-Temesy (1980), and Boulos (2000, 2009).

Morphological characters

Each specimen (flowers and leaves) was soaked in water for a few seconds before measurements were taken (the number of measured specimens is not constant for each feature). Only mature plants were chosen for morphometric analysis on the basis of differences among species in the vegetative and reproductive parts. The investigated species constituted OTU (Operational Taxonomic Units, Table 1).The morphological traits were given codes ranging between 1 and 5 depending on the variation in the average value for the measured traits. A total of 37 characters were measured for each specimen: 11 quantitative (measured) and 26 qualitative. Fifteen of the qualitative characters were scored as binary and the rest were scored as multi-state characters. The measurements of all specimens of a taxon were averaged into one OTU score for each of the characters. OTU scores for quantitative characters were the average measurements of at least 10 specimens (wherever possible). The macro-morphological characters were studied under stereoscopic microscope, and some parameters were measured with a digital caliper or just with a ruler.

Analysis of the morphological data was conducted using NTSYS-pc version 2.01a (Rohlf, 1998). Morphological data were converted into a similarity matrix, using the simple matching coefficient (Sneath and Sokal, 1973), with the SIMQUAL function. A dendrogram was generated from the similarity matrix by the unweighted pair-group method using arithmetic averages (UPGMA) (Sokal and Michener, 1958) with the SAHN function. The cophenetic correlation coefficient was calculated with a Mantel test (Mantel, 1967) by comparing the matrix of cophenetic values with the similarity matrix, in order to estimate how well the dendrogram represents its corresponding pairwise distance matrix. This was done with the COPH and MXCOMP modules of NTSYS-pc (Rohlf, 1998). Principal Components Analysis (PCA) was used to identify then most variable characters among the studied species. SPSS version16.0 release 16.0.0 (2007) software was applied for statistical analysis, and Multivariate Statistical Package (MVSP) software version 3.1 for Windows (Kovach, 1999) was used for multivariate analysis.

Soil sampling and analysis

Three soil samples were collected from each stand at a depth of 0-50cm. These samples were then pooled, forming one composite

sample, air-dried, thoroughly mixed and passed through a 2mm sieve to remove gravel and debris, then packed in paper bags ready for physical and chemical analysis. Three replicates were analyzed for each sample measurement. The portion finer than 2mm was kept for physical and chemical analyses according to Jackson (1967) and Allen and Stainer (1974).

Soil texture was determined by the hydrometer analysis (Bouyoucos, 1962), and the results used to calculate the percentages of sand, silt and clay. Soil reaction (pH) values of the soil samples were determined by an electric pH meter (Jenway 3020 pH-meter) with a glass electrode in a soil/distilled water suspension (1:2). The electric conductivity (EC) of soil samples was determined using an electrical conductivity meter.

Estimation of chlorides in the soil extract was carried out by titration methods against silver nitrate ($AgNO_3$) using potassium chromate ($K_2Cr_2O_7$) as indicator (Hazen, 1989; Kolthoff and Stenger, 1974), and the soluble bicarbonate was determined according to Allen et al. (1974) and Maff (1986). Sulphates were determined using photocolour reaction to measure the turbidity of barium sulphate at 470 nm (Verma et al., 1977). Calcium, magnesium, sodium and potassium ions were determined by atomic absorption spectroscopy technique using PERKIN ELMER A Analyst (100/300). Total hardness (TH) was estimated by titration with EDTA using Eriochrome Black T (EBT) as indicator. Percentages of C, H and N were measured using Vario ELΔIII Elementar C, H, N, S analyzer (Germany).

To determine the soil characteristics of the studied species of *Galium*, Principal Components Analysis (PCA) was carried out using PC-ORD version 4.14 for Windows (McCune and Mefford, 1999). All data variables were assessed for normality (SPSS for windows version 16.0 release 16.0.0) prior to the PCA analysis, and appropriate transformations were performed when necessary to improve normality according to Zar (1984). Seventeen environmental variables were included; soil reaction (pH), electric conductivity (EC), chlorides (Cl^-), bicarbonates (HCO_3^-), sulphates (SO_4^{--}), calcium (Ca^{++}), magnesium (Mg^{++}), sodium (Na^+), potassium (K^+), organic carbon content (C), nitrogen content (N), hydrogen content (H), total hardness (TH), coarse sand (CS), fine sand (FS), silt and clay. The cluster species groups were subjected to ANOVA (One-Way Analysis of variance) based on soil variables to find out whether there were significant variations among groups.

