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International Journal of Biodiversity and Conservation

Table of Contents: Volume 6 Number 5 May, 2014

ARTICLES

Genetic variation of striped snakehead (*Channa striatus* Bloch, 1793) populations using random amplified polymorphic DNA (RAPD) markers
Ajaz Ali Bhat, M. A. Haniffa, M. James Milton, Bilal Ahmad Paray, P. R. Divya and A. Gopalakrishnan

The composition and diversity of net zooplankton species in a tropical water body (Bhoj Wetland) of Bhopal, India
Najeeb Ahmad Bhat , Ashwani Wanganeo and Rajni Raina

Floristic composition, diversity and vegetation structure of woody plant communities in Boda dry evergreen Montane Forest, West Showa, Ethiopia
Fikadu Erenso, Melesse Maryo and Wondaweka Abebe

Genetic diversity among *Asparagus* species and cultivars of *Asparagus officinalis* L. using random amplified polymorphic DNA (RAPD) markers
Muhammad Irshad, Anwar Saeed, Muhammad and Muhammad Idrees

Impact of the cement dust emitted from the South Cement Factory in Tafila/Jordan on plant diversity of the surrounding area
Sawsan A. Oran and Hamad M. F. Abu Zahra

Distribution and diversity of small mammals In Borena-Sayint National Park, South Wollo, Ethiopia: Implications of habitat specialization
Meseret Chane and Solomon Yirga

Planning the priority protected areas of *Chosenia arbutifolia* due to climate change in Northeastern China
Jinghua Yu, Jizhong Wan, Chunjing Wang, Shijie Han and Qinggui Wang

Full Length Research Paper

Genetic variation of striped snakehead (*Channa striatus* Bloch, 1793) populations using random amplified polymorphic DNA (RAPD) markers**Ajaz Ali Bhat¹, M. A. Haniffa^{1*}, M. James Milton¹, Bilal Ahmad Paray¹, P. R. Divya² and A. Gopalakrishnan²**¹Centre for Aquaculture Research and Extension, St. Xavier's College, Palayamkottai Tamilnadu-627 002, India.²National Bureau of Fish Genetic Resources (NBFGR), Cochin Unit, CMFRI Campus, Kochi- 682 018, India.

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Random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) was applied to analyze the genetic variation among *Channa striatus* collected from four geographically distant locations in India. After initial PCR screening, nine random oligodecamers viz. OPA10, OPA11, OPA15, OPAC01, OPAC03, OPAC05, OPAC07, OPAC09 and OPAC19 which generated the RAPD profile for the four *C. striatus* populations were selected. Amplification using these nine primers resulted in fragments ranging in length between 309-3029 bp assigned to 87 loci. Estimates of Nei's (1978) unbiased genetic distance (D) values ranged from 0.3242-0.6320. Unweighted pair group method with arithmetic mean (UPGMA) dendrogram constructed on the basis of genetic distance revealed very close genetic relationship among *C. striatus* populations of river Tamirabarani (Tamil Nadu) and river Periyar (Kerala). Both populations from river Tamirabarani and Periyar were found to be genetically closer to fish populations from Kolleru Lake (Andhra Pradesh) whereas *C. striatus* population from Brahmani River (Orissa) was found to be genetically distant from the rest of the populations.

Key words: Indian snakehead, *Channa striatus*, genetic diversity, random amplified polymorphic DNA (RAPD).

INTRODUCTION

Murrels commonly called snakeheads belonging to genus *Channa* comprise one of the most important groups of freshwater food fish in tropical Asia (Benziger et al., 2011) having a wide natural distribution extending across the continent from Iran in the West, to China in the East, and parts of Siberia in the Far East (Berra, 2007). They are one of the most common staple food fish in Thailand,

Cambodia, Vietnam and other South East Asian countries where they are extensively cultured (Sinh and Pomeroy, 2010). Murrels are characterized by elongated body, round caudal fin, elongated dorsal and anal fins which are supported only by rays, large scales on their heads and dorso lateral position of eyes on the anterior part of the head (Figures 1 and 2). Scales are cycloid or

*Corresponding author. E-mail: sxccare@gmail.com. Tel: 09443157415 or (+91)462-2560670. Fax: (+91) 462-2560670.



Figure 1. Elongated body of *Channa striatus*.

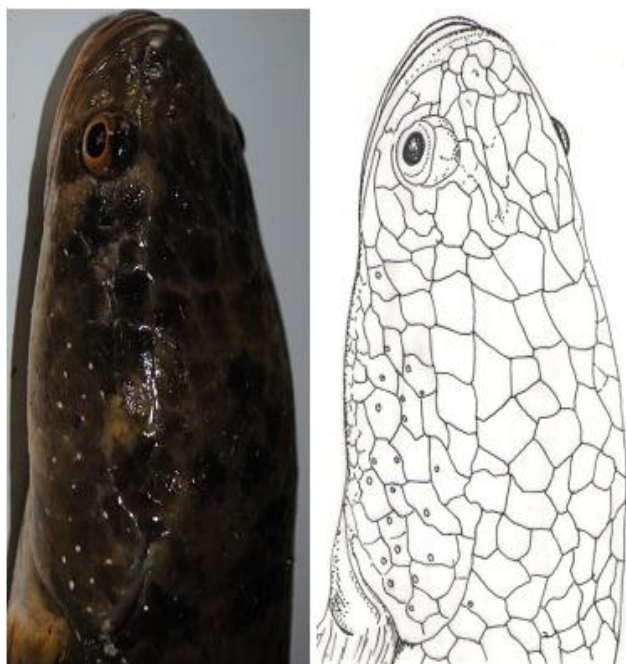


Figure 2. Presence of large scales on head and dorso lateral position of eyes of snakehead.

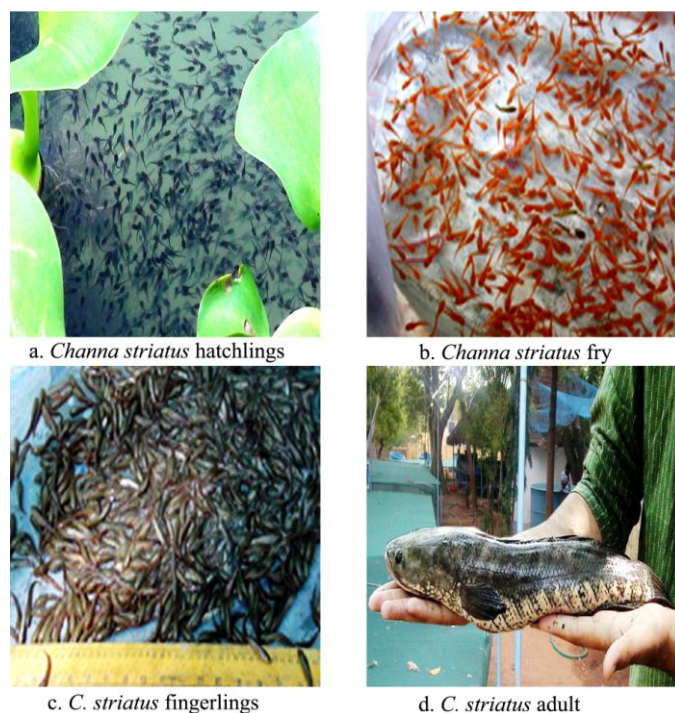


Figure 3. Color variations shown by *C. striatus* during life stages.

ctenoid (Musikasinthorn, 1998, 2003). The mouth is terminal and large with a protruding lower jaw, which is toothed, often containing canine-like teeth. Murrels can also be distinguished based on coloration, meristic, morphometric characteristics, distribution of scales on the underside of the lower jaw, the shape of the head and the morphology of the supra branchial organs.

Channa striatus is commonly called Chevron snakehead, striped murrel or striated murrel and is one of the most economically important Channid species inhabiting freshwater as well as brackishwater (Bloch, 1793). *C. striatus* displays dramatic colour changes (Haniffa et al., 2013) between early, late juvenile and adult stage as the hatchlings are black followed by a critical red color stage from post hatchling to fry, again turn black during fingerling stage and finally the adults show white striations all over the body (Figure 3).

This Channid species is regarded as highly prized food fish in Asia (Hossain et al., 2008; Haniffa, 2010) and is ubiquitous and abundant throughout India. *C. striatus* possesses peculiar characteristics like air breathing ability, hardiness and high tolerance to adverse environmental conditions and the medicinal properties make it suitable for post surgical, convalescence and arthritis patients (Ali, 1999; Mat Jais et al., 2009). Morphologically, the body is cylindrical, elongated dorsal and anal fins supported by rays only, plate like scales on head and a round caudal fin (Xia, 2006; Bhat et al., 2012). Upper body pigmentation is greenish brown to almost black; oblique bars/stripes give the impression of forward facing V shaped pattern consisting of dark streaks and blotches.

The earlier studies presented various meristic and morphometric characters like dorsal fin rays 42-45, anal

fin rays 26-29, lateral line scales 55-65, large mouth with lower jaw having 4-7 canines behind a single row of villiform teeth, dorsal and anal fins slightly darker in color than the maxilla and premaxillary process extending to vertical level beyond posterior margin of orbit, presence of a sharp pointed ridge at the mid-ventral part of isthmus and anterior to it many longitudinal striae are present; total vertebrae count 54 and branchial tooth plate count 13 (Vishwanath and Getakumari 2009). According to Nelson (1994), this group of teleostean fishes known as snakeheads is classified as:

Class: Actinopterygii
Subclass: Neopterygii
Order: Perciformis
Suborder: Channoidei
Family: Channidae

Genetic relationship between populations has been studied in different fish species having enormous evolutionary significance with regard to local adaptation, micro-evolutionary changes and maintenance of genetic variation (Slatkin, 1987). In finite populations, drift may lead to a loss of genetic variability in neutral markers which is usually applied in population studies, as well as allele compositional functional loci. Heterozygosity yields fitness advantage (Mitton, 1994) whereas loss of genetic variation will lead to reduced heterozygosity and ultimately lower fitness.

Random amplified polymorphic DNA (RAPD) is a simple and quick PCR-based technique, which uses arbitrary primers for amplification of discrete regions of genome (Williams et al., 1990) and most importantly, no prior knowledge of the genetic make-up of the organism is required (Hadrys et al., 1992).

RAPD markers have been used to evaluate the genetic diversity and conservation of numerous fish populations (Almeida et al., 2001, 2003; Dergam et al., 2002). The present investigations deals with genetic variation among four geographically distant populations of *C. striatus* collected from different Indian water bodies by RAPD-PCR.

MATERIALS AND METHODS

Sample collection

The *C. striatus* samples were collected from Kolleru lake, Andhra Pradesh (16° 30'N and 81° 15'E), River Periyar, Kerala (10° 10'N and 76° 13'E), Brahmani River, Orissa (22° 48'N and 84° 14'E) and River Tamirabarani, Tamil Nadu (80° 44'N and 77° 44'E) (Figure 4).

DNA isolation

Approximately 100 mg of fin tissue from 18 individuals of each population was preserved in 95% ethanol. DNA was isolated from preserved samples following Ruzzante et al. (1996) with minor

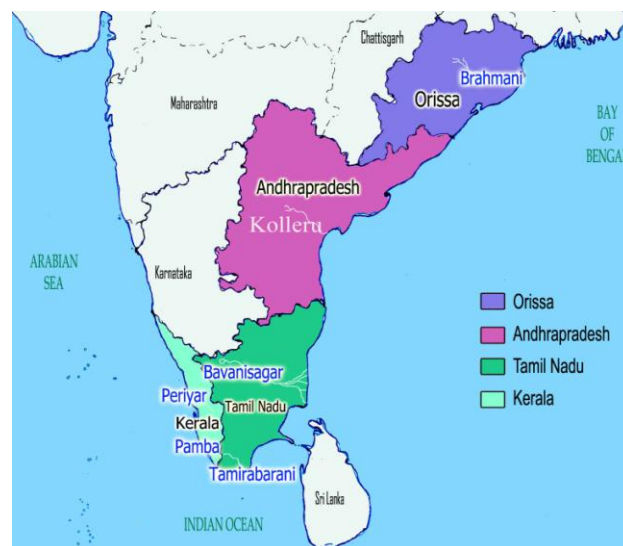


Figure 4. Map showing the collection sites of samples for the analysis of stock structure of *C. striatus*.

modifications.

Screening of primers and PCR amplification

A total of 30 arbitrary primers (OPAC and OPA series Operon Technologies Ltd. USA) with random sequence were screened (Nagarajan et al., 2006). Nine primers OPA10, OPA11, OPA15, OPAC01, OPAC03, OPAC05, OPAC07, OPAC09 and OPAC19 which gave reproducible results were selected. The PCR amplifications were carried out using Veriti 96 well Thermal Cycler Applied Biosystems in a reaction volume of 25 µl containing 50 ng genomic DNA, 10X PCR buffer (10 mM Tris-HCLpH 9.0, 50 mM KCl and 0.01% gelatin), 2.5 mM of each dNTP, 5 pmol of primer and 0.7 units of Taq DNA polymerase. The amplification conditions were 94°C for 5 min followed by 29 cycles at 94°C for 1 min, 40°C for 1 min and 72°C for 2 min with a final extension at 72°C for 10 min.

Agarose gel electrophoresis and visualization of bands

After amplification 8 µl of PCR products were electrophoresed in 1.5% agarose gel containing ethidium bromide and 1X TBE buffer to visualize the band patterns generated by each primer. The molecular weight of each band was estimated using a standard molecular marker (Lambda DNA/Eco RI Hind III Double Digest) with Image master 1D Elite Ver.3.01 (GE Amersham Biosciences USA) (Saini et al., 2010).

Statistical analysis and dendrogram

Statistical analysis was carried out for the RAPD band pattern of all the four *C. striatus* populations used for the present study. Using eight selected arbitrary primers viz, OPA10, OPA11, OPA15, OPAC01, OPAC03, OPAC05, OPAC07, OPAC09 and OPAC19, the molecular characterization of *C. striatus* populations and comparative analysis were made. RAPD band pattern was visually analyzed and scored from photographs. The well defined, prominent,

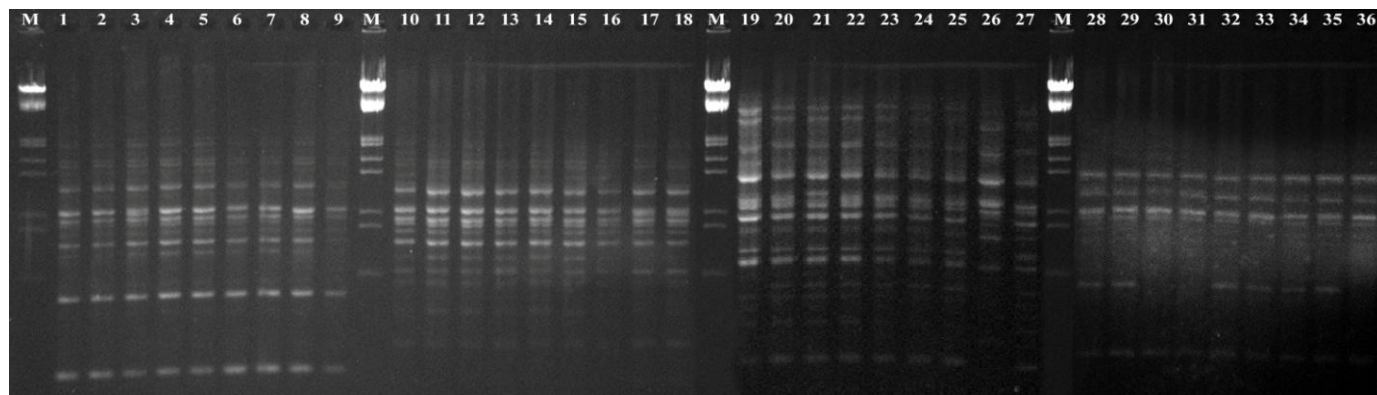


Figure 5. RAPD bands amplified by primer OPA-10 in four *Channa striatus* populations collected from various Indian locations (Lane M - standard molecular weight marker; lanes 1 to 9 - Kolleru population; lanes 10 to 18- Periyar population; lanes 19 to 27- Brahmani population and lanes 28 to 36 - Tamirabarani population).

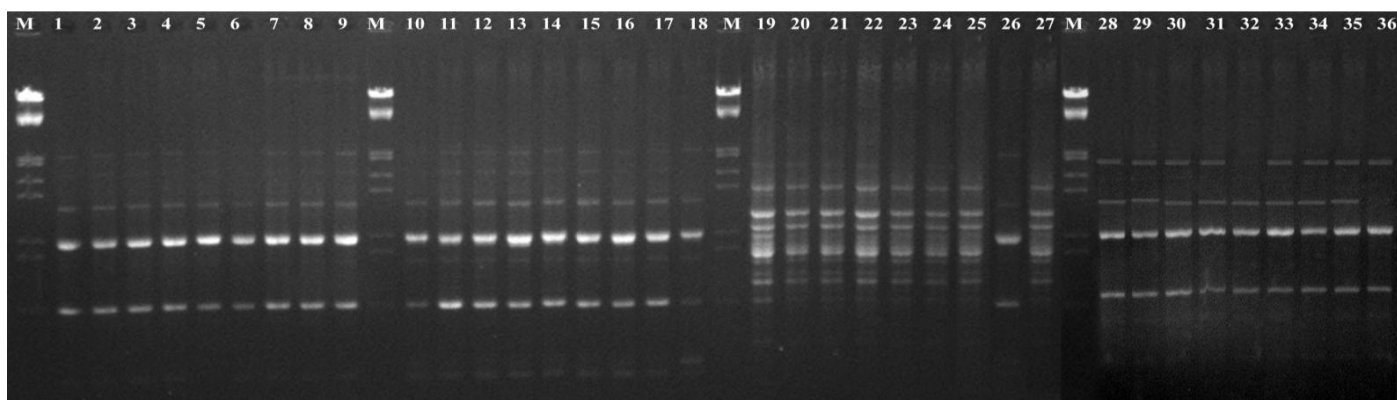


Figure 6. RAPD bands amplified by primer OPA-11 in four *Channa striatus* populations collected from various Indian locations. (Lane M - standard molecular weight marker; lanes 1 to 9 - Kolleru population; lanes 10 to 18- Periyar population; lanes 19 to 27- Brahmani population and lanes 28 to 36 - Tamirabarani population).

distinct and well separated bands were selected for the comparative analysis. The genotypes were determined by recording the presence (1) or absence (0) of the bands and neglecting the weak and unresolved bands. Nei's (1978) unbiased genetic identity (I) and genetic distance (D) values between *C. striatus* populations were calculated using the data generated from RAPD profiles using POPGENE 1.31 (Yeh et al., 1999). Genetic distance values were utilized to construct a dendrogram through clustering analysis (UPGMA) to determine the relationship between *C. striatus* populations.

RESULTS AND DISCUSSION

Out of 30 decamer primers screened 9 primers *viz*, OPA10, OPA11, OPA15, OPAC01, OPAC03, OPAC05, OPAC07, OPAC09 and OPAC19 showed reproducible results with good resolutions in banding patterns whereas the other 21 primers produced highly inconsistent amplification products or did not amplify at all and hence they were excluded from further analysis. The RAPD band

profile for four geographically distant *C. striatus* populations for various selected oligodecamers are depicted in Figures 5 to 13. The nine oligodecamer primers that generated amplification fragments ranging in length between 309-3029 bp in length were assigned to 87 loci. The number of stable and clear RAPD bands generated per primer varied between 5 and 13. The performance of Operon random primers on *C. striatus* populations collected from different Indian water systems which highlight the primer code, number of bands amplified, number of polymorphic bands, number of unique (monomorphic) bands and polymorphism percentage is shown in Table 1.

The RAPD amplification observed for the four different populations with the oligodecamers highlighted were consistent and showed population specific bands. Of the 87 RAPD bands, 11 were population-specific bands (unique bands) among the eleven population-specific bands, the Kolleru population had four bands, Periyar

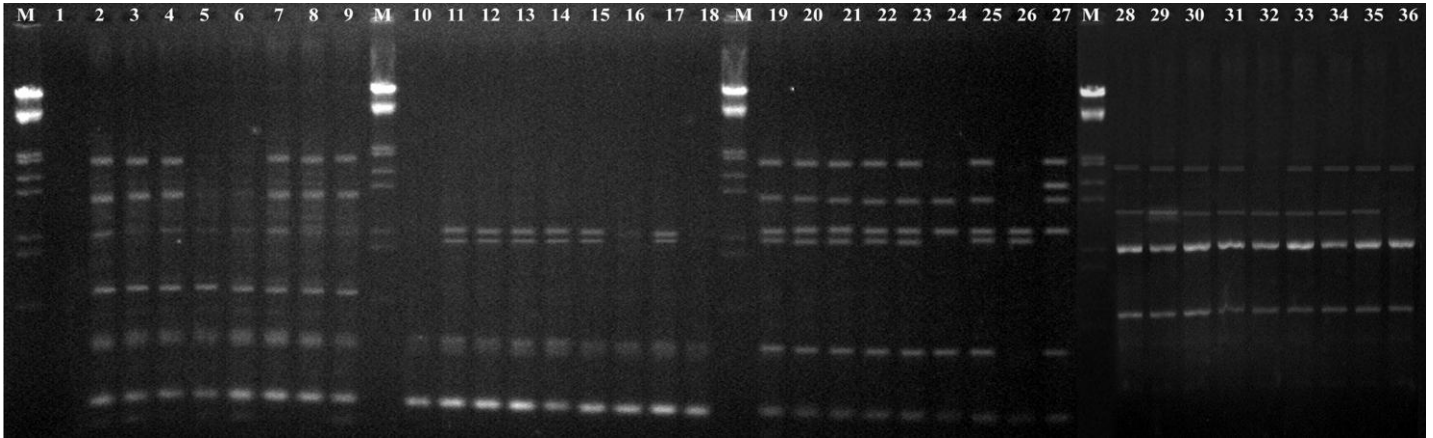


Figure 7. RAPD bands amplified by primer OPA-15 in four *Channa striatus* populations collected from various Indian locations. Lane M - standard molecular weight marker; lanes 1 to 9 - Kolleru population; lanes 10 to 18 - Periyar population; lanes 19 to 27 - Brahamani population and lanes 28 to 36 - Tamirabarani population.