RESULTS

Numerical analysis

All characters showed normal distributions except peduncle length (PL), flower diameter size (FS) and mericarp size (Msz), which were highly asymmetric. Normality for these three traits was obtained after square root transformation. Figure (1) illustrated the UPGMA phenogram of the studied species. The cophenetic correlation of the distance matrix and tree matrix was 0.92 (Mantel test, $p<0.0002$), indicating good fit of the phenogram to the distance matrix (Rohlf, 1998). Five species-specific clusters were distinguished at 63% similarity level. At the first hierarchical level, the species were classified into major groups according to significant differences in leaf length (LLg; p=0.028), petal colour (PTCo; p=0.11), ovary shape (OS; p=0.003), ovary surface (OSr; p=0.49), mericarp shape (MS; p=003), mericarp size (Msz; p=0.002) and seed shape (SDS; p=0.001). The first major group included Sp5, Sp6 and Sp8, while the other included the remaining species. At the second hierarchical level, each of the major group was split into 2 subgroups giving 4 end subgroups based on the significant differences in petiole width (PTW; p=0.014), petiole colour (PTCo; p=0.008), style length (SYLg; p=0.007), mericarp shape (MSh; p=0.004), mericarp surface (MSr; p=0.001), and plant height (PLg; p=0.015). At the third hierarchical level, 5 subgroups were finally split based on the significant differences in petal colour (PTCo, p=0.026), style length (SYLg, p=0.17),

mericarp shape (MSh; p=0.009), plant height (PLg; p=0.017), leaf margin (LM; p=0.011) and inflorescence flower number (IFN; p=0.011). These subgroups including Sp5 and Sp8 in subgroup (A) which represents populations of *Galium mrale*, Sp6 in subgroup (B) that represents *G. setaceum*, Sp9, Sp10 and Sp11 in subgroup (C) that represents *G. verrucosum*, Sp2, Sp3 and Sp7 in subgroup (D) which represents *G. tricornutum,* and Sp1, Sp4 & Sp12 in subgroup (E) that represents *G. aparine.*

Factor analysis based on PCA of morphological characters among the species revealed that the first three factors comprise about 68% of the total variance. The first PCA axis (comprising about 26.1% of the total variance) that includes leaf shape (LSr), petal length (PTLg), petal width (PTW), petal colour (PTCo), style length (SYLg), ovary shape (OS), ovary surface (Osr), mericarp shape (MSh), mericarp size (Msz), mericarp surface (MSr) and seed surface (SDS) showed the highest correlation. The second PCA axis (comprising about 23.3% of the total variance) included plant height (PLg), leaf length (LLg), leaf width (LW), leaf margin (LM), pedicel length (PLg), diameter shape (PdLg), pedicel surface (PdSr), pedicel shape (PdS) and flower diameter size (FS), and the third PCA axis (comprising about 18.7% of the total variance) were the plant nature (PN), plant surface (PS), inflorescence flower number (IFN), peduncle length (PL), peduncle diameter shape (PS), and peduncle surface (PSr). Thus, the characters mentioned in the first three factors can be considered as the most variable morphological characters among the studied *Galium* species.

Ordination of the species collected from various localities based on the first two axes of principal components of their studied characters (Fig. 2) showed that *G. murale* (Sp5 and Sp8) was affected by mericarp shape (MSh) and seed shape (SDS); *G. setaceum* (Sp6) was correlated with petal colour (PTCo) and style length (SYLg) ;*G. aparine* (Sp1, Sp4 and Sp12) was strongly affected by leaf width (LW), leaf shape (LS) and pedicel length (PLg); *G. verrucosum* (Sp9, Sp10 and Sp11) was affected by the mericarp size (MSz); and *G. tricornutum* showed correlations with petal length (PTLg), flower diameter size (FS) and petal width (PTW).

Soil characteristics of the studies species

Table (2) demonstrated the mean values, standard deviations (SD) and ANOVA values of the studied soil variables in each of the five species group. Calcium and chloride ions content exhibited the most significant difference (p=0.05) among the five species groups, while the other examined soil variables showed no significant differences.

Clearly, the soil inhabited with *Galium verrucosum* group was characterized by its rich concentrations of of Mg, Na, K, Cl and HCO_3 ions and the highest percentages of carbon, and hydrogen as well. Also, soil reaction (pH) values in *G. verrucosum* group exhibited slight alkalinity (7.23), but it was neutral to slightly acidic (6.9-6.8) in the other species groups. *Galium aparine* group was found on sandy (59.77 ± 23.50) soil with high salinity levels (EC=109.93 ± 46.03), calcium (17.63 ± 2.90), sulphates (23.33 ± 13.61) and total hardness (75.93 ± 24.84). The soil of *Galium tricornutum* group exhibited high nitrogen content with the lowest levels of calcium, potassium chloride ions concentrations and the lowest salinity contents. In case of *G. setaceum* group, soil was rich in its clay contents with lowest amounts of coarse sand, magnesium, carbon and nitrogen concentrations. On soil with high percentages of silt and lower contents of fine sand, sodium, chlorides and bicarbonates, *G. murale* was found.

Species-soil relationships

The relationship between the examined soil variables and the studied populations of *Galium* species was assessed by Principal Components Analysis (PCA; Fig. 3). In the upper left corner, *Galium verrucosum* was affected by potassium, carbon and coarse sand contents, and *G. murale* with the amount of silt. In the lower left corner, *G.tricornutum* was highly attributed with concentrations of nitrogen and clay, while *G. setaceum* and *G.*

murale were highly affected by hydrogen content. In the lower right corner, *G. aparine* was highly related with sulphates, sodium, fine sand and total hardness.

Results of PCA ordination were demonstrated in Table (3). Axis 1 (Eigenvalue=5.90) was accounted for 34.726%, while Axis 2 (Eigenvalue=3.38) was accounted for 19.907% of the total variation. The first three axes were accounted for 68.880% of cumulative variance. This relatively high percentage showed that the data set was well-structured. Along Axis 1, Ca, Na, EC, TH, SO_4, FS were the highest positive loadings, while silt and clay were the highest negative loadings. So, this axis can be shaped by fine sand and silt which constituted the two highest gradients. Therefore this axis can be inferred as fine sand-silt gradient. Along Axis 2, Mg, K, C, pH and CS were the highest positive loadings, and clay was the highest negative loading. Therefore, this axis can be inferred as K–clay gradient.

DISCUSSION

This study was concerned with 5 species dominating various habitats in Libya: mountainous areas, coastal wadis, inland wadis, canal banks and the farmlands. The populations of *Galium tricornutum* and *G. setaceum* showed consistency to certain habitats: the farmlands for the former and inland desert wadis for the latter. However, populations of the other 3 species showed wide range of distribution (growing in more than one habitat), e.g., *Galium aparine* in mountainous areas and coastal wadis; *G. murale* in the inland desert wadis and canal banks; *G. verrucosum* in the coastal wadis, farmlands and the inland desert wadis.

Based on 37 morphological characters, numerical taxonomic analysis was achieved using classification and ordination techniques. At the third hierarchical level of classification, 5 subgroups were finally split based on the significant differences in petal color, style length, mericarp shape, plant height, leaf margin and inflorescence flower number. These subgroups included Sp5 and Sp8 in subgroup (A) which represents populations of *Galium mrale*, Sp6 in subgroup (B) that represents *G. setaceum*, Sp9, Sp10 and Sp11 in subgroup (C) that represents *G. verrucosum*, Sp2, Sp3 and Sp7 in subgroup (D) which represents *G. tricornutum,* and Sp1, Sp4 & Sp12 in subgroup (E) that represents *G. aparine*. Factorial analysis based on PCA of morphological characters among the species has revealed that the first PCA axis included highest correlation of leaf shape, petal length, petal width, petal colour, style length, ovary shape, ovary surface, mericarp shape, mericarp size, mericarp surface and seed surface. The second PCA axis included plant height, leaf length, leaf width, leaf margin, pedicel length, diameter shape, pedicel surface, pedicel shape and flower diameter size, while the third PCA axis included high correlations of the plant nature, plant surface, inflorescence flower number, peduncle length, peduncle diameter shape, and peduncle surface. Thus, the afore-mentioned characters can be considered as the most variable morphological characters among the studied *Galium* species. It was also indicated that *G. murale* (Sp5 and Sp8) was affected by mericarp shape and seed shape, *G. setaceum* (Sp6) was correlated with petal colour and style length, *G. aparine* (Sp1, Sp4 and Sp12) was strongly affected by leaf width, leaf shape and pedicel length, *G. verrucosum* (Sp9, Sp10 and Sp11) was affected by the mericarp size, and *G. tricornutum* showed correlations with petal length, flower diameter size and petal width. Generally, these results demonstrated congruence between the UPGMA clustering and PCA analyses in suggesting 5 subgroups, each was assigned to one of the studied species. Our results were in line, in some characters, with those of irises (Iridaceae) from Lebanon (Saad and Mahy, 2009), and *Plantago* from Turkey (Doğan et al., 1992). For the Egyptian materials of *Galium*, Abdel Khalik et al. (2008) showed high similarity between the differential characters obtained by PCoA, and that for the Libyan materials of the studied *Galium.*