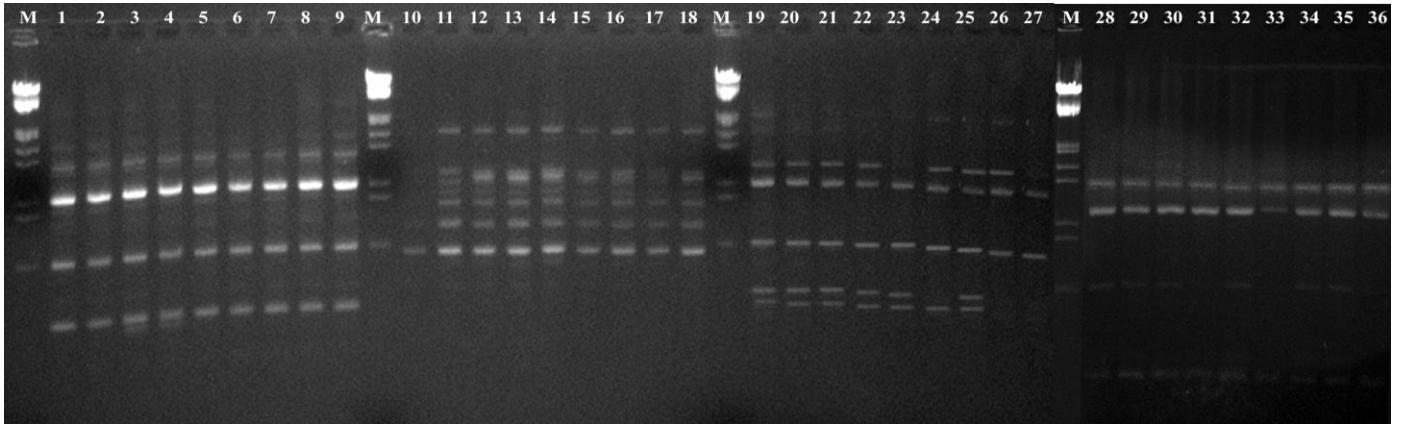


Figure 8. RAPD bands amplified by primer OPAC-01 in four *Channa striatus* populations collected from various Indian locations. Lane M - standard molecular weight marker; lanes 1 to 9 - Kolleru Population; lanes 10 to 18 - Periyar Population; lanes 19 to 27 - Brahamani Population and lanes 28 to 36 - Tamirabarani Population.

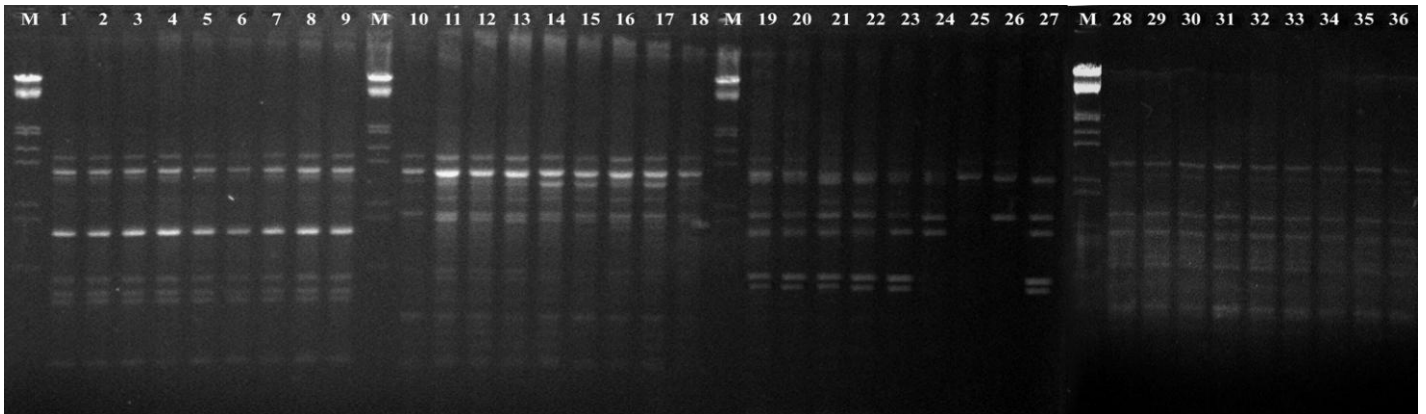


Figure 9. RAPD bands amplified by primer OPAC-03 in four *Channa striatus* populations collected from various Indian locations. Lane M - standard molecular weight marker; lanes 1 to 9 - Kolleru population; lanes 10 to 18 - Periyar population; lanes 19 to 27 - Brahamani population and lanes 28 to 36 - Tamirabarani population.

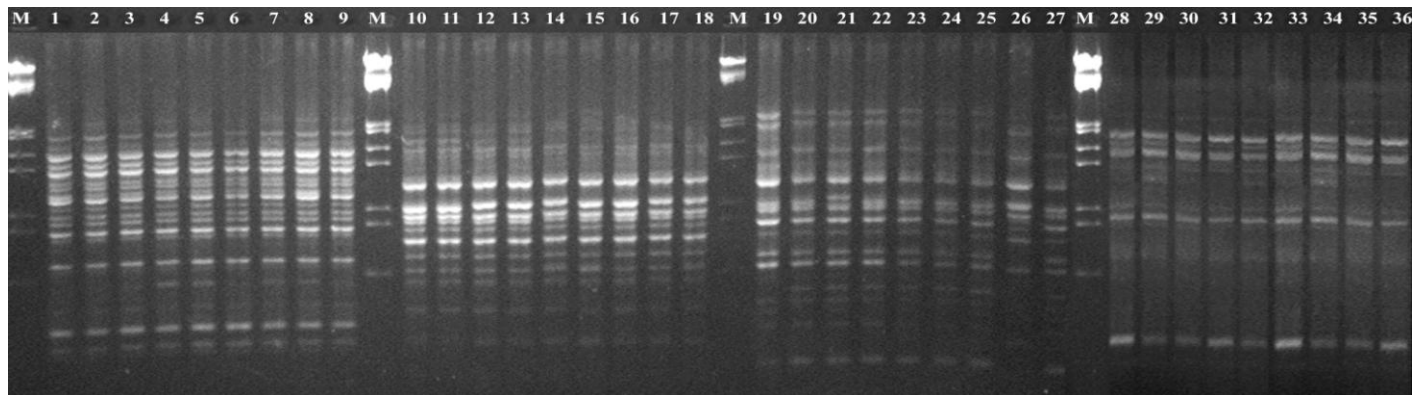


Figure 10. RAPD bands amplified by primer OPAC-05 in four *Channa striatus* populations collected from various Indian locations. Lane M - standard molecular weight marker; lanes 1 to 9 - Kolleru population; lanes 10 to 18 - Periyar population; lanes 19 to 27 - Brahamani population and lanes 28 to 36 - Tamirabarani population.

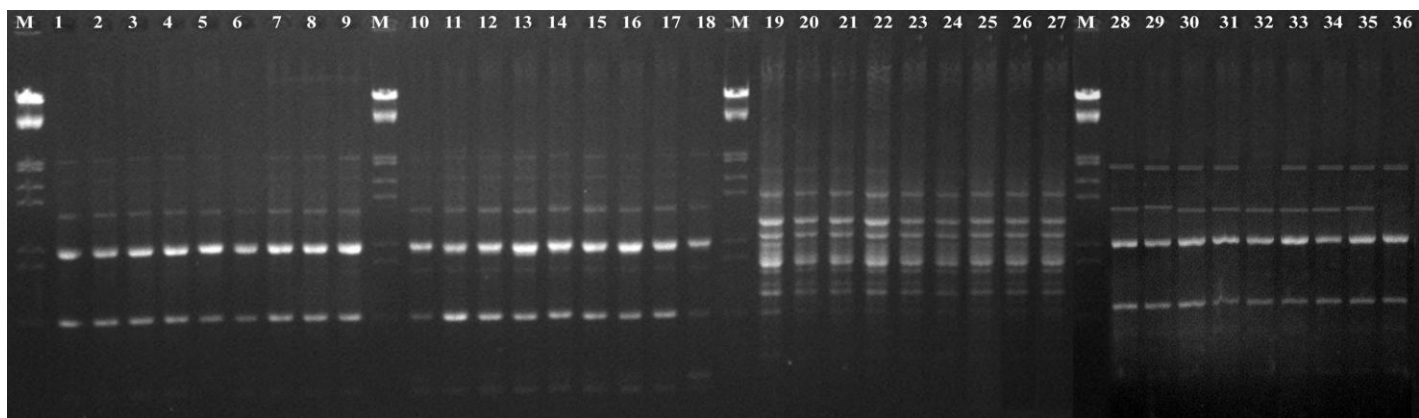


Figure 11. RAPD bands amplified by primer OPAC-07 in four *Channa striatus* populations collected from various Indian locations. Lane M - standard molecular weight marker; lanes 1 to 9 - Kolleru population; lanes 10 to 18 - Periyar population; lanes 19 to 27 - Brahamani population and lanes 28 to 36 - Tamirabarani population.

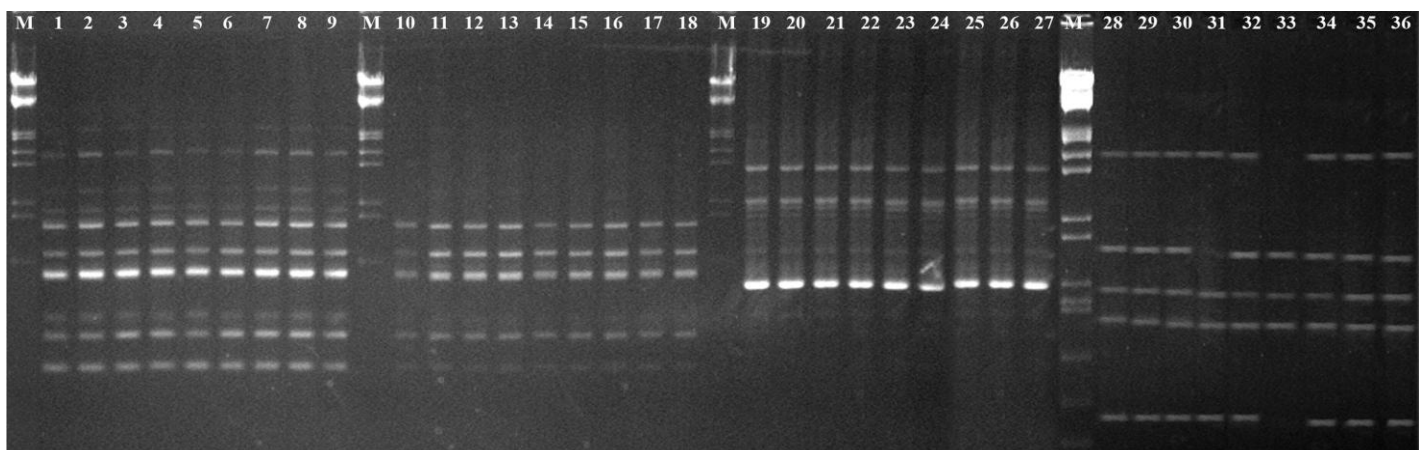


Figure 12. RAPD bands amplified by primer OPAC-09 in four *Channa striatus* populations collected from various Indian locations. Lane M - standard molecular weight marker; lanes 1 to 9 - Kolleru population; lanes 10 to 18 - Periyar population; lanes 19 to 27 - Brahamani population and lanes 28 to 36 - Tamirabarani population.

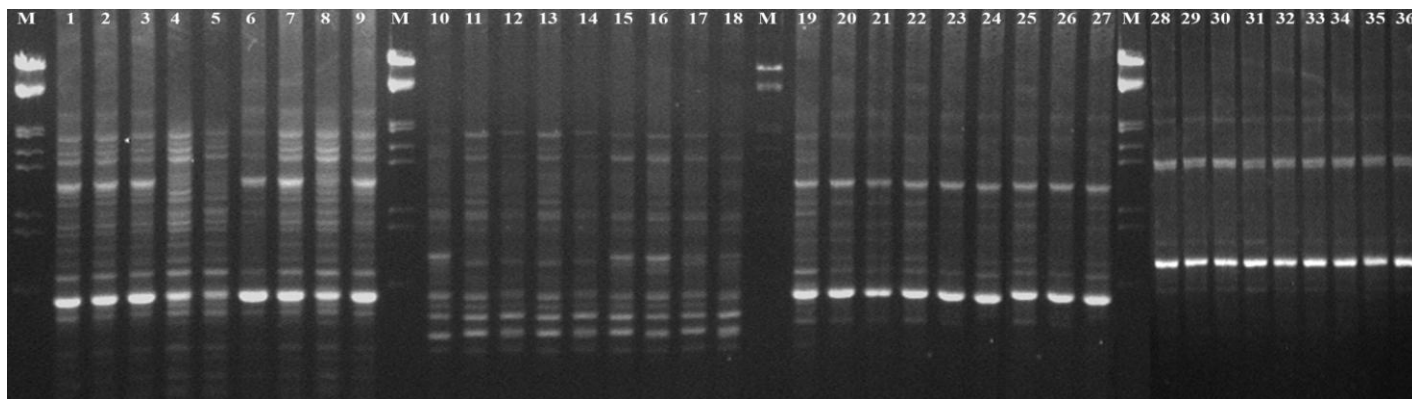


Figure 13. RAPD bands amplified by primer OPAC-19 in four *Channa striatus* populations collected from various Indian locations. Lane M - standard molecular weight marker; lanes 1 to 9 - Kolleru population; lanes 10 to 18 - Periyar population; lanes 19 to 27 - Brahmani population and lanes 28 to 36 - Tamirabarani population.

Table 1. Performance of operon random primers on four *C. striatus* populations.

Primer code	No. of band	Polymorphic band	Unique band	Polymorphism (%)	Description of unique/population specific bands (bp)			
					Kolleru	Periyar	Brahmani	Tamira- b arani
OPA 10	13	11	2	84.62		1(790)	1(3530)	-
OPA 11	10	9	1	90.0	-	-	(1375)	-
OPA15	8	7	1	87.50		(860)	-	-
OPAC 01	5	4	1	80.00	(1950)	-	-	-
OPAC 03	9	8	1	88.89	(376)	-	-	-
OPAC 05	8	7	1	87.50	-	-	-	(1820)
OPAC 07	13	12	1	92.31	-	-	(2102)	-
OPAC 09	9	8	1	88.89		-	(947)	-
OPAC 19	12	11	1	91.67	(1716)	-		-

population had two bands, Brahmani population had four bands whereas the Tamirabarani population had only one population specific band.

The polymorphic and unique DNA bands can be used as genetic markers to select the breeders from the desired population for the purpose of selective breeding programmes and to monitor the level of DNA variability in the wild or cultured population. Taking into account the amplification results of the individual primers used in the present study especially the population specific bands OPA-10 amplified single unique band for each of the population of Periyar and Brahmani waterbodies, whereas OPA-11 amplified a single unique band for Brahmani population and OPA-15 oligodecamer amplified a single unique band for periyar population. The primers OPAC -01 and OPAC -03 each amplified one specific band for Kolleru population. OPAC-05 oligodecamer amplified single unique band for Tamirabarani and OPAC-07 amplified single unique band for Brahmani population. OPAC-09 oligodecamer amplified single unique band for Brahmani population whereas OPAC-19 oligodecamer amplified a single unique bands for Kolleru

population.

The overall estimate of gene diversity, average pair wise similarity index, number of polymorphic loci and percentage of polymorphism in *C. striatus* populations is given in Table 2. Estimates of Nei (1978) unbiased genetic distance (D) demonstrated the genetic distance to discriminate the different *C. striatus* populations (Table 3) and the values ranged from 0.3242-0.6320. UPGMA dendrogram constructed on the basis of genetic distance revealed that the genetic relationship was very close among *C. striatus* populations of Tamirabarani (Tamilnadu) and Periyar (Kerala). Both these populations were found to be genetically closer to Kolleru (Andhra Pradesh) population whereas *C. striatus* population of Brahmani (Orissa) was found to be genetically distant from the rest of the populations (Figure 14).

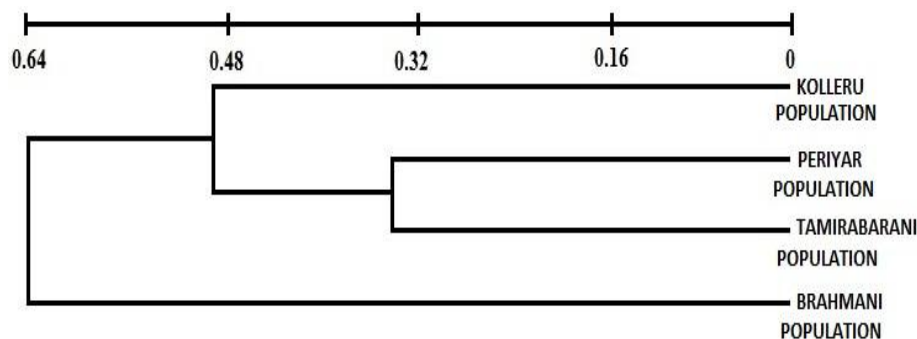
The presence of variability among populations as well as individuals within a population is essential for their ability to survive and successfully respond to environmental changes (Ryman et al., 1995). Excessive exploitation by capture fishery and loss of aquatic ecosystems combined with poor fishery management result in the

Table 2. Overall estimate of gene diversity, average pair wise similarity index, polymorphic loci and percentage of polymorphism in *Channa striatus* populations.

Parameter	Kolleru	Periyar	Brahmani	Tamirabarani
Gene diversity	0.0684	0.1005	0.1465	0.0969
Shannon information index	0.0972	0.1426	0.2063	0.1368
No. of polymorphic loci	13	19	27	18
Percentage polymorphism	14.94	21.84	31.03	20.69

Table 3. Nei's 1978 genetic distance among four populations of *C. striatus*.

Population ID	Kolleru	Periyar	Brahmani	Tamirabarani
Kolleru	****			
Periyar	0.3922	****		
Brahmani	0.6320	0.4812	****	
Tamirabarani	0.4668	0.3242	0.4546	****

**Figure 14.** UPGMA dendrogram of four *C. striatus* populations based on RAPD profiles.

depletion of the fishery stocks. Such depletions can result in the loss of total gene pool (Smith et al., 1991). Conservation of the genetic diversity has emerged as one of the central issues in conservation biology (Bickham et al., 2000), considering its value for the sustainability of populations (Avisé, 2004). Simultaneously, advances in molecular techniques increased the availability of different DNA-based markers, which have become efficient tools in conservation genetic studies, gene mapping, population genetics, molecular evolutionary genetics and plant and animal breeding (Haig, 1998; Ertas and Seker, 2005).

RAPD markers have been found to have a wide range of applications in gene mapping, population genetics, molecular evolution and genetic breeding programmes (Bardakci and Skibinski, 1994; Wasko et al., 2004; Leuzzi et al., 2004; Matoso et al., 2004). For instance, genetic variation has been well studied between 4 different populations of Hilsa Shad from Ganga, Yamuna, Hoogly

and Narmada rivers of India using RAPD technique (Brahmane et al., 2006). Morphometric and ISSR marker systems used for categorization of five Channid species revealed the closeness of *C. striatus* and *Channa marulius* (Haniffa et al., 2014).

In the present study, the polymorphic and unique DNA bands detected can be used as genetic markers to select the breeders from the desired population for the purpose of selective breeding programmes and to monitor the level of DNA variability in the wild or cultured population of the species. Besides these, population-specific unique bands can be used to detect any possible mixing of the populations, especially during selective breeding programmes (Ferguson et al., 1995). D'Amato and Corach (1996) reported that a single RAPD fraction present in one of the two populations of *Macrobrachium borellii* was considered as a genetic marker for stock identification.

In the present study, based on clustering analysis of

C. striatus populations, Tamirabarani and Periyar populations were found to be genetically closer. Among Kolleru and Brahmani populations the former was found genetically closer to Periyar and Tamirabarani populations. The population structure of freshwater organisms is dependent on the distributions of river systems (Ikeda et al., 1993; Hara et al., 1998). Among the four water bodies studied, river Tamirabarani (Tamilnadu) and Periyar (Kerala) originate from Western Ghats, South India and are geographically closer to each other than to Lake Kolleru of Andhra Pradesh. The river Brahmani (Orissa) is located more than 1500 km (East India) geographically away from Tamirabarani, Periyar and Kolleru lake. Based on Nei's genetic distance values, it is possible to conclude that Tamirabarani, Periyar and Kolleru populations are closer to each other when compared to Brahmani population. The genetic differentiation is primarily dependent on geographical isolation as the present study showed a significant correlation between genetic closeness and geographical distance. RAPD has been used in population studies in fisheries and can be used efficiently for variation analysis of populations with differential degrees of geographic isolation. The present study will be helpful to understand genetic relationship between different *C. striatus* populations inhabiting Indian waters, their effective management, conservation and effective scientific captive breeding programmes. The study will also be useful as the reference investigation for the future molecular as well as morphological studies on snakeheads.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Full Length Research Paper

The composition and diversity of net zooplankton species in a tropical water body (Bhoj Wetland) of Bhopal, India

Najeeb Ahmad Bhat ^{1*}, Ashwani Wanganeo¹ and Rajni Raina²¹Department of Environmental Sciences and Limnology, Barkatullah University, Bhopal (M.P) India-462026.²Department of Zoology, Government Science and Commerce College, Bhopal (MP) India.

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The aim of the present study was to determine the species diversity and abundance of net zooplankton in samples collected from Bhoj wetland, Bhopal, India. A total of 82 species of zooplankton were identified, among them, 66 species were recorded during the first year (2008-09) and 70 species were documented during the second year (2009-10) of the study period. In the first year, Rotifera recorded the highest number of species (53%) followed by Cladocera (29%), which in turn was followed by Copepoda (8%), Protozoa (6%) and Ostracoda (5%) in the second year of study, Rotifera recorded the highest number of species (47%) followed by Cladocera (37%), which in turn was followed by Protozoa (7%), Copepoda (6%) and Ostracoda (3%). Cumulative 24 months density in the present study ranged from 760 to 11050 Ind.l⁻¹, with an overall mean of 3307 Ind.l⁻¹. A major peak of 11050 Ind.l⁻¹ was observed in June 2009, with 47 and 43% contribution from Copepoda and Rotifera. Among Copepoda, *Cyclops* sp. and nauplii were major contributors to this peak while amongst Rotifera, *Brachionus caudatus* and *Keratella tropica* were dominant contributors. Cladocera was comparatively less represented group, being chiefly represented by *Diaphanosoma* sp. Shannon-index ranged between 0.96 and 2.75 during the two years of study period.

Key words: Zooplankton, diversity, Cyclops, Brachionus, Keratella, Shannon-Wiener index, Bhoj wetland.

INTRODUCTION

Tropical wetlands have played an important role for humankind in all continents (Junk, 2002). These are characterized by a large number of ecological niches and harbour a significant percentage of world's biological diversity. Wetlands are among the most productive ecosystems in the world, comparable to rainforests and coral reefs (Thomas and Deviprasad, 2007). Zooplankton

are microscopic organisms which do not have the power of locomotion and move at the mercy of the water movements. Zooplankton community is cosmopolitan in nature and they inhabit all freshwater habitats of the world. Zooplankton diversity and density refers to variety within the community (Jalilzadeh et al., 2008). These are often an important link in the transformation of energy

*Corresponding author Email: najahbhat@gmail.com. Tel: 09419425309.

from producers to consumers due to their large density, drifting nature, high group or species diversity and different tolerance to the stress. Zooplankton plays an important role in lake ecosystem, as grazers that control algal and bacterial populations, as a food source for higher trophic levels and in the excretion of dissolved nutrients. The organization of biological communities in aquatic ecosystems is closely dependent on the variations of physical and chemical conditions linked to natural and anthropogenic factors (Pourriot and Meybeck, 1995). The zooplankton communities, very sensitive to environmental modifications, are important indicators for evaluating the ecological status of these ecosystems (Magadza, 1994). They do not only form an integral part of the lentic community but also contribute significantly, the biological productivity of the fresh water ecosystem (Wetzel, 2001). The presence and the relative predominance of various copepod species have been used to characterize the eutrophication level of aquatic ecosystems (Park and Marshall, 2000; Bonecker et al., 2001). Herbivorous zooplankton is recognized as the main agent for the top-down control of phytoplankton, and the grazing pressure exerted by cladocerans and copepods on algae and cyanobacteria is sometimes an important controlling factor of harmful algal blooms (Boon et al., 1994).

The objectives of this study are i) to study the seasonal fluctuations of zooplankton abundance of the Bhoj wetland, ii) to understand the impact of pollution on zooplankton community in the Bhoj wetland. In this investigation, the data of zooplankton density and diversity in a tropical wetland system (Bhoj wetland) was studied for two years.

Study area

Bhopal, the capital city of the state of Madhya Pradesh, India is famous for its numerous lakes. Of these, the most important are the Upper and Lower Lakes, which have commonly been designated as Bhoj Wetland. The Bhoj Wetland is a wetland of international importance. The Upper Lake basin comprises of a submergence area of about 31.0 sq km and a catchment area of 361 sq km., whereas the Lower Lake basin comprises of a submergence area of 0.9 sq km and catchment area of 9.6 sq km. While Lower Lake is surrounded on all sides by dense urban settlements, only about 40% of the fringe area of Upper Lake has dense human settlement and the rest is sparsely populated having cropping as the major land use. The Upper Lake spread over longitude 77°18'00" to 77°24'00" E and latitude 23°13'00" to 23°16'00" N, whereas the considerably smaller Lower Lake is spread over 77°24'00" to 77°26'00" E and latitude 23°14'30" to 23°15'30" N. The Upper Lake was created in the 11th century by constructing an earthen dam across Kolans River, the main feeding channel of the lake with the objective of supplying potable water to the city

dwellers. The wetland also supports a wide variety of flora and fauna. Several species of phyto and zooplankton, macrophytes, aquatic insects, amphibians, fishes and birds (resident as well as migratory) are found in these wetlands. Considering its ecological importance, Ramsar site was declared by the Government of India in 2002. Increase in anthropogenic activities in the catchment during the second half of the last century resulted in environmental degradation of the lakes.

Investigations on the ecology of Bhoj wetland of Madhya Pradesh indicate that this man-made wetland is under severe degradation pressure. Siltation, solid waste disposal and weed infestation, dumping of agricultural waste, hospital waste disposal and idol immersion in the wetland during the festival season pollutes the wetland ecosystem beyond the tolerable limits of any aquatic system Figure 1.

MATERIALS AND METHODS

Water samples were collected on monthly basis for a period of two year. For the present study nine sampling points in the wetland were selected and each point, taking into account the human activities such as washing, bathing, fishing, boating, the outlets, inlets, morphometric features and growth of aquatic vegetation etc., and other important factors were considered during the selection of the sampling sites. Some of the features of the sampling sites are as follows: Station I (Kamla Park) is situated on eastern end of the wetland. It is subjected to maximum anthropogenic pressure. The Idol immersion activity at this site has been reduced after developing Prempura Ghat particularly for immersion activity. Station II (Gandhi Medical College) is situated close to the inlet of Shaheed Nagar Nallah adjacent to Gandhi Medical College. Station III (Koh e Fiza) has an intake point for water supply in this area. This station is also the site of Tazia immersion. Station IV (Van Vihar) represents the area that comes under protected forest (Van Vihar). The station is comparatively free from human intervention and other anthropogenic activities. Station V (Yatch Club) is the boating station, where maximum human interaction takes place. Tourists start their motor and paddle boats from this station, and a crowd of tourists can be observed from morning till evening at this station. Station VI (Bairagarh), a station of Bhoj wetland is situated near Bairagarh where substantial inflow of domestic sewage can be seen. The area has become shallow due to high density of free floating, emergent and submerged macrophytes. Station VII (Sehore side) has a lot of agricultural land surrounds this station in Bhoj Wetland. Most of the catchment area consists of agricultural land. Because of this all the fertilizers, pesticides and agricultural residues used in the fields find their way as run off into the wetland waters. Station VIII (Prempura Ghat) is the idol immersion station. During the Hindu religious festivals, lots of idols are immersed in water. Station IX (Nehru Nagar) is highly influenced by anthropogenic and cattle activities. The run-off from the catchment area adds nutrients to the wetland. The region is covered with high density of emergent/submerged macrophytes. The run-off from the catchment area also adds considerable quantities of nutrients to the wetland.

The water samples have been collected in one liter polyethylene canes of the surface waters by the boat between 8 am to 12 pm from the selected sites of the Bhoj wetland. For the quantitative analysis of zooplankton, water was collected from the surface with minimal disturbance and filtered through a No. 25 bolting silk cloth, net of mesh size 63 µm. Ten liters of water were filtered and con-

centrated to 100 ml and were preserved by adding 2 ml of 4% formalin simultaneously. The quantitative analysis of zooplankton was done by using Sedgwick-Rafter cell with dimensions of 50 x 20 x 1 mm, following the method given in APHA (2000). 1 ml of concentrated sample was taken in a Sedgwick-Rafter counting cell and the entire contents were counted. The identification of aquatic biota (zooplankton) have been done following the standard works and methods of Edmonson (1959), Needham and Needham (1962), Pennak (1978), Victor and Fernando (1979), Michael and Sharma (1988), Battish (1992) and Sharma (1999). The results have been expressed as individuals/l (Wanganeo and Wanganeo, 2006).

$$\text{Number of zooplankton "n"} = \frac{C \times 1000 \text{ mm}^2}{A \times D \times E}$$

C = Number of organisms recorded; A = area of field of microscope; D = depth of field (SRC depth) in mm; E = number of fields counted.

$$\text{Number of zooplankton/l} = \frac{n \times \text{Vol. of concentrate (ml)}}{\text{Vol. (litres) of water filtered}}$$

Shannon diversity index

This index is an index applied to biological systems derived from a mathematical formula used in communication area by Shannon in 1948.

$$H' = -\sum [(n_i / N) \times (\ln n_i / N)]$$

H': Shannon Diversity Index; n_i: Number of individuals belonging to i species; N: total number of individuals.

RESULTS AND DISCUSSION

Zooplankton are the central trophic link between primary producers and higher trophic levels. The freshwater zooplankton comprises Cladocera, Rotifera, Copepoda, Ostracoda and Protozoa. Most of them depend to a large extent, on various bacterioplankton and phytoplankton for food. Many of the larger forms feed on smaller zooplankton, forming secondary consumers. Some of them are detritivore feeders, browsing and feeding on the substrate attached organic matter. Many of these organisms are also fish food organisms and are consumed by the other aquatic macrofauna.

In the two years of the study period, a total of 82 species of zooplankton were identified, among them, 66 species were recorded during the 1st year (2008-09) of study, while 70 species of Zooplankton were documented during the 2nd year (2009-10) of study period. At all the nine stations total 66 species were identified, group Rotifera recorded the highest number of species (53%) followed by Cladocera (29%), which in turn was followed by Copepoda (8%), Protozoa (6%) and Ostracoda, 5% (Table 1 and Figure 1 and 2).

Similarly in the second year of study at all the nine stations a total of 70 species were identified, group Rotifera recorded the highest number of species (47%) followed by Cladocera (37%), which in turn was followed

Table 1. Qualitative enumeration of zooplankton species, 2008-09.

Group	First year (2008-09)	Second year (2009-10)
	Number of species (%)	Number of Species (%)
Rotifera	35 (53%)	33 (47%)
Cladocera	19 (29%)	26 (37%)
Copepoda	5 (8%)	4 (6%)
Ostracoda	3 (5%)	2 (3%)
Protozoa	4 (6%)	5 (7%)
Total	66	70

by Protozoa (7%), Copepoda (6%) and Ostracoda (3%) (Table 1 and Figure 3).

The relative abundance was maximum (3.83%) for *Bosmina* sp. and minimum (0.01%) for *Chydorus ventricosue*, *Diaphanosoma excisum*, *Diaphanosoma sarsi*, *Sida crystallinein* Cladocera while the maximum (8.43%) for *Brachionus caudatus* and minimum (0.01%) for *Ploesoma* sp. *Triploceros limnias* in Rotifera; maximum (32.20%) for *Cyclops* sp. and minimum (0.03%) for *Mesocyclops* sp. in Copepoda and Ostracoda and protozoa are least groups (Table 2).

Furthermore, the frequency of occurrence was maximum (42.15 and 47.38%) in the month of October during the first and second year and minimum (8.54 and 5.94%) in the month of November 2008 and September 2009 from the group Cladocera. Similarly in the Rotifera group, frequency of occurrence was maximum (39.51 and 47.49%) in December 2008 and September 2009 and minimum (14.05% and 3.13%) in October 2008 and January 2010 during first and second year of study. While in Copepoda, it was maximum (66.54 and 81.04%) in the March 2008 and January 2009 and minimum (36.47% and 29.65%) in the January 2009 and October 2009. Nevertheless, the frequency of the occurrence in Ostracoda and Protozoa (each of these groups were represented by least species density) was maximum having 2.26 and 3.73% respectively (Table 3 and Figure 4).

In the present study, the zooplanktonic mean density during 1st year was 2484 Ind.l⁻¹ which increased to 4130 Ind.l⁻¹ in the 2nd year (Table 3). There was variation in zooplankton density during two years which may be attributed to low water volume caused by drought conditions in the second year. The maximum population density recorded in the 2nd year also reflected a positive relationship with temperature, nitrate and phosphate concentrations. Similar observations were recorded by Paliwal (2005). The maximum population density of zooplankton in the 2nd year may also be attributed to greater availability of food viz., phytoplankton. The factors like temperature, dissolved oxygen play an important role

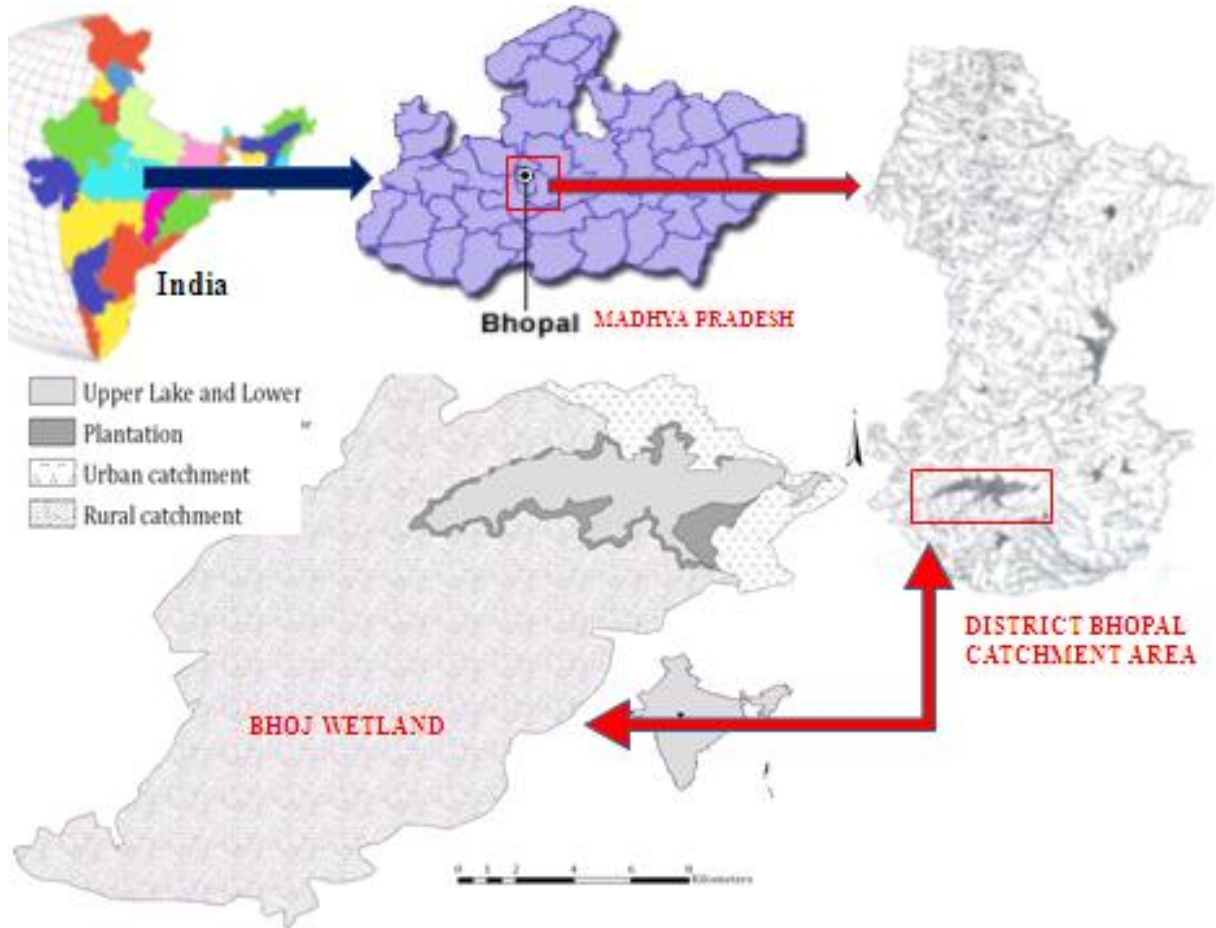


Figure 1. Map of India indicating location of Madhya Pradesh state and also indicating location of study area (Bhoj wetland), Bhopal (Source MPCST 2009).

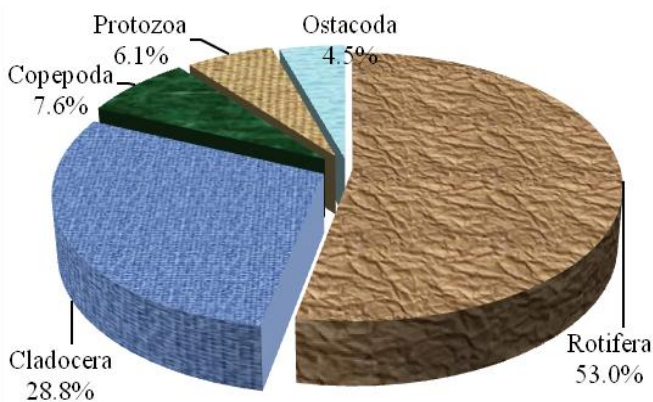


Figure 2. Group wise percent contribution of zooplankton (2008-09).

in controlling the diversity and density of zooplankton (Edmondson, 1965; Baker, 1979).

According to Kurbatova (2005) and Tanner et al. (2005),

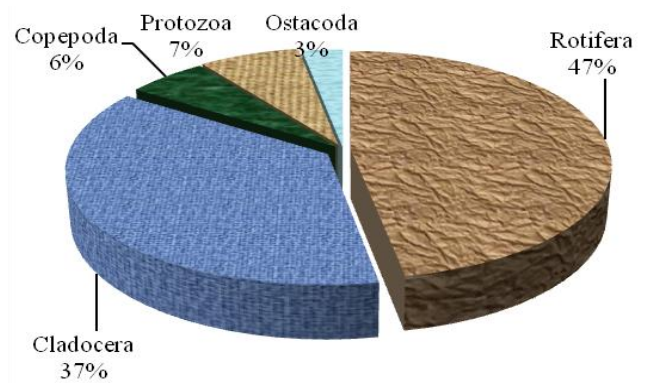


Figure 3. Group wise percent contribution during 2009-10.

pH more than 8 means highly productive nature of a water body, in the present study, the average pH recorded was 8.3 units, indicating water highly productive for zooplankton population. Cumulative station (24

Table 2. Net zooplankton species recorded from the surface water of the Bhoj wetland from February 2008 to January 2010.

Groups with species	Number of individuals	Relative frequency (% by number)	Groups with species	Number of individuals	Relative frequency (% by number)
Cladocera (28 species)		16.75	<i>Mytilina</i> sp.	70	0.09
<i>Alona</i> sp.	400	0.50	<i>Philodina</i> sp.	20	0.03
<i>Alonella</i> sp.	260	0.33	<i>Platylas</i> sp.	90	0.11
<i>Alonella dentifera</i>	20	0.03	<i>Ploesoma</i> sp.	10	0.01
<i>Bosmina</i> sp.	3040	3.83	<i>Polyarthra</i> sp.	1020	1.28
<i>Bosmina longirostris</i>	200	0.25	<i>Rotaria</i> sp.	60	0.08
<i>Bosminopsis deitersi</i>	30	0.04	<i>Scaridium</i> sp.	80	0.10
<i>Ceriodaphnia</i> sp.	700	0.88	<i>Synchaeta</i> sp.	40	0.05
<i>Chydorus (space)</i> sp	1950	2.45	<i>Tetramastixapoliensis</i>	60	0.08
<i>Chydorus sphaericus</i>	390	0.49	<i>Trichocerca</i> sp.	710	0.89
<i>Chydorus ventricosue</i>	10	0.01	<i>Trichocercalongiseta</i>	140	0.18
<i>Conochiloides</i> sp.	40	0.05	<i>Trichotria</i> sp.	20	0.03
<i>Daphnia</i> sp.	130	0.16	<i>Triploceros limnias</i>	10	0.01
<i>Diaphanosoma</i> sp.	910	1.15	<i>Trochosphaera</i> sp.	20	0.03
<i>Diaphanosoma brachyurum</i>	110	0.14	Copepoda (5 species)		51.23
<i>Diaphanosoma excisum</i>	10	0.01	<i>Cyclopoid copepod</i>	40	0.05
<i>Diaphanosoma sarsi</i>	10	0.01	<i>Cyclops</i> sp.	25590	32.20
<i>Leydigia</i> sp.	280	0.35	<i>Diaptomus</i> sp.	1010	1.27
<i>Macrothrix</i> sp.	80	0.10	<i>Mesocyclops</i> sp.	20	0.03
<i>Moina</i> sp.	1010	1.27	Nauplius larvae	14050	17.68
<i>Moina macrocopa</i>	140	0.18	Ostracoda (3 species)		0.33
<i>Moina micrura</i>	70	0.09	<i>Cyprinotus</i> sp.	60	0.08
<i>Moinadaphnia</i> sp.	1310	1.65	<i>Cypris</i> sp.	140	0.18
<i>Pleuroxus aduncus</i>	180	0.23	<i>Stenocypris</i> sp.	60	0.08
<i>Scapholebris</i> sp.	40	0.05	Protozoa (8 species)		0.99
<i>Sida</i> sp.	70	0.09	<i>Actinophyrus</i> sp.	20	0.03
<i>Sida crystallina</i>	10	0.01	<i>Arcella</i> sp.	20	0.03
<i>Simocephalus</i> sp	1780	2.24	<i>Centropyxix</i> sp.	590	0.74
<i>Streblocerus</i> sp.	130	0.16	<i>Climacostomum</i> sp.	10	0.01
Rotifera (38 species)		30.69	<i>Coleps</i> sp.	80	0.10
<i>Asplanchna</i> sp.	200	0.25	<i>Colpidium</i> sp.	30	0.04
<i>Asplanchnopsis</i> sp.	60	0.08	<i>Oxytricha</i> sp.	30	0.04
<i>Ascomorpha</i> sp.	40	0.05	<i>Verticella</i> sp.	10	0.01
<i>Brachionus angularis</i>	1110	1.40	<i>Filinia</i> sp.	890	1.12
<i>Brachionus angulosum</i>	50	0.06	<i>Gastropus</i> sp.	110	0.14
<i>Brachionus calyciflorus</i>	1960	2.47	<i>Harringia</i> sp.	70	0.09
<i>Brachionus caudatus</i>	6700	8.43	<i>Hexarthra</i> sp.	130	0.16
<i>Brachionus falcatus</i>	2290	2.88	<i>Keratella</i> sp.	120	0.15
<i>Brachionus forficula</i>	400	0.50	<i>Keratella cochlearis</i>	1560	1.96
<i>Brachionus quadridentata</i>	180	0.23	<i>Keratella tropica</i>	4000	5.03
<i>Brach. urceus</i>	40	0.05	<i>Lecane</i> sp.	1010	1.27
<i>Cephalodella</i> sp.	70	0.09	<i>Lepodella</i> sp.	170	0.21
<i>Colurella</i> sp.	40	0.05	<i>Monostyla</i> sp.	780	0.98
<i>Conochilus</i> sp.	60	0.08	Total	79460	100.00 %

months) density in the present study ranged from 760 to 11050 Ind. l⁻¹, with an overall mean of 3307 Ind.l⁻¹ (Table 3). A major peak of 11050 Ind. l⁻¹ was observed in June

2009, with 47 and 43% contribution from Copepoda and Rotifera, respectively. Among Copepoda, *Cyclops* sp. and nauplii were major contributors to this peak while

Table 3. The net zooplankton assemblages across different months in surface water of the Bhoj wetland (frequency of occurrence (%)).

Month	Frequency of occurrence (%) Cladocera	Frequency of occurrence (%) Rotifera	Frequency of occurrence (%) Copepoda	Frequency of occurrence (%) Ostracoda	Frequency of occurrence (%) Protozoa	Net zooplankton
Feb. '08	27.61	29.10	40.30	1.49	1.49	1340
Mar	16.92	15.77	66.54	0.38	0.38	2600
Apr	9.21	33.89	56.07	0.84	0.00	2390
May	32.36	19.24	47.52	0.87	0.00	3430
Jun	17.62	26.64	54.92	0.41	0.41	2440
Jul	15.74	29.70	54.57	0.00	0.00	3940
Aug	18.42	25.00	56.58	0.00	0.00	760
Sep	33.71	26.97	38.20	0.00	1.12	890
Oct	42.15	14.05	41.32	0.83	1.65	1210
Nov	8.54	35.68	55.28	0.50	0.00	1990
Dec	12.65	39.51	45.37	0.62	1.85	3240
Jan. '09	24.37	37.81	36.74	0.36	0.72	5580
Feb	8.48	29.70	60.91	0.15	0.76	6600
Mar	10.48	37.90	50.97	0.00	0.65	6200
Apr	14.00	33.00	50.00	0.00	3.00	2000
May	17.65	42.41	38.70	0.31	0.93	3230
Jun	9.59	42.53	47.15	0.18	0.54	11050
Jul	17.22	38.28	44.50	0.00	0.00	2090
Aug	8.55	30.38	58.70	0.88	1.47	3390
Sep	5.94	47.49	46.58	0.00	0.00	2190
Oct	47.38	21.80	29.65	0.29	0.87	3440
Nov	29.32	24.06	42.86	2.26	1.50	1330
Dec	32.84	29.85	33.58	0.00	3.73	1340
Jan. '10	12.99	3.13	81.04	0.00	2.84	6700

amongst Rotifera, *Brachionus caudatus* and *Keratella tropica* were dominant contributors.

The two minor peaks of 6600 and 6700 Ind.l⁻¹ were recorded in February 2009 and January 2010, respectively. Among Copepoda, *Cyclops* sp. alone contributed significantly to the February 2009 and January 2010 peaks, to the tune of 61 and 81%, respectively (Table 3 and Figure 4).

On monthly basis, maximum zooplankton density

was observed in summer and winter months during both years. Winter peak months in both years were mainly represented by Copepoda, summer peak was represented by Copepoda. In summer months, low flow of water brings stability to the ecosystem and more availability of food due to production and decomposition of organic matter.