The relationship between the soil variables and the studied populations of *Galium* species was assessed by application of Principal Components Analysis (PCA). It was revealed that, *Galium verrucosum* was affected by potassium, carbon and coarse sand contents, and *G. murale* with the amount of silt. *Galium tricornutum* was highly attributed with concentrations of nitrogen and clay, *G. setaceum* and *G. murale* were highly affected by hydrogen content, and *G. aparine* was highly related with sulphates, sodium, fine sand and total hardness. El-Khatib et al. (1998) studied the distribution of the Egyptian species of *Convolvulus* in relation to the prevailing soil conditions, and revealed that $CaCO_3$, moisture content, SO_4 and soil texture were the most effective soil parameters controlling the distribution of *Convolvulus*. Moreover, Abd El-Ghani et al. (2011a) pointed out that gravel, coarse sand, ions of Na, SO_4, Cl and NO_3 were the most important factors for the distribution of the vegetation patterns of the studied desert vines. In Slovakia and eastern Czech Republic, Janišová et al. (2012) studied the ecology and distribution of the endemic *Tephroseris longifolia* subsp. *moravica* in relation to environmental variation at a micro-scale. They concluded that pH, ions of Na, K, Ca, P and NH_4 were of significant importance in the distribution of this taxon. Results of the present study, however, shared several soil factors that indicated these investigations

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Table 1. Location and date of collections of the studied species of *Galium*. Figures between parentheses refer to the population numbers. NA=Not Available.

Species	Location	Habitat	Elevation (m ASL)	Coordinates
Galium aparine L.	Gasr Libya (4) Shahat Ruins (1) Wadi Qaam (12)	Mountainous areas Mountainous areas Coastal wadis	280.7 560.2 20.7	32° 37'N, 21° 23'E 32° 50'N, 21° 55'E 32° 28'N, 14° 25'E
G. murale L.	Wadi Elkouf (5) Sharshara (8)	Inland desert wadis Canal banks	264.6 327.4	32° 41'N, 21° 33'E 32° 27'N, 13° 37'E
G. setaceum Lam.	Wadi Derna (6)	Inland desert wadis	85.3	32° 42'N, 22° 36' E
G. tricornutum Dandy	Lamluda (2) Almansoura (3) Stwa (7)	Farmlands (barley fields) Farmlands Farmlands	668.1 NA 566.9	32° 44'N, 22° 06'E 32° 50'N, 21° 55'E 32° 49'N, 22° 09'E
G. verrucosum Huds.	Wadi Qaam (11) Almansoura (9) Wadi Derna (10)	Coastal wadis Farmlands Inland desert wadis	20.7 NA 85.3	32° 28'N, 14° 25'E 32° 50'N, 21° 55'E 32° 42'N, 22° 36' E

Table 2. Mean values, standard deviations and ANOVA F values of the soil variables in the populations supporting the five species of *Galium*. EC=electric conductivity, TH=total hardness, CS=coarse sand, FS=fine sand. $^{*}= P \leq 0.05$.

Soil Variables	Total Mean	Species Groups					F-ratio
		G. murale	*G. setaceum*	*G. verrucosum*	*G. tricornutum*	*G. aparine*	
Ca	12.31 ± 4.44	11.75 ± 0.35	9.9 ± 0.0	13.13 ± 1.60	7.33 ± 3.66	17.63 ± 2.90	6.03*
Mg (ppm)	1.18 ± 1.09	0.43 ±0.01	0.38 ± 0.0	1.96 ± 1.76	0.7 ± 0.78	1.67 ± 0.58	1.08
Na	2.79 ± 2.45	0.8 ± 0.28	0.93 ± 0.0	3.47 ± 2.50	1.44 ± 1.01	5.4 ± 2.52	2.47
K	2.18 ±1.56	3.15 ±1.63	3.3 ± 0.0	3.43 ± 1.83	0.97 ± 0.48	1.11 ± 0.78	2.48
C	6.37 ± 7.34	10.0 ± 0.01	2.5 ± 0.0	12.4 ± 0.001	3.9 ± 3.42	1.68 ± 0.83	1.15
H (%)	2.63 ± 1.19	2.45 ±0.64	1.3 ± 0.0	3.63 ± 0.63	2.8 ± 1.71	2.02 ± 1.14	1.1
N	0.7 ± 0.64	0.62 ± 0.11	0.5 ± 0.0	0.66 ± 0.30	1.22 ± 1.20	0.34 ± 0.31	0.67
pH	6.98 ±0.22	7.0 ± 0.01	6.9 ± 0.0	7.23 ± 0.21	6.8 ± 0.17	6.9 ± 0.17	2.67
EC (µs/cm)	83.22 ± 31.29	78.95 ± 7.00	67.0 ± 0.0	91.3 ± 1.58	56.63 ± 29.30	109.93 ± 46.03	1.38
TH	58.53 ± 15.37	53.25 ±6.72	58.0 ± 0.0	50.1 ± 2.77	53.27 ± 4.75	75.93 ± 24.84	1.65
HCO_3 (mg/l)	64.96 ±15.45	59.0 ± 8.48	67.98 ± 0.0	68.9 ± 18.17	65.03 ± 23.65	63.93 ± 17.98	0.09
SO_4	12.58 ± 8.71	9.0 ± 0.01	9.0 ± 0.0	9.0 ± 1.0	9.0 ± 0.001	23.33 ± 13.61	2.17
Cl	17.2 ± 3.55	13.8 ± 0.02	20.6 ± 0.0	20.6 ± 0.001	13.8 ± 0.02	18.33 ± 3.93	6.12*
CS	10.2 ± 11.56	15.45 ± 18.17	2.7 ± 0.0	16.17 ± 13.96	10.5 ± 13.41	2.93 ± 2.50	0.60
FS (%)	38.47 ± 21.43	21.65 ± 9.83	25.2 ± 0.0	39.2 ± 27.85	32.07 ± 6.03	59.77 ± 23.50	1.38
Silt	18.37 ± 8.45	29.9 ± 3.25	27.3 ± 0.0	16.43 ± 7.80	17.27 ± 1.94	10.73 ± 7.40	3.76
Clay	32.97 ±11.29	33.0 ± 11.60	44.8 ± 0.0	28.2 ± 6.44	40.17 ± 11.58	26.57 ± 14.66	0.93

Table 3. The extracted and cumulative variance of PCA ordination, and correlation coefficients between soil variables coefficients and ordination axes. Bold figures indicate statistical significance at $p<0.05$. For soil variables abbreviations, see Table (2).

Factors	Axis 1	Axis 2	Axis 3
% Extracted variance	34.726	19.907	14.247
% Cumulative variance	34.726	54.633	68.880
Eigenvalue	5.90	3.38	2.42
Ca	**0.8090**	0.2376	0.2028
Mg (ppm)	0.0115	**0.4908**	**0.5955**
Na	**0.6216**	-0.1026	**0.6333**
K	-0.2166	**0.8098**	-0.2733
C	-0.3404	**0.7047**	0.2815
H (%)	-0.3741	-0.1315	**0.5712**
N	**-0.4803**	-0.3747	0.2562
pH	0.3554	**0.6711**	0.0845
EC (µs/cm)	**0.8559**	0.4163	-0.1578
TH	**0.7874**	-0.1574	-0.3320
HCO_3 (mg/l)	-0.0318	-0.1766	**0.5796**
SO_4	**0.8162**	-0.0985	-0.3861
Cl	0.3184	0.3438	**0.5697**
CS	-0.3402	**0.7760**	-0.1239
FS (%)	**0.8892**	-0.3114	0.1445
Silt	**-0.7685**	0.2890	-0.2935
Clay	**-0.7646**	**-0.4195**	0.0722