The high density of zooplankton recorded in

summer months may be related to high phytoplankton density during this period. It was documented that nutrient availability influence the abundance of Rotifera and Copepoda (particularly *Cyclops* sp.) (Kumar et al., 2004). The net zooplankton abundance increased during summer, probably corresponding to the water quality, decaying vegetation, increased levels of organic matter in the sediment and higher

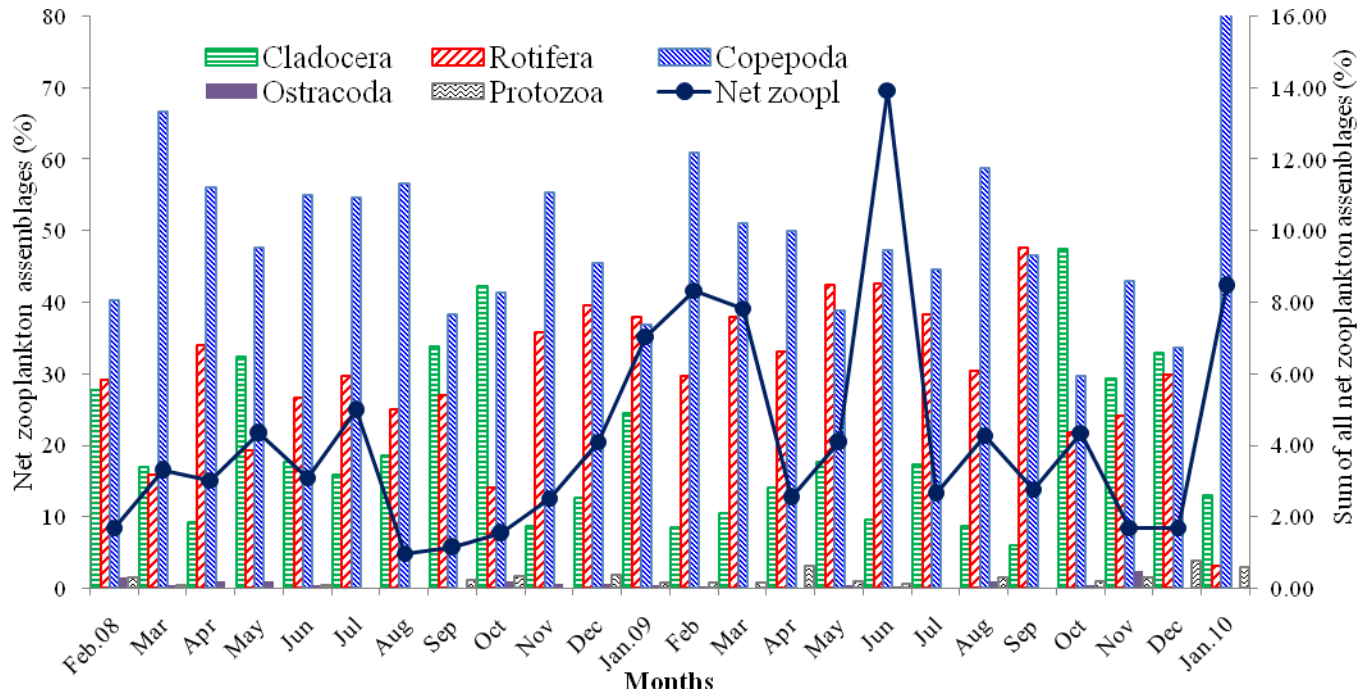


Figure 4. Net zooplankton abundance across different months in surface water of the Bhoj wetland (frequency of occurrence (%)).

abundance of bacteria in the wetlands (Coman et al., 2003; Chattopadhyay and Barik, 2009). Copepods develop better in warm periods (Dar and Dar, 2009). Copepoda population dominated numerically in the zooplankton populations in Dal Lake, Kashmir (Zutshi and Vass, 1982). The dominance of Copepods in flood-plain lakes of Kashmir has already been established by Khan (2002). The significant density of Copepoda nauplii in Bhoj wetland was recorded during the summer months, indicating the role of high temperature in promoting the egg production and development. This is in agreement with the work of Makino and Ban (2000), in Lake Toya who reported that higher water temperature causes more rapid development and higher egg production while increased food density results in larger body size and higher egg production.

During the present investigation, the summer population of total zooplankton fell significantly in monsoon season (July to September) as was also observed (Sadguru et al., 2002 and Pandey et al., 2004). Sudden reduction in the zooplankton population density during the rainy season as noticed in the present findings could also be due to fall of temperature and dilution in concentration of minerals and salts in wetland water (Chakraborty, 2004; Dutta et al., 2010 and Okogwu et al., 2010). The population in winter as a result of favorable environmental conditions, including temperature, dissolved oxygen and the availability of abundant food in the form of bacteria, nanoplankton and suspended detritus as reported by Edmondson (1965) and Baker (1979).

In the present study, it has been observed that Copepoda followed by Rotifera were well represented groups quantitatively throughout the study period. Cladocera was comparatively less represented group being chiefly represented by *Diaphanosoma* sp. Cladocera which followed Rotifera was represented by *Diaphanosoma* sp. Jana and Pal (1984) reported the abundance of *Diaphanosoma excisum* in water bodies having high organic content. Therefore, presence of *Diaphanosoma* sp. at all the stations in the present study can also be considered as an indication of increased organic content in the water, from sewage and other agricultural effluents.

Copepoda during the entire period was mainly represented by *Cyclops* sp. and nauplii. This was attributed to alkaline nature of waters. Verma et al. (1984) and Ahmad et al. (2011) observed that *Cyclops* sp. and nauplii were sensitive to pollution (organic matter) and increase with an increase in nutrients. Copepods (density, species composition) were directly related to nitrogen and phosphorus and showed tolerance to different physico-chemical characteristics (Kulshreshta et al., 1992). Syuhei (1994) stated that individual growth rate of Copepoda may also depend on temperature conditions. The occurrence of nauplii throughout the study period in the present wetland indicated extended reproductive phase of the cyclopoid, which is in agreement with the reports of Sharma (2011) and Sharma and Sharma (2011). *Brachionides* (*Brachionus* sp.) and *Keratella* spp. were the most dominant genera in

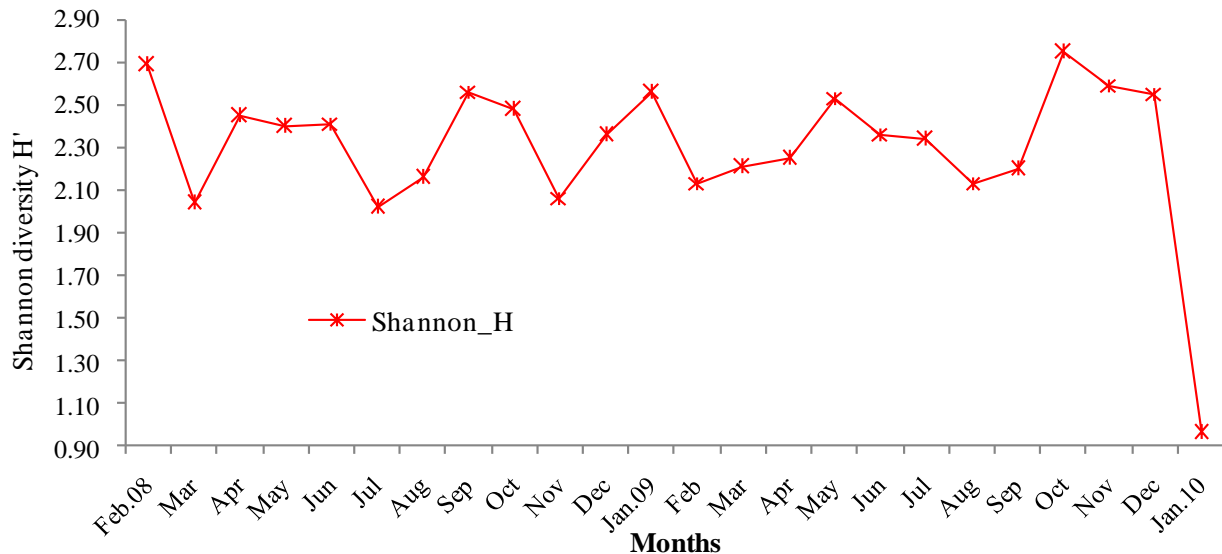


Figure 5. Shannon-Weiner diversity index of net zooplankton species during 2008-10.

the present study. Abundance of such species is considered as biological indicator for eutrophication (Nogueira, 2001). Mulani et al. (2009) reported *Brachionus* spp. to be present in typical tropical conditions while Sampaio et al. (2002) reported *Brachionus* spp. to be indicator of eutrophication.

Diversity of net zooplankton species

The diversity indices are all based on two assumptions: (a) stable communities have a high diversity value and unstable ones have a low diversity, and (b) stability in diversity is an index of environmental integrity and wellbeing (Magurran, 1988). As a consequence, the diversity value decreases with environmental degradation. Shannon-Weaver Index is a combination of the number of species and the evenness of distribution of individuals among taxa. It may function as a sensitive indicator for pollution (Klemm et al., 1990). In the present investigation, Shannon-Wiener diversity index ranged between 0.96 and 2.75 during the two years of study (Figure 5). The above trend can be attributed to the surrounding disturbances in the riparian zone and also increasing anthropogenic interaction in the wetland. Bhoj wetland can be classified as less diverse as Shannon-Wiener index (H') is > 2 ; it also indicates poor quality in the water body. McDonald (2003) stated that the value of the index ranging from 1.5 to 3.4 has low diversity and species richness while value above 3.5 has high diversity and species richness. The present study shows that limnological processes affecting net zooplankton species diversity operated almost equally throughout the surface waters of the water body and across all seasons.

Zooplankton assessment is an important indicator of

aquatic community structuring and water conditions. Zooplankton is directly or indirectly influenced by seasonal variation of complex limnological factors. The annual quantitative study of zooplankton population depends on the succession, appearance and disappearance of component species. Periods of quantitative increase and decrease of individuals do not coincide with seasonal minima and maxima of the total zooplankton. Three main zooplankton groups were identified in the study (Rotifers, Cladocera and Copepoda) which constitute the zooplankton population and contributed significantly to secondary production of the wetland. Some species increases slowly and more or less uniformly to the maximum while others show an almost starting burst of development from an apparent absence to a numerical dominance of the whole net zooplankton within a very short period of time.

The nature of wetland is closely related to the fluctuations of the zooplankton density. The analysis of species richness and diversity indices revealed clearly the status of the water body. The rapid modification of the planktonic communities in response to environmental stress confirms the strong instability of tropical shallow water ecosystems and reinforces the interest of their ecological monitoring, particularly, as for Bhoj wetland; they have multipurpose and potentially conflicting uses (drinking water, irrigation and fishing).

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Full Length Research Paper

Floristic composition, diversity and vegetation structure of woody plant communities in Boda dry evergreen Montane Forest, West Showa, Ethiopia

Fikadu Erenso*, Melesse Maryo and Wondaweka Abebe

Department of Biology, Dilla University, P.O Box 419, Ethiopia.

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This study was conducted on Boda Forest in West Showa Zone, Oromia National Regional State, west Ethiopia with the objective of determining the floristic composition, species diversity and the vegetation structure of the woody plant communities in Boda natural forest. Systematic sampling method was used to collect the vegetation data. Accordingly, 60 plots of 20 x 20 m (400 m²) quadrats were laid at every 50 m along five transect lines from south to north direction using compass. Vegetation parameters such as diameter at breast height (DBH), height and density of the study woody species were recorded, and all the collected vascular plant species were brought to National Herbarium of Ethiopia for identification. Vegetation classification was performed using R program Version 2.15.2 software vegan and labdsv packages. The Sorensens's similarity coefficient and Shannon-Wiener diversity index were also used to detect similarities among communities and to compute species diversity and evenness between the plant communities, respectively. A total of 95 species belonging to 76 genera and 58 families were identified from the forest. The collected species were composed of 34.7% trees, 45.2% shrubs, 13.6% liana, 3% epiphyte, 1% trees/shrubs and 1% tree/liana. Five community types, *Galiniera saxifrage-Maesa lanceolata*, *Juniperus procera-Myrsine Africana*, *Carissa spinarum-Helichrysum citrispinum*, *Osyris quadripartite-Rhus ruspolii* and *Acacia abyssinica-Gomphocarpus fruticosus* were recognized from the hierarchical cluster analysis. Generally, the forest was dominated by the small sized trees and shrubs indicating that it is in the stage of secondary regeneration. The presence of strong anthropogenic disturbance in the area necessitates the need for an immediate conservation action in order to ensure the sustainable utilization and management of the forest.

Key words: Anthropogenic disturbance, diversity, floristic composition, plant community, population structure.

INTRODUCTION

Ethiopia is one of the countries in the world endowed with rich biological resources. One of these resources is natural vegetation where floristic and faunistic life forms

dynamic ecosystems (Balcha, 2002). The major ecological systems in the country support large and highly varied genetic resources along with its extremely

*Corresponding author. E-mail: fikaduerenso@gmail.com.

variable agro-climatic conditions and the altitudinal ranges (-110 to 4,620 m a.s.l at Ras Dejen) (FAO, 1996). The varied topography, the rift valley and the surrounding lowlands have given Ethiopia a wide spectrum of habitats and a large number of endemic plants and animals (Teketay, 1999; Woldu, 1999). The size of Ethiopian flora is estimated to be over 6500-7000 species of vascular plants, of which about 12% are considered endemic (Tewolde, 1991).

According to Kelbessa et al. (1992), 120 threatened endemic plant species are known from Ethiopia. Thirty five of these species were from the dry afro-montane forests of the country. Dry evergreen montane forest has a very complex type of vegetation, roughly above 1500 m a.s.l. and below 3200 m a.s.l., with an average annual temperature and rainfall of 14-25°C and 700-1100 mm, respectively (Friis, 1992; Woldu, 1999). The Boda dry evergreen montane forest is one of the remnant dry afro-montane forests that are found in the high lands of West Showa next to Chilimo National forest. Settlements, illegal cutting, small patches of farmland, substitution by the exotic species and open pasture fields are challenges that are facing this forest. Overgrazing and continuous human interference are believed to lead to an irreversible change in the function of forests (Bishaw, 2001). It also frequently leads to loss of forest cover and biodiversity, erosion, desertification and reduced water resources (Kelbessa and Soromessa, 2008). Indigenous knowledge on medicinal and other useful plants is also eroded with destruction of these forests (Hundera and Gadissa, 2008).

However, forest resources are the fruits of evolution that are developed through the combined influence of physical environment and people, and play important economic, social and cultural roles, particularly in the lives of many local communities. For instance, the multitude of uses, which could be obtained for trees and shrubs have been categorized as timber, fuelwood and charcoal, food, forage, medicine, raw materials as well as protection and soil improvement (Teketay and Bekele, 2005).

In order to maintain ecological equilibrium and to meet the forest product requirements, of the species diversity, floristic composition and vegetation structure are important to judge the success of the conservation efforts of the natural forests for their sustainability. No such study was done on Boda natural forest. There is a lack of knowledge on the sustainable forest management in the local community. Currently, intensive use of land for agriculture, and the high demand of wood for different purposes are leading to the loss of plant species. People were attracted only to temporary yields obtained at the initial stage without realizing the outcome they would be facing in the long term (Abebe, 2007). The present trend of forest management needs a step forward progress that depends on scientific data or information to minimize uncontrolled exploitation and restrict the conversion of

forest into agricultural land, and the substitution by the exotic species. Otherwise, the small remnants of natural forest left will be gone in the very near future. Based on this background and baseline, this study was undertaken aiming at describing and providing available floristic information of Boda natural forest, including some impacts on the vegetation of the study area.

MATERIALS AND METHODS

The study area

The study area, Dendi district, is one of the eighteen districts of the West Showa zone of Oromia Regional State. The district capital city, Ginchi, is located 77 km west of Addis Ababa, on the Addis Ababa-Naqamte Road. Geographically, the district lies within the coordinates of 8°43'N-9°17' N and 37°47'E-38°20' E. The district covers about a total area of 104,680 ha. Of which 72, 836 ha is covered by farm land, 19,080 ha grazing-land, 9,685 ha forest and shrubs and others 3,079 ha with the population of 192,784 (99,475 males and 93309 females). The district has 48 farmers associations and five urban, out of which Ghinchi and Olanкоми have municipal governments (Dendi District Report, 2011).

Boda natural forest at Boda Bosoka has Farmers associations, 22 km away from the district's capital city, along Ginchi- Busa Road Figure 1. It covers around 20 ha.

The physiographic region of the district is characterized by one major escarpment running from east to west direction. The steepness of the escarpment varies from place to place being generally steeper at the central part of the district. Both on the top and bottom, the escarpment merges with flat lands largely used for farming. The altitudinal range of the district is between 2,000 to 3,288 m a.s.l. Besides, the relief feature of the area is characterized by rugged topography, which provides a variety of hills having interesting scenes. The district is an important watershed area for Awash and Nile river basin (Bekele, 1994).

Climate

The district has three traditional agro-climatic regions namely: Dega (10%), Woina-Dega (60%) and Kolla (30%). The annual average temperature of the study area is 17.5°C. The mean minimum and maximum temperature of the district is 9.3 and 23.8°C respectively. The study area has two rainy seasons with an average annual precipitation of 1,225 mm where the minor rainy season extends from March to May, and the major rainy season from June to September (Dendi District Report, 2011).

Soil

The majority of the soil of the district range from sandy to sandy-loams and clay-loams. Generally, the soils are reddish brown and shallow at higher altitudes, while at lower altitudes they tend to become dark-gray and deep-gray. The soils in the surrounding low plains are vertisols black soils with characteristics of high clay content (CNRASD, 1999; cited in Tamrat, 1993).

Vegetation and wildlife

Dendi District is covered with ever green forests with various types of vegetation. The entire high land of the district is believed to have been covered once with dense forest. According to Friis (1992) the

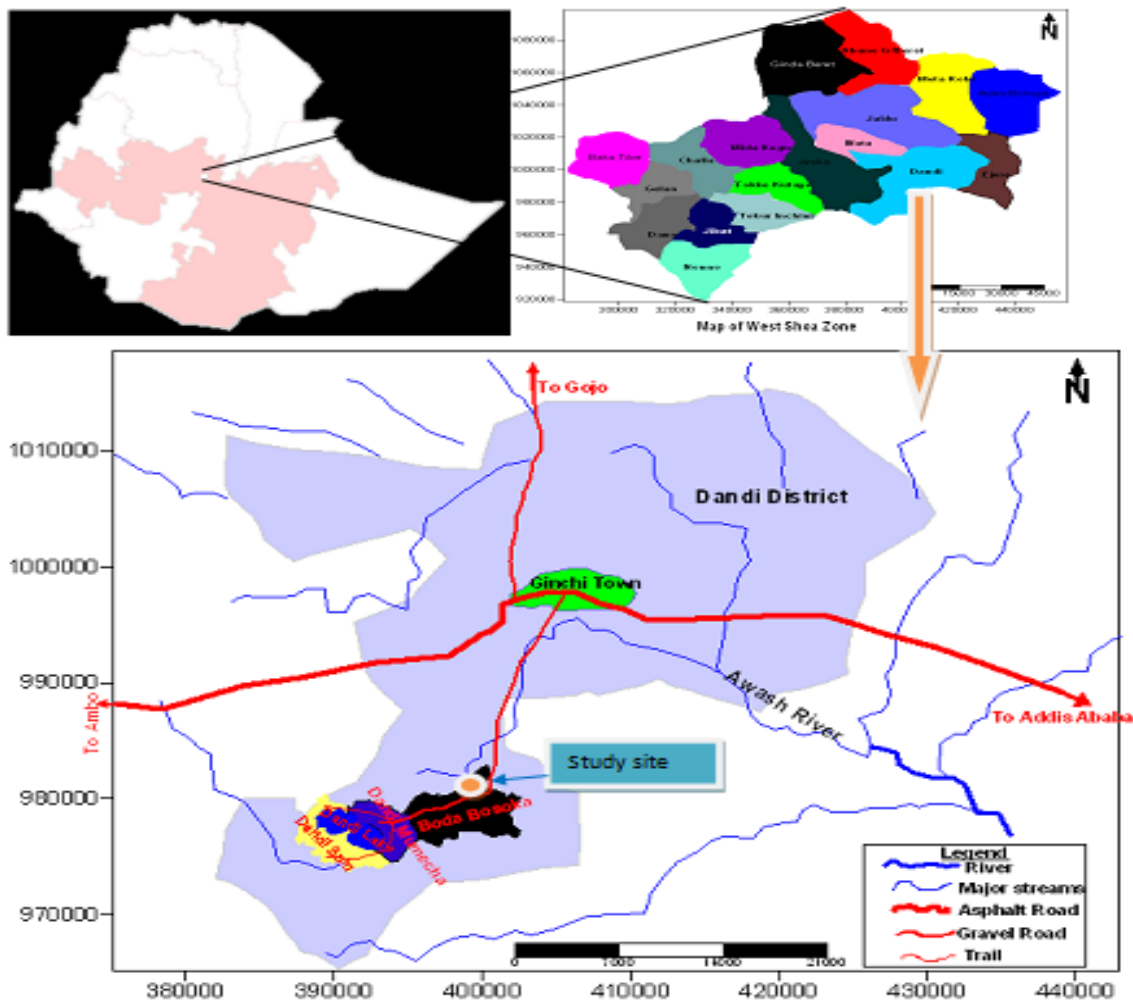


Figure 1. Location map of Dendi District and study site (ODA, 2011).

forest in the study area is considered as one of the remnant dry evergreen Afro-montane forests of Ethiopia. The major tree species in the canopy are *Junipers procera*, *Podocarpus falcatus*, *Prunus africana*, *Olea europaea* subsp. *cuspaata*, *Hagenia abssinica*, *Ficus* spp., *Croton macrostachyus* and *Eucalyptus globulus*. Besides, in the study area small indigenous flowering grasses, herbs as well as bushes such as *Carissa edulis* and *Rosa abyssinica* are very common.

Wildlife species *Phacochoerus africanus*, *Redunca redunca*, *Cercopithecus aethiops*, *Sylvicapra grimmia*, *Crocuta crocuta* and various types of bird species including *Bostrychia carunculata*, *Cyanochen cyanoptera*, *Serinus nigriceps*, *Poicephalus flavifrons*, *Agapornis taranta*, *Tauraco leucotis*, *Alcedo semitorquata* are common.

Sampling design

The vegetation data were collected systematically from 60 plots of 20 x 20 m (400 m²) quadrats laid at every 50 m along 5 transect lines from south-north direction using compass following the Braun-Blanquet approach of phytosociology as modified by vander Maarel (1979). The distance between each transect line was 100 m in a zigzag form of starting point of laying plot. This is to include as

much vegetation as possible that can represent the vegetation of the study area. Plant species in each plot was counted and recorded at individual level, and voucher specimens was also collected, numbered, pressed and taken to the National Herbarium of Ethiopia (ETH), Addis Ababa University, for identification and storage following standard taxonomic method (Bridson and Forman, 1992).

Floristic data collection and identification

Additional plant species occurring outside the quadrats, but inside the forest within 10 m distance was also recorded only as 'present' for floristic composition, but they were not used in the subsequent vegetation data analysis (Bekele, 1994). The vernacular (local) names were used when available. The altitude of each quadrat was recorded by using global Positioning System (Garmin 12 channel GPS). Specimens with height >2 m were identified, counted and measured using a clinometers and mater tape. Where topographic features make it difficult to measure their height were estimated visually. All individuals of the trees with a circumference > 7 cm at breast height (DBH 2.50 m) were also measured and recorded. The reported specimens of the woody species were collected, identified and deposited at the National Herbarium, Addis Ababa University.