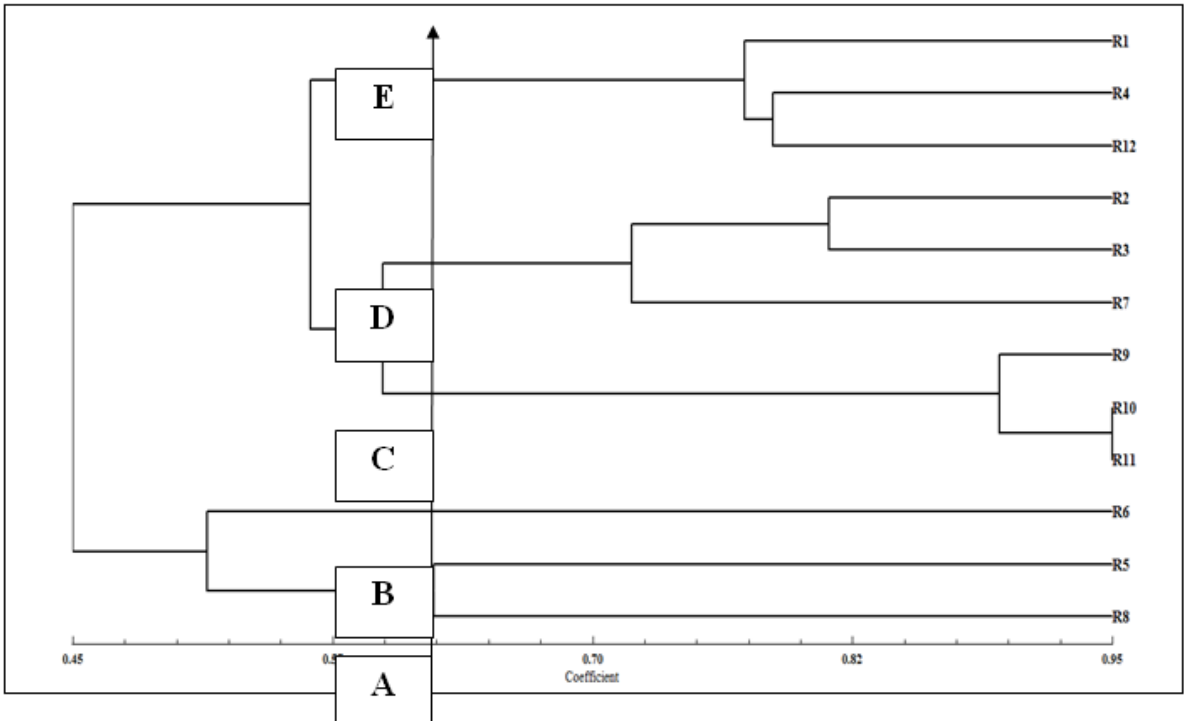

Figure 1. UPGMA phenogram showing the relationships within the studied species of *Galium*, with the 5 groups (A-E). R1, R4 & R12= *G. aparine*, R6= *G. setaceum*, R5 & R8= *G. murale*, R2, R3 & R7= *G. tricornutum*, R9, R10 & R11= *G. verrucosum*.

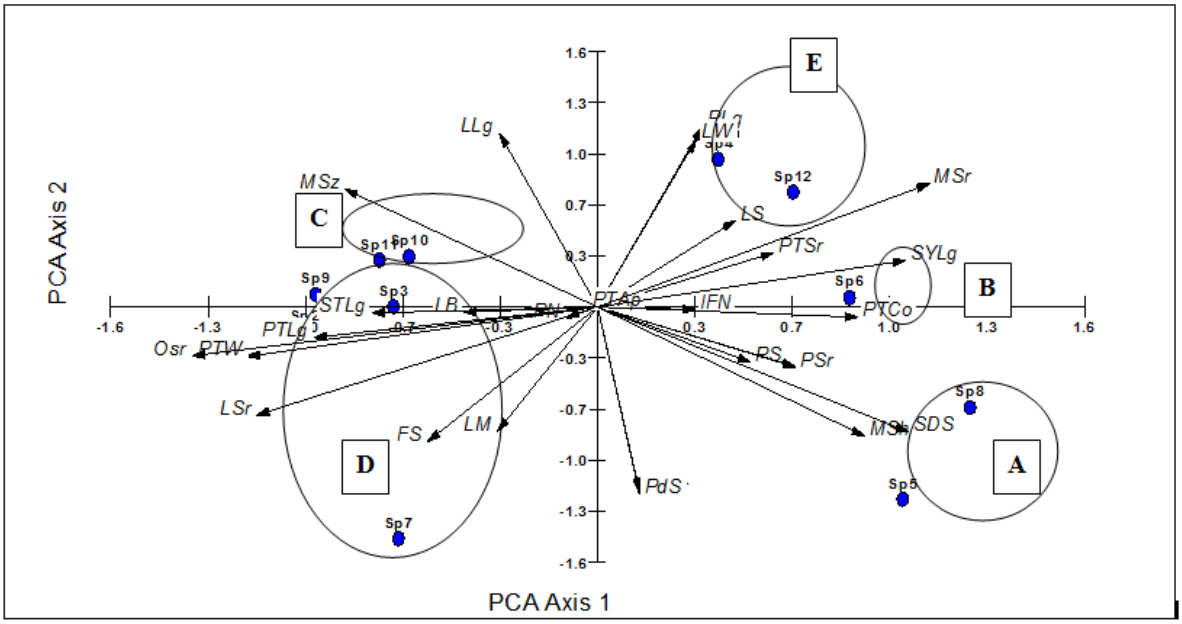

Figure 2. Principal components analysis (PCA) of the studied species of *Galium* based on 37 morphological characters (arrows), together with their species groups (A-E). See text for abbreviations.

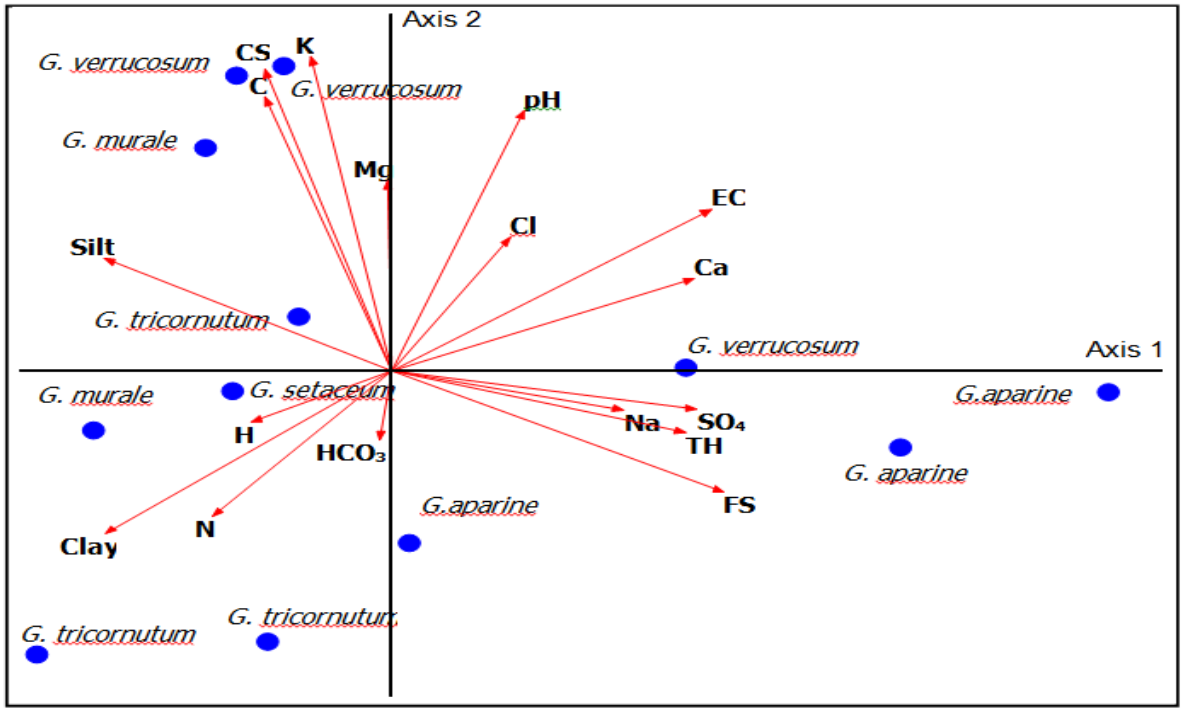

Figure 3. PCA biplot showing the relation between the examined soil variables and the studies species of *Galium*. For soil abbreviations, see Table (2).