Plant diversity and population structure data analysis

Biological diversity could be quantified in different ways. Shannon-Wiener diversity index and species richness were computed to describe species diversity, and the population size of each of the species present (Mueller-Dombois and Ellenberg, 1974). Shannon - Wiener diversity index is the most popular measure of species diversity because it accounts both for species richness and evenness, and it is not affected by sample size (Kent and Coker, 1992; Krebs, 1999). Shannon-Wiener diversity index is calculated as follows:

$$H' = \sum_{i=1}^s p_i \ln p_i$$

Where, H' = Shannon diversity index; S = the number of species; P_i = the proportion of individuals or the abundance of the i^{th} species expressed as a proportion of total cover; \ln = logbase _{e} .

The minimum value of H' is 0, which is the value for a community with a single species, and increases as species richness and evenness increases (Manuel and Molles, 2007).

Evenness (Equitability) is measured as the relative abundance of the different species making up the richness of an area and when compared the similarity of the population size of each of the species present. The species evenness that measures the equity of species in a given sample area is represented by 0 and 1, where 0 indicates the abundance of few species and 1 indicates the condition where all species are equally abundant (Whittaker, 1975). Shannon's equitability (J) or Evenness is calculated as follows:

$$J = H/H_{\max} = H/\ln s$$

Where, J = Evenness, H' = Shannon-Wiener diversity index and $H'_{\max} = \ln s$, where s is the number of species.

Sorensen's similarity index is used to evaluate woody species composition (tree/shrubs) and species distribution among the plant communities. It was described using the following formula (Kent and Coker, 1992):

$$S_s = 2a/(2a+b+c),$$

Where: S_s = Sorensen's similarity coefficient; a = number of species common to both communities; b = number of species in community 1; c = number of species in community 2.

The diameter at breast height (DBH), basal area, tree density, height, frequency and importance value index were used for description of vegetation structure. These vegetation data were computed and summarized using Microsoft Office Excel (2007) spread sheet using the following formulae (Mueller-Dombois and Ellenberg, 1974; Kent and Coker, 1992). The frequency distribution of tree species was calculated as:

Frequency (F): The probability or chance of finding a species in a given sample area or quadrat. It is dependent on quadrat size, plant size and patterning in the vegetation (Kent and Coker, 1992). It is calculated with this formula:

$$F = \frac{\text{Number of plots in which a species occur}}{\text{total number of plots laid out in the study site}} * 100$$

Relative Frequency (RF): It is the frequency of species A /sum of frequencies of all species $\times 100$.

Density of a species = is a count of the numbers of individuals of a species within the quadrat (Kent and Coker, 1992). It is closely related to abundance but more useful in estimating the importance

of a species. Counting is usually done in quadrats placed several times in the plant communities under study. Afterwards, the sum of individuals per species is calculated in terms of species density per convenient area unit such as a hectare (Mueller-Dombois and Ellenberg, 1974).

$$D = \frac{\text{Number of above ground stems of a species counted}}{\text{sampled area in hectare (ha)}}$$

Relative density = Density of species A /total density of all species $\times 100$.

Basal area (BA): Basal area is the area outline of a plant near ground surface. It is expressed in square m/hectare (Mueller-Dombois and Ellenberg, 1974). It is measured through diameter, usually at breast height (DBH) that is 1.3 m above ground level. It is also used to calculate the species dominance. DBH values were calculated from circumference measurements and used in the formula for the estimation of basal area as follows:

$$BA = \pi d^2/4$$

Where, BA = Basal Area in m^2 per hectare; d = diameter at breast height in meter. $d = C/\pi$, where c = circumference, $\pi = 3.14$.

Dominance: It is the mean basal area per species time's abundance of the species.

Relative dominance (RDO): It is basal area of a species /total basal area of all species $\times 100$

Importance values index (IVI) was analyzed for woody species. IVI of a species was calculated from the sum of relative dominance (RDO), relative density (RD) and relative frequency (RF) (Kent and Coker, 1992).

$$IVI = RDO + RD + RF$$

Plant community determination

The vegetation data analysis was made based on presence absence data. The R program Version 2.15.2 software *vegan* and *labdsv* packages (The R Core Team, 2012) was used to classify the vegetation into communities. The community name was derived based on the tree and/or shrub with high synoptic value.

RESULTS AND DISCUSSIONS

Floristic plant species composition

A total of 95 specimens of woody plants (shrubs, trees, and lianas) were identified from the forest. The identified species belong to 76 genera and 58 families. Two species were observed outside the sampling plots in the study area within the ranges of ten meters distance from the plot boundary. These were *Geranium arabicum* and *Opuntia ficus indica*. The collected species were composed of 34.7% trees, 45.2% shrubs, 13.6% liana, 3% epiphyte, 1% trees/shrubs and 1% tree/liana.

Plant community classification

Cluster analysis was used to identify groups of sites (vegetation samples) that are similar in terms of their

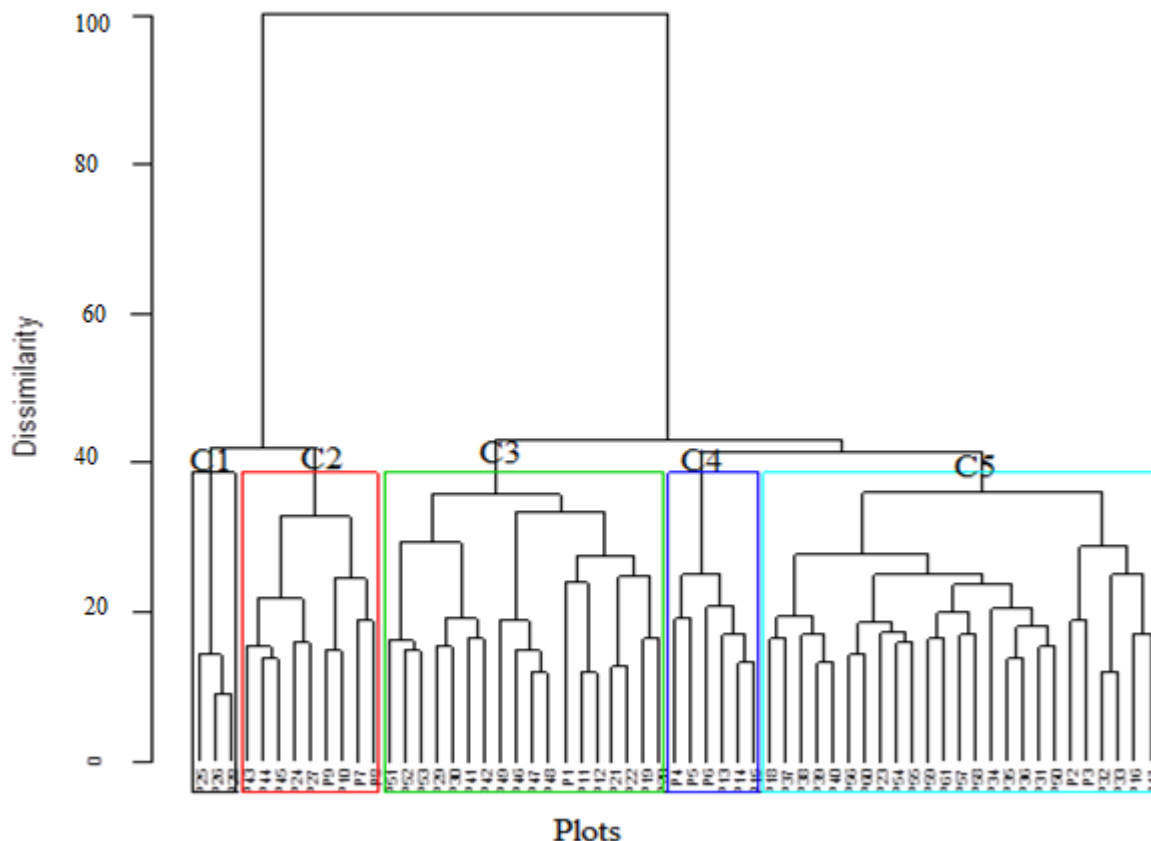


Figure 2. Dendrogram showing plant community types of the study area.

woody species composition. The abundance data of a species were used for the analysis. R program Version 2.15.2 software (The R Core Team, 2012) was used to perform a hierarchical cluster dendrogram, which depicted the vegetation community of woody species. The five plant community types (clusters) at Boda natural forest (Figure 2) and the five communities and distributions of the sample plots in the communities were identified (Table 1).

The indicator values for each species in each group and tests for statistical significance ($P < 0.05$) were analyzed to name the vegetation community (Table 2). The values are based only on the species abundance and frequency comparisons. In order to obtain an effective description of community types and their environmental relations, both classificatory and ordination techniques were employed.

***Galiniera saxifrage-Maesa lanceolata* community type**

This community was represented by three plots and 53 woody plant species. *Rhamnus staddo*, *Premna schimpehi*, *Juniperus procera*, *Maytenus heterophylla* and *Hypericum quartineanum* were the importance

species in the tree layer of the community. *Canthium oligocarpum*, *Abrus schimperi*, *Sparmannia ricinocarpa* and *Myrsine africana* were important species in the shrub layer. The common Lianas in this community include *Toddalia asiatica* and *Gladiolus dalenii*. *Salvia nilotica*, *Pentas lanceola* and *Peperomia abyssinica* were also importance herb species in this community.

***Juniperus procera-Myrsine africana* community type**

This community was represented by nine plots and 61 plant species. *Maytenus addat*, *Hagenia abyssinica* and *Rhus vulgaris* were the importance species of the tree layer of the community.

In the shrub layer *Hibiscus panduliformis*, *Myrsine africana* and *Carissa spinarum* were the importance species.

***Carissa spinarum-Helichrysum citrispinum* community type**

This community was represented by 18 plots and 46 plant species. *Acanthus polystachius*, *Sida schimperiana*,

Table 1. The communities and distributions of the sample plots in the communities.

Community	Number of plots	Plots in the community
I	3	25, 26, 33
II	9	43, 44, 45, 24, 27, 9, 10, 7, 2
III	18	51, 52, 53, 29, 30, 41, 42, 49, 45, 47, 43, 1, 11, 12, 21, 22, 19, 20
IV	6	4, 5, 6, 13, 14, 15
V	24	18, 37, 38, 39, 40, 56, 60, 23, 54, 55, 59, 57, 53, 34, 35, 31, 50, 2, 3, 32, 33, 16, 17

Table 2. Indicator plant species for each community and the test of significance (P*value) observed for each indicator species.

Indicator species	Local name	Communities					P*value
		I	II	III	IV	V	
<i>Galiniara saxifraga</i>	Mixoo	0.49	0.00	0.00	0.06	0.08	0.00
<i>Maesa lanceolata</i>	Abayyii	0.42	0.00	0.00	0.00	0.00	0.01
<i>Juniperus procera</i>	Gaatiraa	0.21	0.41	0.10	0.21	0.00	0.21
<i>Myrsine africana</i>	Qacama	0.16	0.37	0.02	0.14	0.00	0.00
<i>Carissa spinarum</i>	Agamsa	0.20	0.20	0.36	0.20	0.20	0.90
<i>Helichrysum citrispinum</i>	Mukaa gaguraa	0.00	0.00	0.65	0.02	0.00	0.00
<i>Osyris quadripartita</i>	Waatoo	0.03	0.00	0.00	0.59	0.00	0.00
<i>Rhus rus polii</i>	Daboobesaa	0.00	0.02	0.00	0.50	0.00	0.00
<i>Acacia abyssinica</i>	Laafto	0.00	0.04	0.10	0.00	0.60	0.03
<i>Gomphocarpus fluticosus</i>	Aanannoo	0.00	0.00	0.00	0.00	0.67	0.00

Table 3. Sorensen's similarity coefficient among the plant communities.

Community	I	II	III	IV	V
I					
II	0.43				
III	0.22	0.35			
IV	0.37	0.38	0.31		
V	0.23	0.23	0.21	0.24	

and *Solanum marginatum* were the importance species of the shrub layer of the community. The common Lianas in this community include *Lagenaria abyssinica*, *Senna septemtrionalis* and *Clematis longicauda*.

***Osyris quadripartite- Rhus ruspolii* community type**

This community was represented by six plots and 38 plant species. *Mimusops kummel*, *Clerodendrum mylicoides*, *Juniperus procera*, *Maytenus addat* and *Maytenus heterophylla* were the importance species of the tree layer of the community. The shrub layer is dominated by *Solanum anguivi* and *Lippia adoensis*. The common climbers in this community include *Clematis*

longicauda and *Dregea abyssinica*.

***Acacia abyssinica-Gomphocarpus fruticosus* community type**

This community was represented by 25 plots and 31 plant species. *Juniperus procera*, *Dovyalis abyssinica*, *Cupressus lusitanica* and *Eucalyptus globulus* were the importance species of the tree layer of the community. The shrub layer is dominated by *Canthium oligocarpum*, *Euphorbia schimperiana*, *Carissa spinarum*, *Ocimum lamiifolium* and *Solanum giganteum*.

Community similarity analysis

The Sorensen's similarity index measures the degree to which the species composition of forest or samples is alike, whereas dissimilarity coefficient assesses which two forest or samples differ in composition. Based on this, similarity in species composition slightly varied among communities Table 3.

The highest similarity was observed between communities I and II (43%). The least similarity was observed between community III and V (21%), followed by community I and III. Overall similarity coefficient ranges

Table 4. Species richness, evenness and Shannon-Wiener diversity index of the plant community types.

Community	Species richness	Diversity index (H')	H'max	Species evenness (J)
I	53	1.72	1.9	0.09
II	61	1.79	2.0	0.09
III	46	1.66	1.8	0.08
IV	38	1.58	1.8	0.08
V	31	1.49	1.4	0.08

from 21-43% among all the communities. Thus, species composition dissimilarities account for 57% of the most similar communities and 79% of those that share least similarity (community III and V)

Species diversity

The five communities have almost the same species distribution (equitability or evenness) but comparatively community V has the least species evenness Table 4.

Vegetation structure

Frequency

We recorded a total of 682 woody plants per ha- from all quadrats. The most frequent of the tree species in this forest found was *J. procera* (93.44%), occurring in almost all of the quadrats sampled, followed by *Maytenus heterophylla* (49.18%) and *Maytenus addat* (44.26%), while species like *Ficus vast*, *Euphorbia ampliphylla*, *Podocarpus falcatus*, *Erithrina brueci* and *Maesa lanceolata* were poorly represented.

Basal area

The total basal area of all tree species in Boda Forest was calculated from DBH data. It was found to be 114.64 m²/ha. *J. procera* has the highest basal area (25.5%) followed by *Podocarpus falcatus* (24.64%). On the other hand the lowest (below 0.56 m²/ha) was recorded for most species like *Rhamnus prinoides*, *Rhamnus staddo*, *Dovyalis abyssinica*, *Maesa lanceolata*. Thus, the species with the largest basal area could be considered the most important species in the forest.

With regard to basal area, the most important species of the study forest includes *Juniperus procera*, *Podocarpus falcatus*, *Ekebergia capensis*, *Olea europaea*, *Ficus vasta*, *Cupressus lusitanica* and *Eucalyptus globulus*.

Importance value index (IVI)

The most leading dominant and ecologically significant

trees in Boda Forest are *J. procera*, *Maytenus heterophylla*, *Ficus vast*, *Mayteus addat*, *Eucalyptus globulus*, *Rhamnus staddo*, *Ekebergia capensis* and *Premna schimpehi* on the basis of their IVI values relative to other species Table 5, but *Bersama abyssinica*, *Acacia abyssinica*, *Cordia africana*, *Rhus rus polii* and *Olea europaea* are species among the lowest relative IVI values.

DISCUSSION

Floristic composition

In this study, a total of 95 woody species, including shrubs, trees, shrubs/trees, epiphyte, liana and tree/liana), were recorded. Overall diversity and evenness were 1.79 and 0.09, respectively. According to Kent and Coker (1992), the Shannon-Weiner diversity index normally varies between 1.5 and 3.5 and rarely exceeds 4.5. In our study area, however, there is high diversity and evenness showing more or less even representation of individuals of most woody species in the sampled quadrats.

Vegetation structure

Vegetation classification is a powerful tool employed for several purposes, including: efficient communication, data reduction and synthesis, interpretation, and land management and planning. It also provides one way of summarizing our knowledge of vegetation patterns (Dalle et al., 2005). The study identified five plant community types (clusters) at Boda natural forest. Plant communities are conceived as types of vegetation recognized by their floristic composition. The species compositions of communities better express their relationships to one another and environment than any other characteristic.

Community types I and II, which is dominated by *G. saxifrage*, *M. lanceolata* *J. procera* and *M. africana*, is found in specialized habitats such as along river courses. The stands sampled in this type are located at the middle of the forest, which is less grazed by cattle and its human impact is found to be low. Regenerating species of *M. addat*, *H. abyssinica* and *R. vulgaris* are common here.

Community types III, is rich in shrub layer species and

Table 5. The high importance value index (IVI) of tree species in Boda Forest.

Botanical name	RDO	RD	RF	IVI	IVI%
<i>Juniperus procera</i>	10.96	41.76	15.7	68.42	22.81
<i>Maytenus heterophylla</i>	1.22	9.16	8.26	18.64	6.21
<i>Ficus vasta</i>	16.37	0.18	0.83	17.38	5.79
<i>Mayteus addat</i>	0.96	7.33	7.44	15.72	5.24
<i>Eucalyptus globulus</i>	11.78	2.81	1.1	15.69	5.23
<i>Ekebergia capensis</i>	7.27	1.95	3.03	12.25	4.08
<i>Rhamnus staddo</i>	1.22	4.33	6.61	12.17	4.05
<i>Cordia africana</i>	7.95	0.85	2.75	11.56	3.85
<i>Cupressus lusitanica</i>	8.66	2.08	0.83	11.56	3.85
<i>Premna schimpehi</i>	1.22	3	7.16	11.37	3.79
<i>Olea europaea</i>	2.54	1.95	4.96	9.45	3.15
<i>Acacia abyssinica</i>	2.95	2.81	3.03	8.79	2.93
<i>Bersama abyssinica</i>	1.82	2.75	1.38	5.94	1.98
<i>Rhus ruspolii</i>	1.22	1.1	2.2	4.52	1.51
Others	23.86	17.94	34.72	76.54	25.53
Total	100	100	100	300	100

RDO, Relative dominance; RD, relative density; RF, relative frequency; IVI, Importance values index.

woody climbers. The stands sampled in this community are located in an area having shallow soils with medium human interference. In few of its stands introduced exotic species of *C. lusitanica* have been observed.

Community types V, is highly influenced by people collecting firewood, charcoal making and grazing animals. During the survey of this study, in this community, illegal cutting by local people and introduced exotic species of *C. lusitanica* and *E. globules* have been observed Plate 1. This is due to its being nearby to Boda town and having species of plants suitable for charcoal making and firewood.

Low species evenness can be attributed to excessive environmental disturbances, variable conditions for regeneration and selective exploitation of some species (Wassie and Teketay, 2006). Kidane (2003) also explained that the highest species numbers are found at low disturbance intensities while there is a drastic decrease at high disturbance intensity. The result of the present study agrees with this regarding species evenness. The five communities have almost the same species distribution (equitability or evenness) but comparatively community V has the least species evenness.

The patterns of plant species diversity have often been noted for prioritizing conservation activities because they reflect the underlying ecological processes that are important for management (Lovett et al., 2000; Senbeta et al., 2007). Based on similarity index measures, similarity in species composition slightly varied among communities. The highest similarity was observed between communities I and II (43%) due to the communities having close altitudinal similarity and

adaptation. The least similarity was observed between community III and V (21%), followed by community I and III. This may be due to conservational variation and variation in disturbance due to anthropogenic activities. that is, one area which is better protected varies from the one which is highly exposed to deforestation resulting in communities' variation. As it was reported by Denu (2007), in addition to altitudinal gradient, other environmental factors such as aspect, slope, and soil physical and chemical properties have sound effects on patterns of vegetation in communities. The present study agreed that high dissimilarity between species to communities may arise from the altitudinal differences, degree of human impact (anthropogenic) action, over grazing and climatic conditions. For all communities, the Sorensen's similarity coefficient values were below 0.5, indicating the existence of low similarities among the recognized communities which implies that all the communities are important in terms of floristic diversity and needs attention from a conservation point of view.

According to Lamprecht (1989), species with the same importance value index (IVI) have the same or at least similar population structure. The result indicates that high IVI was attributed to few species. These species are those which are well adapted to the high pressure of disturbance, natural and environmental factors, and the effect of local communities. In contrast to this idea, almost all species in this study showed variation in terms of their IVI, showing different ecological importance of each species in the forest. In our study, basal area analysis across individual species revealed that very few species had high dominance. *J. procera* was the leading dominant and other dominant species in terms of basal



Plate 1. Some areas of Boda natural forest (Photo by Fikadu, September, 2013G .C).

area were *M. heterophylla*, *F. vast*, *M. addat*, *E. globulus*, *R. staddo*, *E. capensis* and *P. schimpehion*. This implies that these eight species are the most ecologically important woody species at Boda forest.

Conclusions

The results of the study indicated that the study forest had relatively high woody species diversity, that is, 95 specimens of plants (shrubs, trees, shrubs/trees, epiphyte, liana and tree/liana) and dominated by small sized tree and shrub species in secondary stage of development, indicating that the forest was heavily exploited and affected in the previous periods, but good regeneration is in process at the present time. Therefore, to improve the natural diversity and structure of the forest, to minimize the influence of the surrounding communities and utilize the forest resources sustainably for present and future generation, the following points were made as recommendations:

1. Initiate enrichment plantation program of those most leading dominant and ecologically significant trees, because of the use of selective cutting by local peoples (e.g. *P. falcatus*, *E. capensis* and *R. staddo*).
2. Raising awareness of local communities on the value of forest resources and ecological consequences of deforestation and device mechanisms by which human

impacts can be minimized through discussion and consultation with the local communities with emphasis on returning the benefits of the protected areas to those communities.

3. Use the cut and carry method for feeding domestic animals than using free grazing method in the forest to enhance the germination capacity of the seeds in the soil and seedling development of woody species.

Finally, further studies on soil properties, land use management system and detailed ethno-botanical studies are also required to explore the wealth of indigenous knowledge on the diversity of plants and their implications in conservation are also recommended

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Full Length Research Paper

Genetic diversity among *Asparagus* species and cultivars of *Asparagus officinalis* L. using random amplified polymorphic DNA (RAPD) markers

Muhammad Irshad¹, Anwar Saeed², Muhammad³ and Muhammad Idrees^{1*}

¹Department of Biotechnology and Genetic Engineering, Kohat University of Science and Technology (KUST), Kohat, Pakistan.

²Department of Biotechnology, Bacha Khan University, Charsadda, Pakistan.

³Centre of Biotechnology and Microbiology, University of Peshawar, Peshawar, Pakistan

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The species of *Asparagus* are very important as they are used for ornamental, vegetable and medicinal purposes since ancient time. In the present study, random amplified polymorphic DNA (RAPD) markers were used to evaluate genetic diversity among nine species of *Asparagus* and six cultivars of *Asparagus officinalis* L. RAPD analysis using seven random oligonucleotide primers yielded a total amplification of 245 bands, among which 220 (89.80%) were polymorphic with an average of 31.4 bands per primers. Highest number of 39 (97.50%) polymorphic bands were obtained with primer OPC-07, while minimum polymorphic bands were 18 (69.23%) with primers OPA-01. Genetic similarity coefficient ranged from 0.75 to 0.96 with an average of 0.85. Phenogram clustered all *Asparagus* species and *A. officinalis* L. cultivars into two clear clusters. One cluster comprised of all cultivars of *A. officinalis* L. while the second cluster comprised of all the *Asparagus* species. The present study reveals that RAPD markers were more convincing for analyzing genetic diversity among *Asparagus* species and cultivars of *A. officinalis* L.

Key words: *Asparagus officinalis* L., genetic diversity, random amplified polymorphic DNA (RAPD), Phenogram.

INTRODUCTION

Asparagus is an herbaceous, perennial plant belonging to the *Asparagaceae* family, comprising of about 150 species and is widely distributed in tropical and subtropical region up to an altitude of about 1500 m (Velvan et al., 2007). In Pakistan, 14 different species of *Asparagus* has been found (Ali and Khan, 2009).

Asparagus officinalis L. is an important vegetable crop, while *Asparagus adscendens* Roxb, *Asparagus capitatus* Baker and *Asparagus racemosus* Willd. are extensively used for various medicinal purposes (Goyal et al., 2003; Sharma and Bhatnagar, 2011), whereas *Asparagus densiflorus* (Kunth) Jessop, *Asparagus setaceus* (Kunth)

*Corresponding author. E-mail: idreesbiotech@yahoo.com

Jessop, *Asparagus plumosus* Baker and *Asparagus monophyllus* Baker have economic importance both for horticultural and ornamental purposes.

Asparagus act as a highly valuable plant species having both therapeutic and nutraceutical importance as well as used for food consumption (Shasnay et al., 2003). *Asparagus* contains saponins that possess antitumor activity while it also contains fructans that help to reduce the risk of disorder such as constipation, diarrhea as well as disease like osteoporosis, obesity, cardiovascular disease, rheumatism and diabetes (Shao et al., 1997). Fruit is eaten to treat pimples. Seeds are also used for blood purification. Its pharmacological activities include anticancer, antioxidant, antifungal, antibacterial, anti-dysenteric, anti-inflammatory, anti-abortion, anti-oxytoxic, antiulcer, hypertensive and anticoagulant effects (Sharma et al., 2000).

The availability of a variety of DNA markers including restriction fragment length polymorphism (RFLP) (Carreel et al., 2002), amplified fragment length polymorphism (AFLP) (Loth et al., 2000), simple sequence repeat (SSR) (Silvana et al., 2003), and inter simple sequence repeat (ISSR) (Rout et al., 2009), has enabled researchers to examine genetic variation among various plant species across natural populations (Archak et al., 2003). Among these, PCR-based techniques of random amplified polymorphic DNA analysis (RAPD) have been successfully used, due to technical simplicity and speed, RAPD methodology have been used for genetic diversity analysis, genotyping and genome mapping in various medicinal plant species such as *Berberis lycium* Royle (Tripathi and Sandhya, 2013), and Rose (Jan and Byrne, 1999). The objective of the present study was used to analyze the genetic diversity among 14 different *Asparagus* species and *A. officinalis* L. cultivars using RAPD markers.

MATERIALS AND METHODS

Plant materials

A total of eight *Asparagus* species and six cultivars of *A. officinalis* L. were collected from different region of Pakistan including Islamabad, Lahore, Kohat and Swat (Table 1). The leaves were collected and stored at -20°C in a freezer until their DNA was extracted.

DNA isolation

Genomic DNA was isolated from fresh and young leaves of *Asparagus* by standard cetyl trimethyl ammonium bromide (CTAB) method with few modifications (Doyle and Doyle, 1987). Modifications were designed to counter the high level of secondary compounds and polysaccharides present in *Asparagus* leaves. These compounds degrading DNA, inhibit subsequent enzyme digests and PCR reactions (Pirttila et al., 2001). The modifications included the use of high concentration of PVP (polyvinylpyrrolidone), repetition of purification step with chloroform : Isoamyl alcohol, DNA pellet wash with wash buffer and ethanol (70%).

Asparagus young leaves samples were crushed in liquid nitrogen, about 100 mg were weight and transfer in a 1.5 mL tube. The powder was then mixed with 800 µL extraction buffer (100 mM Tris HCl pH (8.5), 50 mM EDTA, 500 mM NaCl and 1% PVP) and 20 µL β-2-mercaptoethanol. The homogenate was incubated in water bath at 65°C for 1 h with periodic gentle vortexing and the DNA was extracted twice with chloroform-isoamyl alcohol (24:1). The DNA was precipitated by adding equal volume (0.6V) of chilled isopropanol and 30 µl of 5 M NaCl was added, the tube was mixed gently to form fibrous DNA, DNA pellet was first washed with 800 µl of wash buffer (5 mM Tris HCl; pH (8), 25 mM NaCl, 75 % ethanol), and again washed with 300 µl of 70% ethanol. The DNA pellet was dissolved in 30 µL TE buffer (10 mM Tris-HCl, pH 8, 2 mM EDTA) and stored at -20°C. DNA concentration was determined by running the DNA samples on 0.8% agarose gel electrophoresis and comparison of band intensities with lambda DNA standards was done.

RAPD primers

A total of 15 RAPD primers (Operon Technologies, Alameda, CA, USA) were used. Seven random primers including OPA-01, OPA-03, OPA-09, OPA-10, OPB-07, OPC-05 and OPC-07 were used (Table 2).

RAPD PCR amplification

DNA amplification was performed for arbitrary polymerase chain reaction (PCR) in an ABI (Applied Biosystem Inc, USA) thermal cycler. PCR was performed in a reaction mixture with a total volume of 25 µl containing 10X buffer, 1.5 mM MgCl₂, 0.2 mM dNTPs, 1U Taq DNA polymerase, 0.2 picomole primers, 40 ng of template DNA and PCR water. After initial denaturation of the DNA at 94°C for 5 min, the thermal cycling was performed with denaturation at 94°C for 45 s, annealing at 37°C for 1 min and extension at 72°C for 1.5 min and final extension at 72°C for 10 min, while hold a temperature at 4°C.

Agarose gel electrophoresis

Amplified RAPD products were size separated by on 1.5% agarose gels electrophoresis at 125 V in 1X TBE buffer for 1 h, stained with ethidium bromide and photographed by gel documentation system (Alpha Innotech, Alpha Imager EP, and U.S.A). All PCR experiments were done at least twice and best gels of the replicates were used for band scoring.

RAPD data analysis and scoring

Electrophoretic patterns of each RAPD primers were scored manually as '1' or '0' for presence and absence of the bands. The results were analyzed on the principle that a band is considered to be 'polymorphic' if it is present in some individuals and absent in others, and 'monomorphic' if present in all individuals. Using Nei and Li genetic similarity coefficient (Nei and Li, 1979), a similarity matrix involving eight *Asparagus* species and six cultivars was generated with NTSYS-pc (Numerical taxonomy system, applied biostatistics, Inc., New York, USA, software version 2.02e (Sonnante et al., 2002). A phenogram was constructed using the Neighbor Joining Method.

RESULTS AND DISCUSSION

The identification is more difficult through vegetative

Table 1. Collection sites and environmental parameters for *Asparagus* species and *A. officinalis* cultivars.













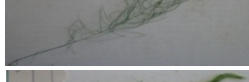

Species and cultivars	Type	Collection sites	Herbarium specimen
<i>A. racemosus</i> Willd.	Species	Charbhage, Swat	
<i>A. capitatus</i> subsp. <i>capitatus</i>	Species	Ghalegay, Swat	
<i>A. capitatus</i> subsp. <i>gracilis</i>	Species	Shamozu, Swat	
<i>A. adscendens</i>	Species	Jerma, Kohat	
<i>A. setaceus</i>	Ornamental	Bhage Jinnah, Lahore	
<i>A. densiflorus</i>	Ornamental	More green, Lahore	
<i>A. plumosus</i>	Ornamental	Mingora, Swat	
<i>A. officinalis</i> L.	Vegetative	NARC, Islambad	
<i>A. officinalis</i> Cv. Abril	Cultivar	ARI, Mingora	
<i>A. officinalis</i> Cv. Apollo	Cultivar	ARI, Mingora	
<i>A. officinalis</i> Cv. Gersengum	Cultivar	ARI, Mingora	
<i>A. officinalis</i> Cv. Huchel	Cultivar	ARI, Mingora	
<i>A. officinalis</i> Cv. Taranga	Cultivar	ARI, Mingora	
<i>A. officinalis</i> Cv. Para selection	Cultivar	ARI, Mingora	

Table 2. Lists of RAPD primers with their sequences and GC (%).

Primer	Sequence (5'-3')	GC%
OPA-01	CAGGCCCTTC	60
OPA-03	AGTCAGCCAC	60
OPA-09	GGGTAACGCC	70
OPA-10	GTGATCGCAG	60
OPB-07	GGTGACGCAG	70
OPC-05	GATGACCGCC	70
OPC-07	GTCCCGACGA	70

characters, although true phenotypic expression showed variation. Beside this, morphological and biochemical character cannot determine genetic differentiation and plasticity in population adaptation and variations (Gepts, 1993). So they lack the resolving power for individual genotype identification. Sometimes in early stage in *Asparagus* species, identification is more difficult from the other member of *Asparagus* family. Beside this, for *Asparagus* species due to the erratic flowering and lack of morphological differences, the recognition of genetic relationship is extremely difficult. Reliable identification of

Table 3. Polymorphism of RAPD Primers for *Asparagus* species and cultivars of *A. officinalis* L.

Primer	Number of bands	Monomorphic bands	Polymorphic bands	Monomorphic (%)	Polymorphic (%)
OPA 01	26	8	18	30.77	69.23
OPA 03	77	7	71	9.09	92
OPA 09	28	6	22	21.42	78.57
OPA 10	34	2	32	5.88	94.11
OPB 07	25	1	24	4	96
OPC 05	15	1	14	6.66	93.33
OPC 07	40	1	39	2.5	97.50
Total	245	26	220	80.32	89.80
Average	35	3.71	31.4	11.40%	88.71

taxa is not only necessary for breeders but also necessary for propagation and consumers. Nowadays, traditional method of species identification by morphological parameters is gradually being replaced by DNA profiling which is more reliable because of various limitations of morphological data. In recent year, DNA based RAPD markers have been widely used due to its rapid and simplicity, for the identification of variety, management of genetic resources, genetic diversity and phylogenetic relationship (Hu and Quiros, 1991; He et al., 1992).

In the present study, RAPD markers have been used for the genetic diversity of eight *Asparagus* species and six cultivars of *A. officinalis* L. from different regions of Pakistan (Table 1). Fifteen RAPD markers were selected for this purpose, to identify DNA polymorphisms and relationships among *Asparagus* species and its cultivars. In the present study, only seven random RAPD primers (OPA-01, OPA-03, OPA-09, OPA-10, OPB-07, OPC-05 and OPC-07) were reproducible and satisfactory, while the rest of the primers gave smear and unreadable band pattern.

A total of 245 bands were produced, among which 26 bands were monomorphic (11.40%), whereas 220 bands were polymorphic (88.71%). Lal et al. (2011) in their studies on five different species of *Asparagus*, utilizing 6 RAPD primers yielded 258 polymorphic DNA fragment. Determination of high level of genetic diversity of *Asparagus* species and cultivars of *A. officinalis* L. is very important to conserve for easy management of genetic resources and high level of variation for the breeding programs.

In the present study, the average numbers of bands per primers were 31.4, which were higher than that reported by Ray et al. (2010) for *Asparagus* species (28.1). These differences might be due to different primer sequences as well as different geographical origin. All RAPD primers showed a wide range of amplicons ranging from 300 to 3000 bp. The highest number of bands was obtained with primers OPC-07 which revealed 39 polymorphic bands and 1 monomorphic band with 97.50% polymorphism, while the lowest number of polymorphic bands was

obtained by the primer OPA-01 which were 18 polymorphic bands and 8 monomorphic bands with 69.23% polymorphism (Table 3). Among *Asparagus* species and cultivars of *A. officinalis*, *A. officinalis* Cv. Huchel showed maximum number of bands (45), whereas *A. racemosus* showed lowest number of bands (37) (Table 4).

Estimation of genetic similarity using genetic fingerprinting data are useful tool in plant breeding which allows plant breeders to create better decisions regarding the selection of germplasm to be used in crossing schemes (Milbourne et al., 1997; Russell et al., 1997). The genetic similarity index obtained from RAPD analysis showed a genetic similarity coefficient ranging from 0.75 to 0.96 with mean genetic similarity of 0.85. The highest genetic similarity was observed between (*A. officinalis* and *A. officinalis* Cv. Apollo) and (*A. officinalis* Cv. Gersengum and Abril) with a value of 0.96, while the lowest genetic similarity value was 0.75 between (*A. officinalis* Cv. Abril and *A. capitatus* subsp. *gracilis*) and (*A. setaceus* and *A. officinalis* Cv. Apollo) (Table 5).

The genetic similarity value were used for cluster analysis using neighbor joining algorithm, grouped *Asparagus* species and cultivars of *A. officinalis* L. into two main clusters (cluster I and cluster II). The cluster I was comprised of *A. officinalis* L., *A. officinalis* Cultivars Abril, Apollo, Gersengum, Huchel, Para selection, Taranga and *A. adscendens*, whereas Cluster II comprised of *A. capitatus* subsp. *capitatus*, *A. capitatus* subsp. *gracilis*, *A. densiflorus*, *A. racemosus*, *A. plumosus* and *A. setaceus*.

The data matrix of genetic similarities and phenogram is illustrated in Table 5 and Figure 2. The clarity of the differentiation for wild species by RAPD in the present work agreement with those of Lal et al. (2011), where they clustered the *Asparagus* species on the basis of their geographical isolation.

RAPD analysis to obtain information on genetic variations among *Asparagus* species was applied for the first time in Persia and this was the beginning of further studies by more powerful markers. To preserve this valuable plant, more *Asparagus* samples should be

Table 4. RAPD primers and total number of bands among *Asparagus* species and cultivars of *A. officinalis* L.

	<i>A. officinalis</i> L.	<i>A. officinalis</i> Cv. Abril	<i>A. officinalis</i> Cv. Apollo	<i>A. officinalis</i> Cv. Gersengam	<i>A. officinalis</i> Cv. Huchel	<i>A. officinalis</i> Cv. Para	<i>A. officinalis</i> Cv. Taranga	<i>A. adscendens</i>	<i>A. capitatus</i> subsp. <i>capitatus</i>	<i>A. densiflorus</i>	<i>A. plumosus</i>	<i>A. racemosus</i>	<i>A. setaceus</i>	<i>A. capitatus</i> subsp. <i>gracilis</i>
OPA 01	9	8	9	9	8	9	9	10	10	10	9	10	10	10
OPA 03	12	12	8	12	12	12	11	11	9	11	11	11	10	8
OPA 09	7	7	5	7	8	8	8	8	6	8	7	7	8	9
OPA 10	4	5	2	4	5	6	3	2	5	5	4		4	4
OPB 07	3	1	3	4	4	3	1	3	3	3	3	3	3	2
OPC 05	3	3	3	3	3	3	3	1	1	1	1	1	1	1
OPC 07	5	5	5	5	5	3	5	3	3	2	2	2	2	4
Total	43	41	35	44	45	44	40	38	37	40	37	34	38	38

Table 5. Genetic similarities index based on Nei and Li coefficient using RAPD primers for *Asparagus* species and cultivars of *A. officinalis* L.

<i>Asparagus</i> species	<i>A. officinalis</i> L.	<i>A. officinalis</i> Cv. Abril	<i>A. officinalis</i> Cv. Apollo	<i>A. officinalis</i> Cv. Gersengum	<i>A. officinalis</i> Cv. Huchel	<i>A. officinalis</i> Cv. Para	<i>A. officinalis</i> Cv. Taranga	<i>A. adscendens</i>	<i>A. capitatus</i> subsp. <i>capitatus</i>	<i>A. densiflorus</i>	<i>A. plumosus</i>	<i>A. racemosus</i>	<i>A. setaceus</i>	<i>A. capitatus</i> subsp. <i>gracilis</i>
<i>A. officinalis</i> L.	1													
<i>A. officinalis</i> Cv. Abril	0.95	1												
<i>A. officinalis</i> Cv. Apollo	0.96	0.96	1											
<i>A. officinalis</i> Cv. Gersengum	0.96	0.94	0.93	1										
<i>A. officinalis</i> Cv. Huchel	0.95	0.93	0.92	0.96	1									
<i>A. officinalis</i> Cv. Para	0.92	0.89	0.90	0.88	0.92	1								
<i>A. officinalis</i> Cv. Taranga	0.94	0.93	0.92	0.90	0.91	0.90	1							
<i>A. adscendens</i>	0.84	0.83	0.80	0.85	0.86	0.85	0.87	1						
<i>A. capitatus</i> subsp. <i>capitatus</i>	0.80	0.79	0.76	0.81	0.82	0.79	0.80	0.88	1					
<i>A. densiflorus</i>	0.79	0.84	0.81	0.81	0.84	0.83	0.85	0.89	0.85	1				
<i>A. plumosus</i>	0.82	0.82	0.79	0.86	0.87	0.84	0.85	0.88	0.86	0.90	1			
<i>A. racemosus</i>	0.84	0.83	0.80	0.85	0.86	0.82	0.84	0.92	0.90	0.94	0.93	1		
<i>A. setaceus</i>	0.79	0.78	0.75	0.80	0.84	0.80	0.82	0.89	0.90	0.92	0.93	0.94	1	
<i>A. capitatus</i> subsp. <i>gracilis</i>	0.76	0.78	0.78	0.78	0.81	0.75	0.79	0.81	0.82	0.84	0.80	0.84	0.84	1

gathered, cultivated and domesticated in collections. Prohens et al. (2008) showed Asia, Africa and Europe as the main centers for the genetic diversity of edible

Asparagus. The results of the current study showed that Pakistan could be considered as another center for genetic diversity of *Asparagus*.

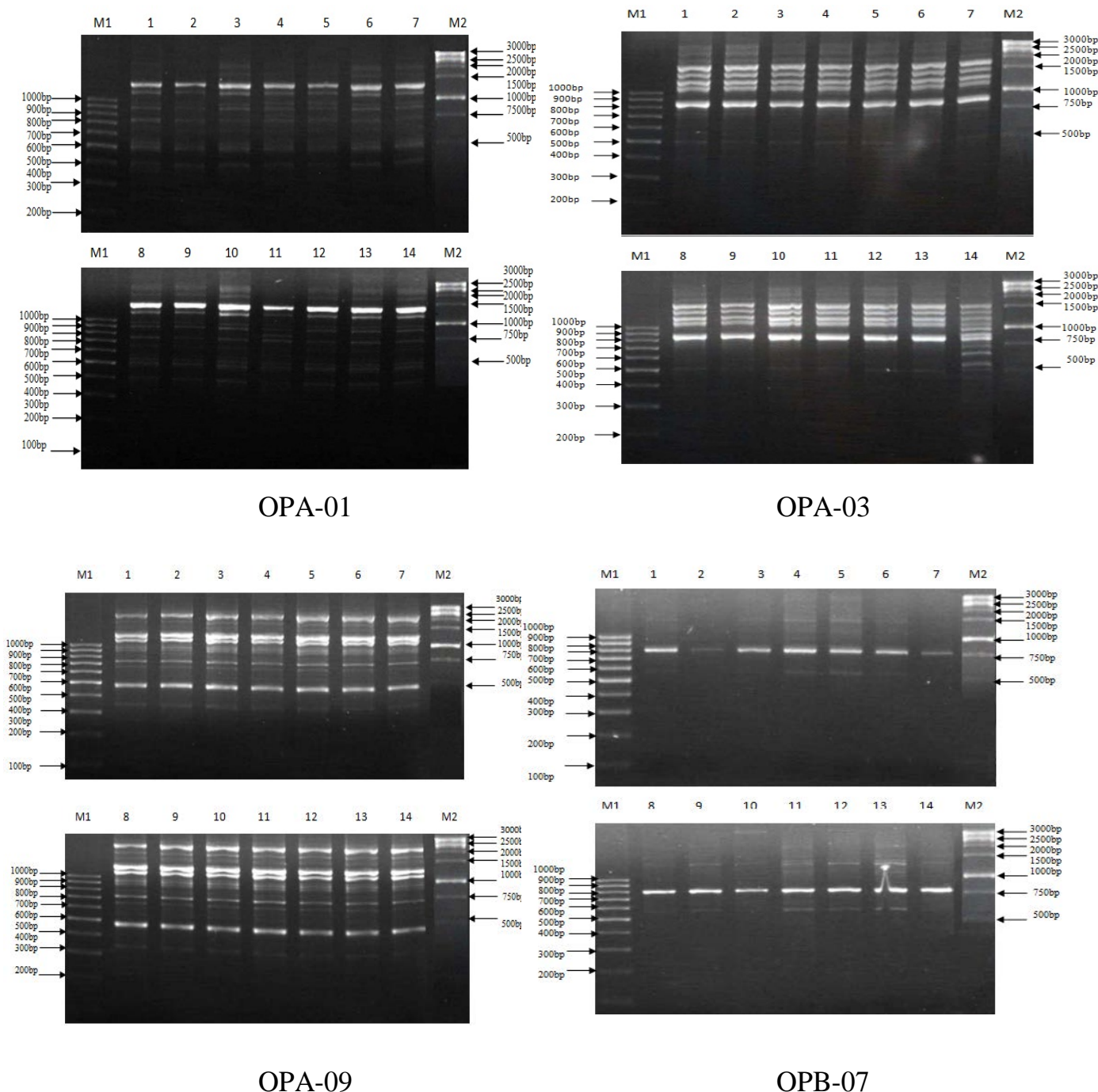


Figure 1. RAPD pattern generated using primer (A) OPA-09 (B) OPA-03 (C) OPA-01 (D) OPB-07: Lane 1-14 represents M1 = 100 bp marker, Lane M2 = 1 kb marker, (1) *A. officinalis* L. (2) *A. officinalis* Cv. Abril, (3) *A. officinalis* Cv. Apollo, (4) *A. officinalis* Cv. Gersengum, (5) *A. officinalis* Cv. Huchel, (6) *A. officinalis* Cv. Para, (7) *A. officinalis* Cv. Taranga, (8) *A. adscendens*, (9) *A. capitatus* subsp. *capitatus*, (10) *A. densiflorus*, (11) *A. plumosus*, (12) *A. racemosus*, (13) *A. setecus*, (14) *A. capitatus* subsp. *gracilis*.

Conclusion

RAPD markers are very useful for analyzing genetic diversity as well as pattern of genetic relationship among *Asparagus* species and cultivars of *A. officinalis* L.

Further, more numbers of primers and large number of samples with wide range of collection sites should be used to obtain a clear picture of genetic diversity. This study will be particularly useful for the conservation, breeding and germplasm management of *Asparagus*.

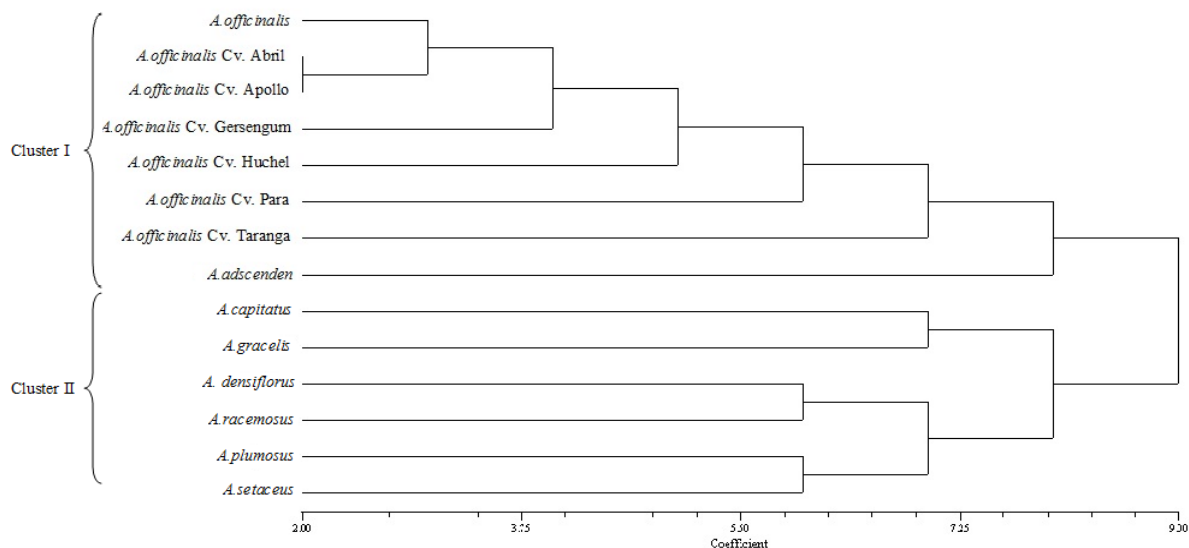


Figure 2. Neighbor Joining method of cluster analysis of *Asparagus* species and cultivars of *A. officinalis* L. using RAPD primer.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Full Length Research Paper

Impact of the cement dust emitted from the South Cement Factory in Tafila/Jordan on plant diversity of the surrounding area

Sawsan A. Oran* and Hamad M. F. Abu Zahra

Department of Biological Sciences, Faculty of Sciences, University of Jordan, Amman- Jordan.

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Observations and analysis based on using scanning electron microscope (SEM) micrographs have been carried out to describe the impact of pollution caused by the emitted cement dust from the cement factory on the plant diversity of the surrounding area of Al-Rashadyah/ Tafila cement factory. Leaves of five selected species of *Crataegus aronia*, *Gundelia tournefortii*, *Anchusa strigosa*, *Lactuca orientalis* and *Astragalus bethlehmiticus*, from five localities within the study area based on their easily sampling and their availability in all localities during the period of collection were used for assessing the impact of the cement dust. A species of *C. aronia* was chosen as control species grown in Wadi Shuaib 200 km to the north of the study area. The results indicate that plants species grown near the cement factory are covered with higher amounts of dust accumulations than those grown at long distance from the factory. The analysis of SEM shows many different elements deposited on the leaves of all plants that have been sampled in the study area such as: calcium, potassium, aluminum and others, and it was found that calcium has the highest concentration than the other mineral salts.

Key words: Plant diversity, pollution, Tafila, Jordan.

INTRODUCTION

Plant diversity in Jordan faces the danger of degradation and loss of many plant species as a result of both adverse human impact and environmental factors. Moreover, these changes are occurring at natural rapid rate as results of human activities, such as land-use, climate change, nitrogen deposition, species introductions, increase in population, over exploitation of plant and animal species, pollution of soil, water and air. Biodiversity in Jordan is exposed to several threats leading to sharp decline in most of the Jordanian flora

and the extinction of several species. Many species have been at risk, or were classified as threatened or endangered or even extinct on the regional and global levels. This situation resulted from various anthropogenic activities, as well as from a general lack of knowledge and awareness (Oran, 2005, 2014; AL-Eisawi, 2000, 2013; Al-Mohaisen et al., 2005). Yet serious attempts have been made to protect and conserve the plant genetic resources of the country. Many reserves have been established, but the laws and regulations governing

*Corresponding author. E-mail: oransaw@ju.edu.jo.

them are not always enforced and dozens of species are facing dramatic pressure (Oran, 1994; EPA, 2001). The plant diversity in Tafila governorate South of Jordan is very rich. A number of 383 species belong to 198 genera and 48 families were recorded (Oran, 2014).

Cement dust is one of the factors that affect plant biodiversity; it can settle on the surrounding vegetation and affect plant growth, directly by covering the leaf surface and indirectly through effects via the soil. The effects of dust on vegetation were reviewed by Farmer (1993) who provided a comprehensive review of the literature of the effects of dust on plants and their communities.

Kamel (1981) pointed out that dust emitted from the chimneys of cement kilns contains major concentrations ($\geq 1\%$) of iron and trace amounts ($\leq 0.1\%$) of manganese, cobalt, nickel, zinc and cadmium, which are known for their potential effect on air pollution. Other similar studies also dealt with the evaluating of the effects on plants growing around cement factories. Misra et al. (2000) investigated the responses of some common plants in Uttar Pradesh state/ India to cement dust pollution and to identify the tolerance to pollution of several species which are good collectors of cement dust and are resistant to pollution. Salami et al. (2002) studied the impact of cement dust emissions from West African Portland Cement Factory at Ewekoro in Southwestern Nigeria on the surrounding vegetation, from this study "it was found that chlorophyll contents leaf abundance, leaf area, woody species density and basal area increased significantly with increasing distance from the factory site". Farmer (1993) explained the effects of dust pollution on vegetation types. Zvereva et al. (2008) investigated the general pattern of changes in species richness and diversity of vascular plants due to environmental contamination, and associated habitat changes imposed by point polluters and identified the sources of variation in the response of plant communities to industrial pollution at a global scale. Lisenko et al. (2012) suggested spectronephelometric methods to determine microphysical characteristics of dust in aspiration air and off-gases in cement plants. Edral and Demirtas. (2010) studied the effects of cement flue dust from a cement factory on stress parameters and diversity of aquatic plants; the toxicity and deficiency caused by cement dust pollution in wild plants growing around a cement factory was investigated by Mutlu et al. (2012); they determined the effects of cement dust pollution on contents of some significant essential elements (P, S, K, Ca, Fe and Cl) in wild plants using wavelength-dispersive spectrometer X-ray fluorescence (XRF) technique. The effects of the emissions of the Mergheb cement factory in North West Libya on the vegetation were evaluated. The impact of stack emissions of the factory on the abundance, frequency, density and number of species at each site surrounding the factory under the effect of wind direction was evaluated by

Okasha et al. (2013). A study by assessed the potential of bioindicator/ biomonitor plants to determining pollution extent of toxic metals in plant leaves from the industrial area of the city Gaziantep in Turkey.

This study is focusing on analyzing and identifying the major pollutants emitted from the cement factory of Al-Rashadeyeh in Tafila governorate, south of Jordan, on the leaf surface of five local wild plant species collected from the surrounding area of the cement factory, and one plant species as a control from Wadi Shuaib area 200 km north of Al-Rashadeyeh cement factory, and its impact on the diversity of plant species.

MATERIALS AND METHODS

Five leaf samples for five plant species were collected randomly from the surrounding area of Al-Rashadeyeh cement factory (Map 1. A), occasional visits to the study area have been made and random collection of the species such as *Crataegus aronia*, *Gundelia tournefortii*, *Anchusa strigosa*, *Lactuca orientalis* and *Astragalus bethlehmiticus* was made. As far the control specimen *Crataegus aronia* leaf was collected from Wadi Shuaib which is 200 km north of the study area that is remote from the pollution area, as shown in (Map 1. B). It is good to know that leaf surface of *C. aronia*, *L. orientalis*, and *A. bethlehmiticus* is smooth and for *G. tournefortii* and *A. strigosa*, is hairy- spiny.

The scanning electron microscope (SEM) was used in this experiment for its ability to analyze small particles on the surface of the leaves. The type of SEM used is FEI Quanta 200 SEM at the Department of Geology in Yarmouk University. From each location, the location of each collected specimen from the factory is shown in Table 1; one leaf was selected at random from specimen collected. A 1 cm² size was cut from each of the leaves by a blade. A small drop of glue was placed on each stub. The leaf samples were placed on top of the glue and were pressed gently to be fixed on the stub.

Stubs with fixed specimens were analyzed under the SEM. Stubs were then placed in the SEM chamber. The high vacuum pressure was used to evacuate the chamber and high voltage was also used to turn the electron beam on for providing the best backscattered electrons (BSE) and X-ray microanalysis.

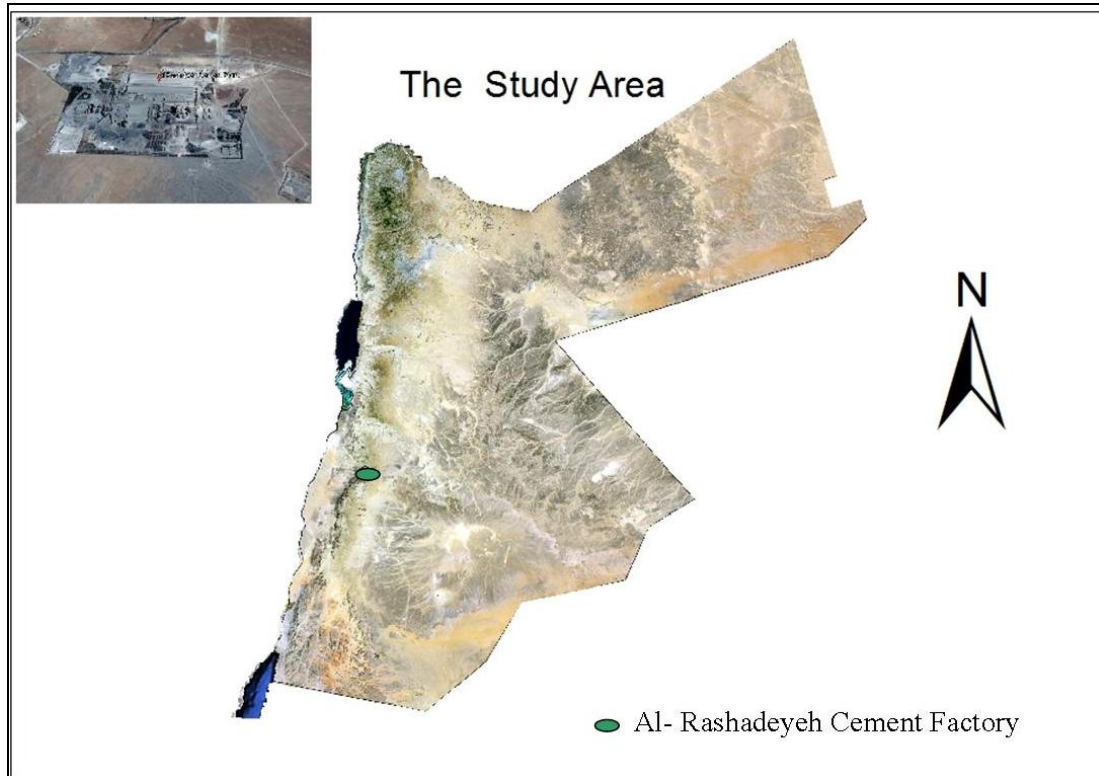
In microanalysis the area of analysis and parameters are set and the software of the SEM controls the data analysis. The X-ray microanalysis of the particles determines which elements are present on the surface of the leaf and places all elements detected in a spectrum. No test has been made to test the elements binding capabilities for the leaves of the different studied plant species; calculations of element composition are then provided for every specimen and finally a digital micrograph is given. In this study different magnifications were set according to the sample used.

RESULTS

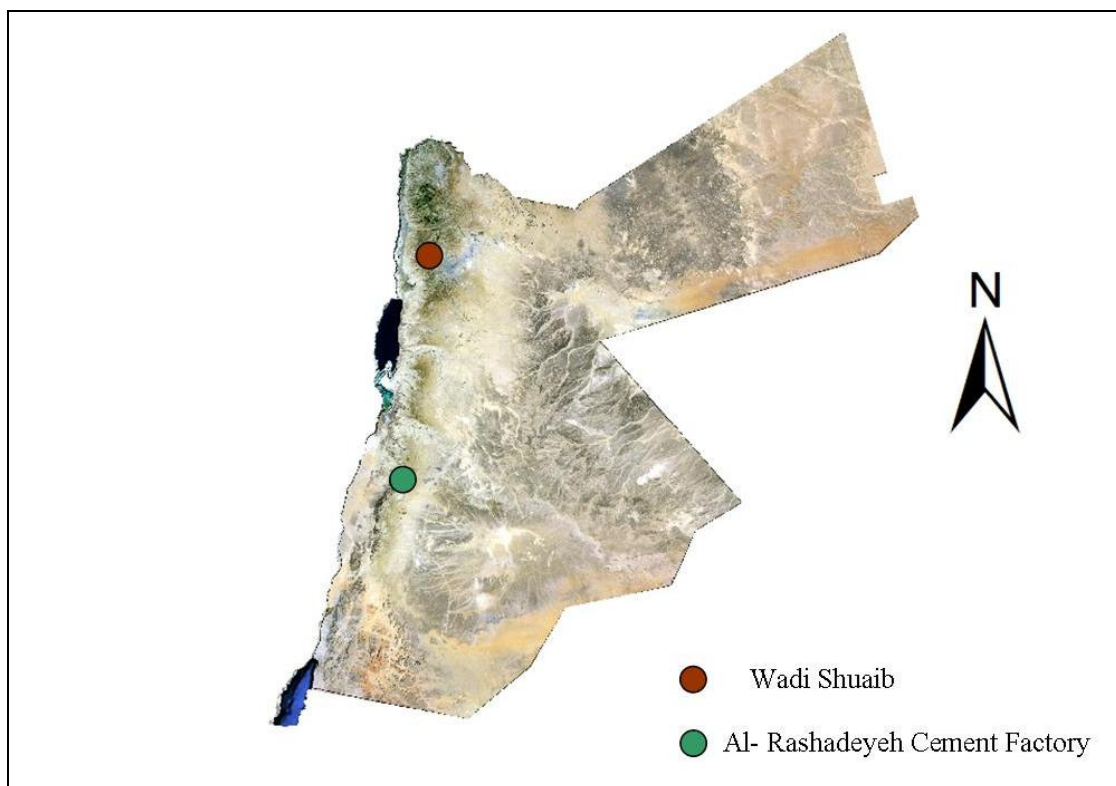
Dust observation

Scanning electron microscopy (SEM) analysis

It can be observed from Figures 1 to 6 that there is a considerable amount of cement dust accumulated in all of the five plant species that have been collected and



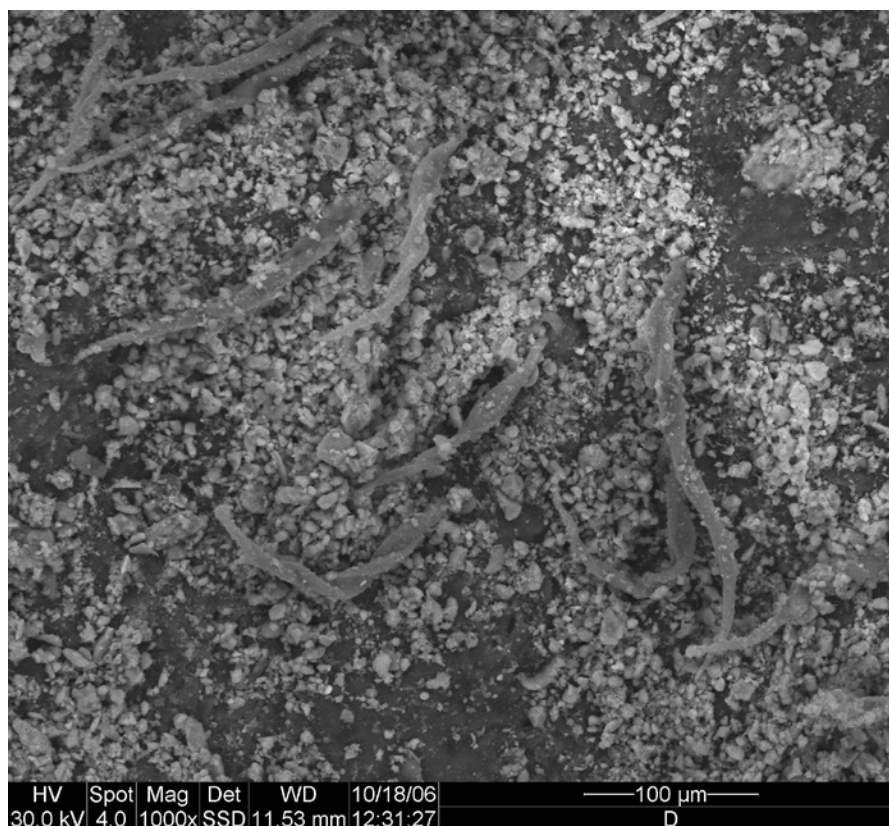
Map 1A. Showing Al- Rashadeyeh cement factory.



Map 1B. Showing Al- Rashadeyeh cement factory and Wadi Shuaib area.

Table 1. Leaf identification and sampling information for the SEM analysis.

Leaf number	Leaf Identification	Location
1	<i>Crataegus aronia</i>	Locality (1) 50 m North of the factory
2	<i>Gundelia tournefortii</i>	Locality (2) 100 m East of the factory
3	<i>Anchusa strigosa</i>	Locality (3) 500 m West of the factory
4	<i>Lactuca orientalis</i>	Locality (4) 750 m South of the factory
5	<i>Astragalus bethlehmiticus</i>	Locality (5) 1000 m North East of the factory
6 (control sample)	<i>Crataegus aronia</i>	Wadi Shuaib

**Figure 1.** SEM micrograph for *Crataegus aronia* (leaf 1) taken from locality 1.

sampled comparing to the control plant *C. aronia* which was collected from Wadi Shuaib and has almost a clean surface.

Localities 1, 2, 3 and 4, which were 50, 100, 500 and 750 m North, East, West and South of the cement plant, respectively tend to have the highest concentration of dust particles, due to the fact they are the closest to the cement factory, and subjected to traffic. This is followed by locality 5 which is about 1000 m North-East of the factory.

Therefore leaf sampling from all these localities shows that particle concentrations on leaves collected from these localities will decrease from locality 1 to 2 to 3 to 4 and to 5.

X-Ray microanalysis

The particles deposited on the leaves are from the air and the surroundings, analysis of the particles will provide information about the particulate air pollutants in the area sampled. The higher the concentration of particles in the area, the higher the concentration of particles on the leaf surface will be. The weight percentage and the chemical composition of the particles on the leaf surface are represented in Tables 2 to 7.

The X-ray microanalysis illustrated that the leaves had a wide range of particle concentrations and they are represented as weight percentage in the tables provided with each graph (Graphs 1 to 6), these are ranged from a

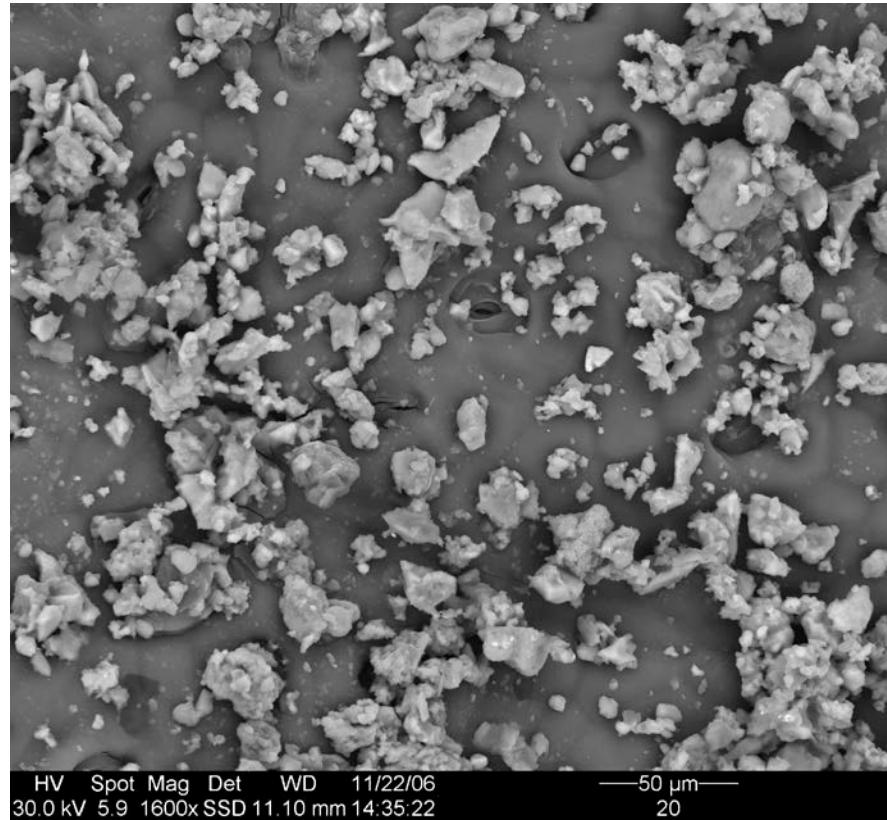


Figure 2. SEM micrograph for *Gundelia tournefortii* (leaf 2) taken from locality 2.

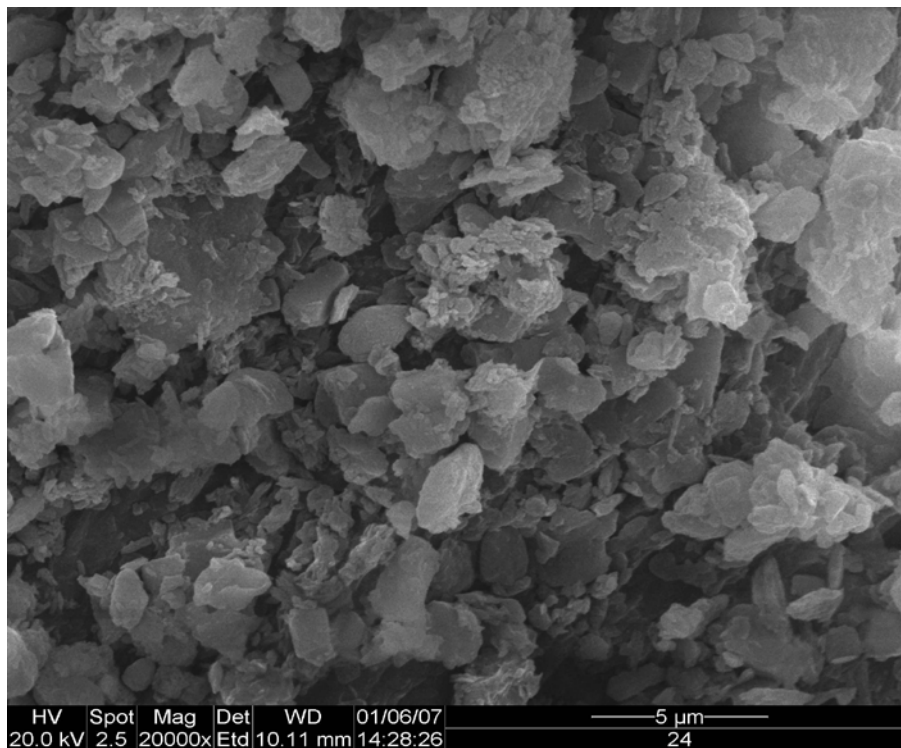


Figure 3. SEM micrograph for *Anchusa strigosa* (leaf 3) taken from locality 3.

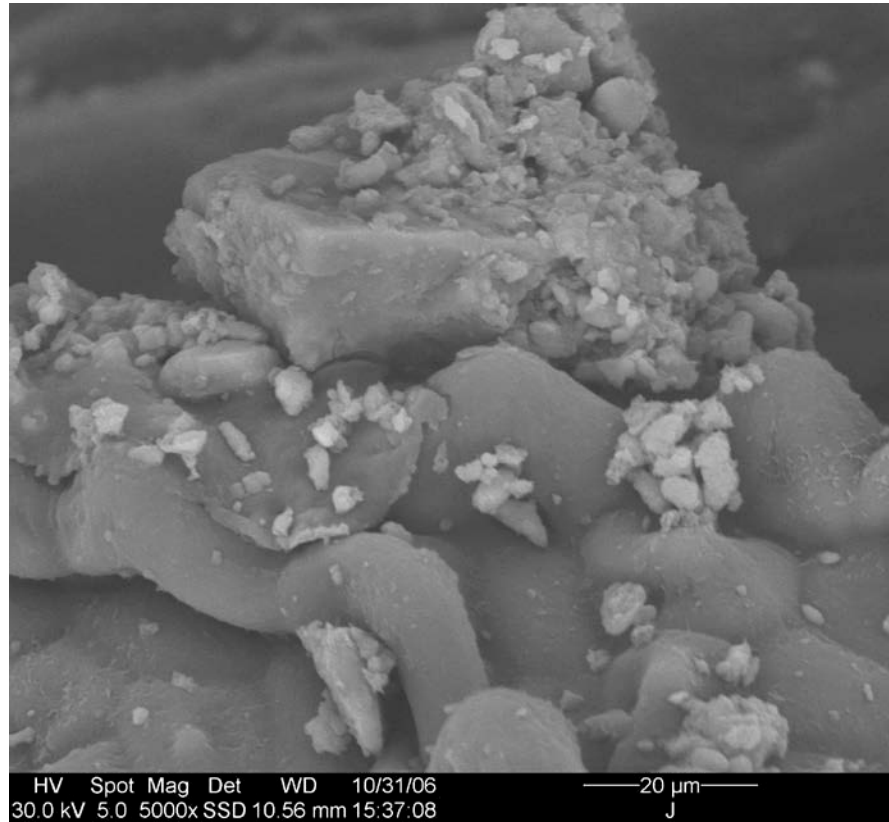


Figure 4. SEM micrograph for *Lactuca orientalis* (leaf 4) taken from locality 4.

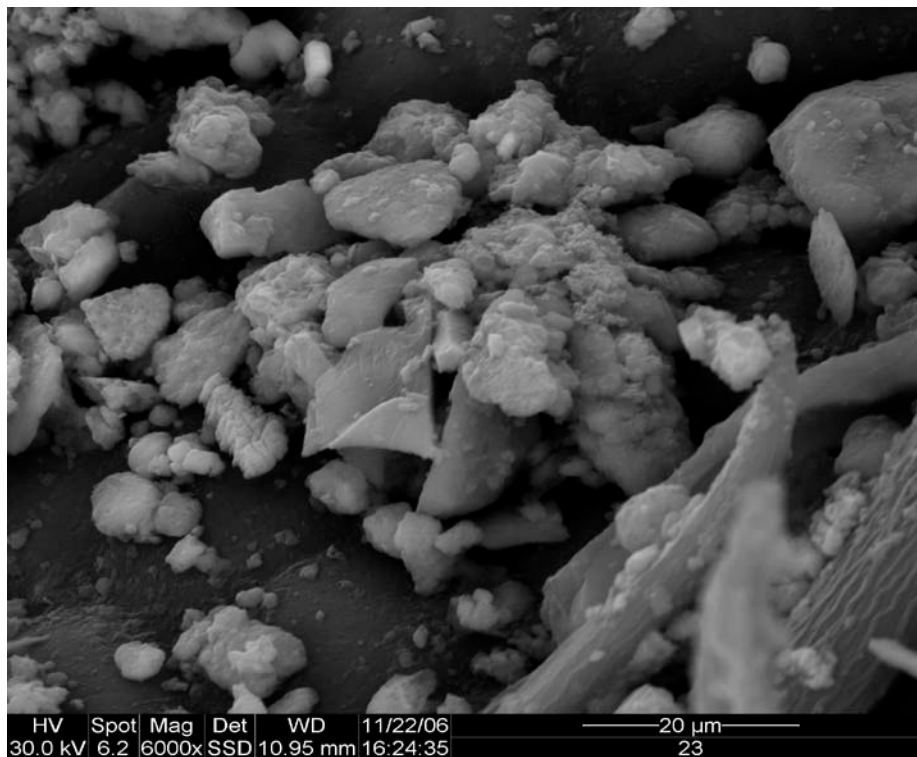


Figure 5. SEM micrograph for *Astragalus bethlehmiticus* (leaf 5) taken from locality 5.

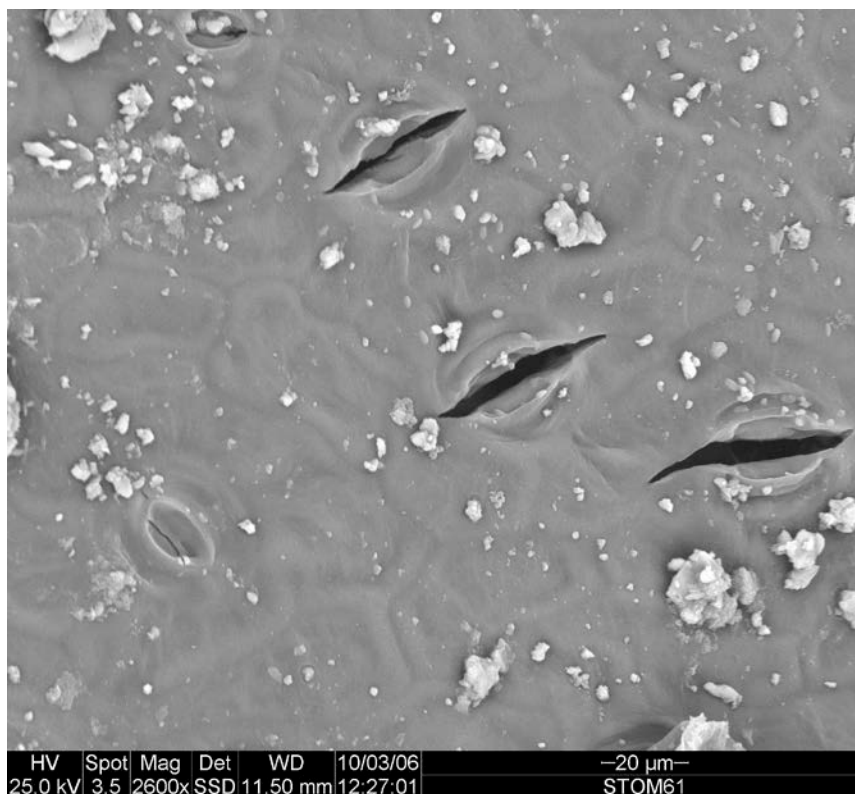


Figure 6. SEM micrograph for *Crataegus aronia* (leaf 6, control sample) collected from Wadi Shuaib.

Table 2. X-ray spectrum of particles for leaf 1.

Element	Weight % (Wt %)
O	53.66
Na	0.51
Mg	1.5
Al	1.85
Si	6.36
S	1.22
K	1.23
Ca	31.91
Fe	1.76
Total	100

Table 3. X-ray spectrum of particles for leaf 2

Element	Weight % (Wt %)
O	55.53
Mg	2.39
Al	3.87
Si	9.74
K	1.08
Ca	23.67
Ti	0.53
Fe	3.19
Total	100

minimum of 0.17% (leaf 4), to 55.53% (leaf 2).

Graph 1 determines which elements are presented in the particles found on the surface of leaf 1 and places them in a spectrum. It is clear from this graph that calcium (Ca) is the highest element found and has a high weight percentage followed by silicon (Si) then sulfur (S), oxygen (O), aluminum (Al), potassium (K) and magnesium (Mg) (Table 2).

Table 3, shows the elemental composition data and provides a spectrum for each element found in dust par-

ticles on the surface of leaf 2. The weight percentage for all of the elements is arranged as follows:

Ca > Si > Al > Mg > K > Fe > Ti.

The elemental composition in Table 4 for leaf 3 based on weight percentage is arranged as follows: Ca > Si > Al > Fe > Mg > K > Cu.

In the same way the graph in Table 5 also shows the arrangement of the elemental composition in leaf 4 as follows: Ca > Si > S > K > Al > Mg > Fe > Na > Ti. Both graphs in Tables 6 and 7 provide the elemental composition and the spectrums for leaves 5 and 6,

Table 4. X-ray spectrum of particles for leaf 3.

Element	Weight % (Wt %)
O	54.14
Mg	2.04
Al	4.83
Si	17.19
K	1.07
Ca	16.27
Fe	3.7
Cu	0.76
Total	100

Table 5. X-ray spectrum of particles for leaf 4.

Element	Weight % (Wt %)
O	51.76
Na	1.24
Mg	2.22
Al	3.78
Si	15.98
S	1.61
K	2.96
Ca	18.49
Ti	0.17
Fe	1.81
Total	100

Table 6. X-ray spectrum of particles for leaf 5.

Element	Weight% (Wt %)
MgO	4.72
Al ₂ O ₃	12.17
SiO ₂	40.39
K ₂ O	0.88
CaO	32.34
TiO ₂	0.84
Fe ₂ O ₃	8.65
Total	100

showing their appearance based on weight percentages as follows respectively:

- (i) Ca > Si > O > Al > Fe > Mg > K > Ti.
(ii) Ca > O > C > P > Si > Al > Mg.

Moreover the results on Tables 2 to 7, shows that the concentration of the different recorded elements were higher on the leaves of smooth surface than the leaves of hairy- spiny surface.

Table 7. X-ray spectrum of particles for the control sample.

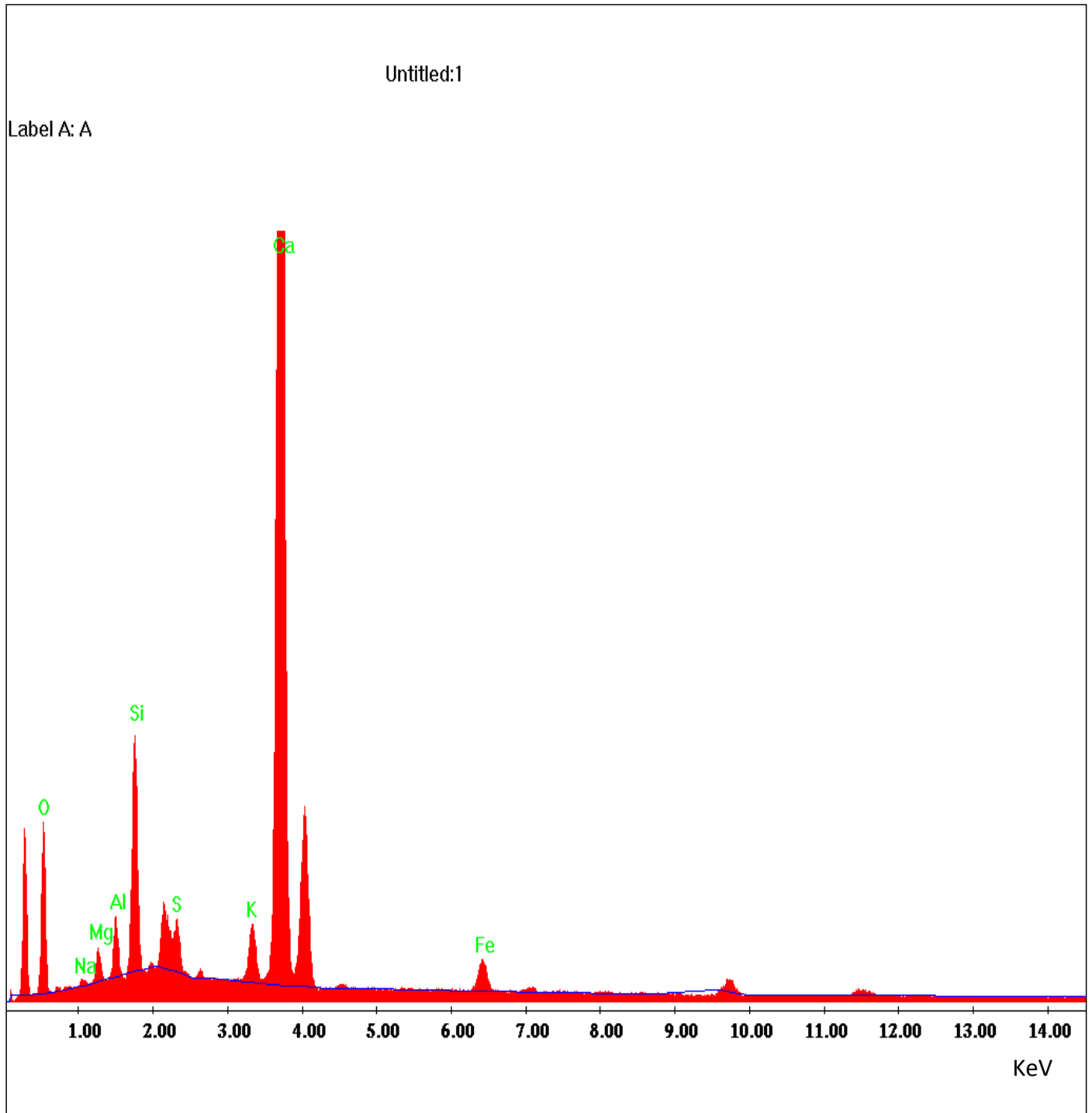
Element	Weight % (Wt %)
C	7.33
O	51.33
Mg	0.9
Al	0.73
Si	1.01
P	0.6
Ca	38.09
Total	100

DISCUSSION

The study area or the area surrounding Al- Rashadeyeh cement factory in Tafila south of Jordan is considered as a Mediterranean mountainous area dominated by *Juniperus phoenicea* degraded forests and a nearby natural reserve called "Dana", which is the safe heaven of many local wild plant species in Tafila area. The establishment of the cement factory in that important natural site was unwise decision made by the authorities; however, the cement dust would be the major pollutant in the area surrounding Al- Rashadeyeh cement factory and would affect the human and other living communities. It can block the stomata of leaf surface, might affect the photosynthesis, respiration, transpiration, and may cause leaf injury symptoms, as a result of that the productivity of these plants would be declined and consequently a reduction in vegetation growth, abundance and species loss. The inhibition of all these processes depending on particle size and concentration of dust that will affect stomata functions.

Dust emitted from the chimneys of the cement factory contains major concentrations of Fe, Co, Ni, Zn, Cd and Pb; these elements are transported with the particulates and are known for their effect on air pollution as reported by previous studies, but this current analysis shows that dust particles deposited on leaf surface contains major concentrations of Ca, Si, O, K, Al and Mg, these elements are known to be the components of the plant cells, but at the same time Si, Al, K and part of O and Ca are the main components of clay and limestone, which are the raw materials for cement production and could come to the plants from the soil itself.

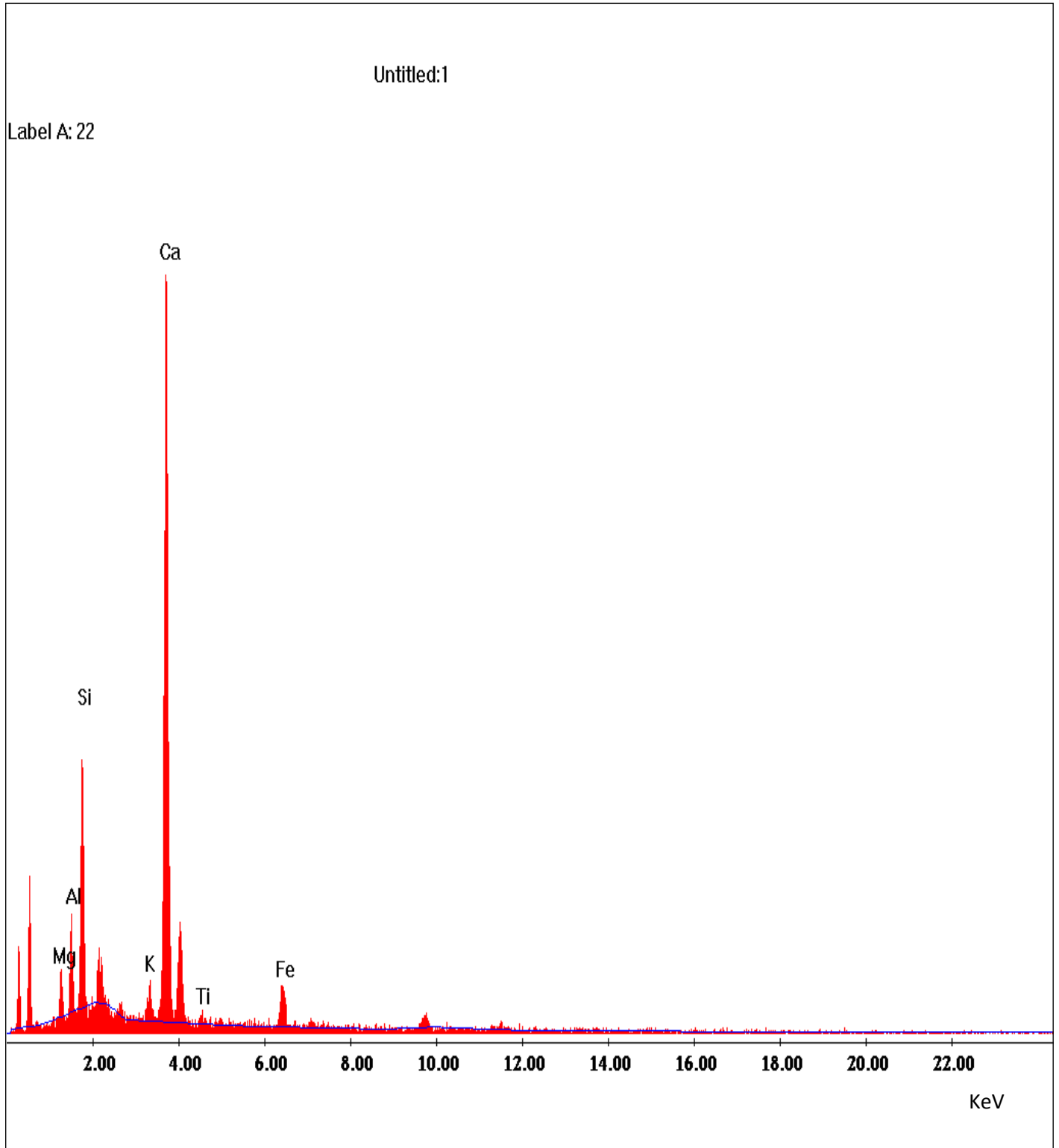
It is worth mentioning that the direction of prevailing wind in Jordan is usually coming from west to east, north to south; occasionally the wind changes direction from east to west and thus easterly wind specially in the cold days of the year or hottest days of the year since the wind is coming from dry- cold desert in winter and dry-hot weather in the summer time, similarly in parts of changing seasons in the year within the period between spring and summer, the prevailing wind is changing direction from south to east carrying lots of dusty winds



Graph 1. X-ray spectrum of particles for leaf 1.

that is known locally and regionally as “*Khamaseen* winds”. As mentioned previously the dominant prevailing wind is westerly and northerly changing occasionally through easterly and southerly. Therefore the directions of prevailing winds have influence on the deposition of dust particles in the study area.

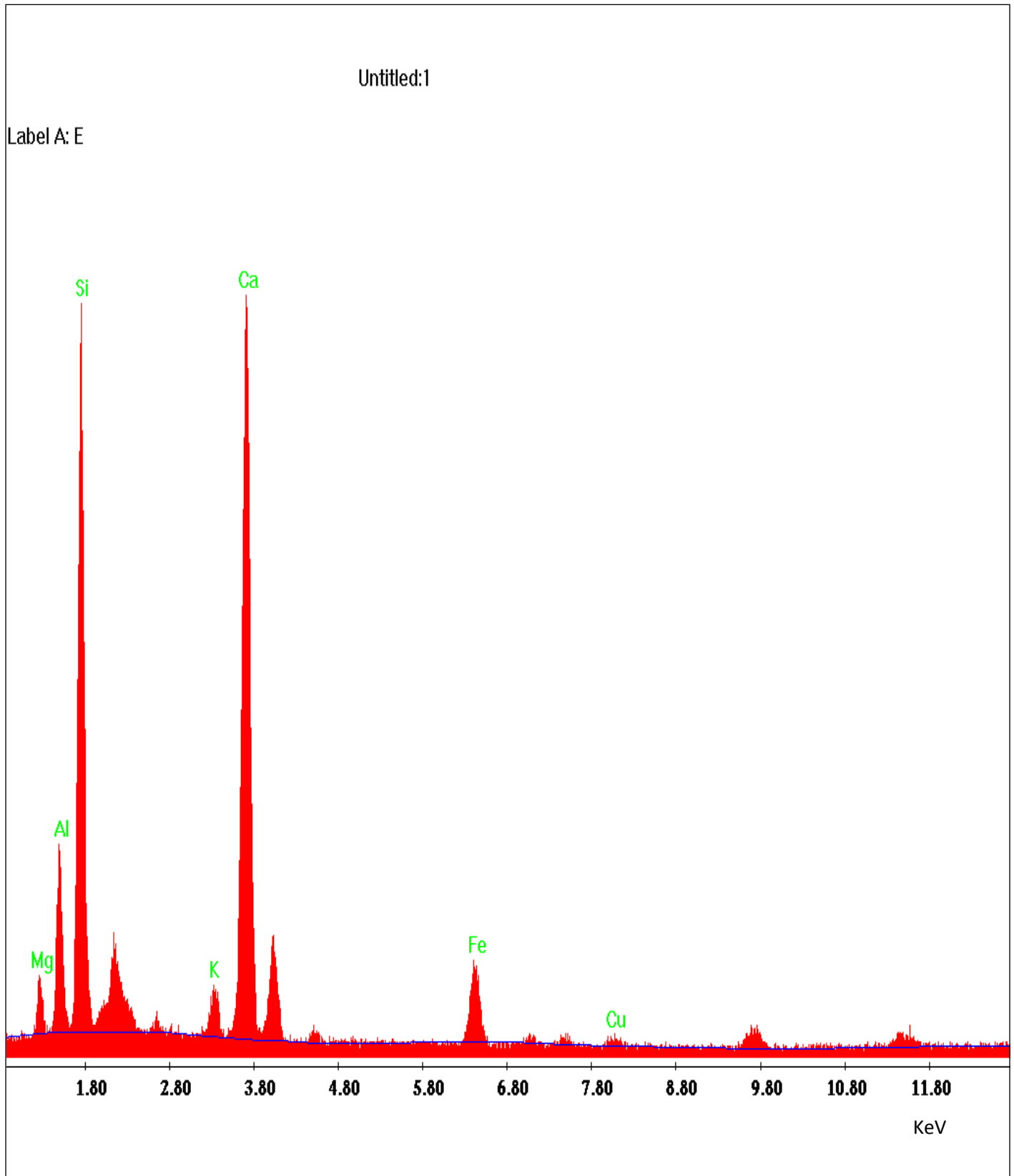
The results of this small scale study would assess the impact of the types of dust emissions or pollutants on leaf surface of local wild plant species that probably affect the survival of wild local plant species and also will be affecting the diversity of endemic species and those of economic value.



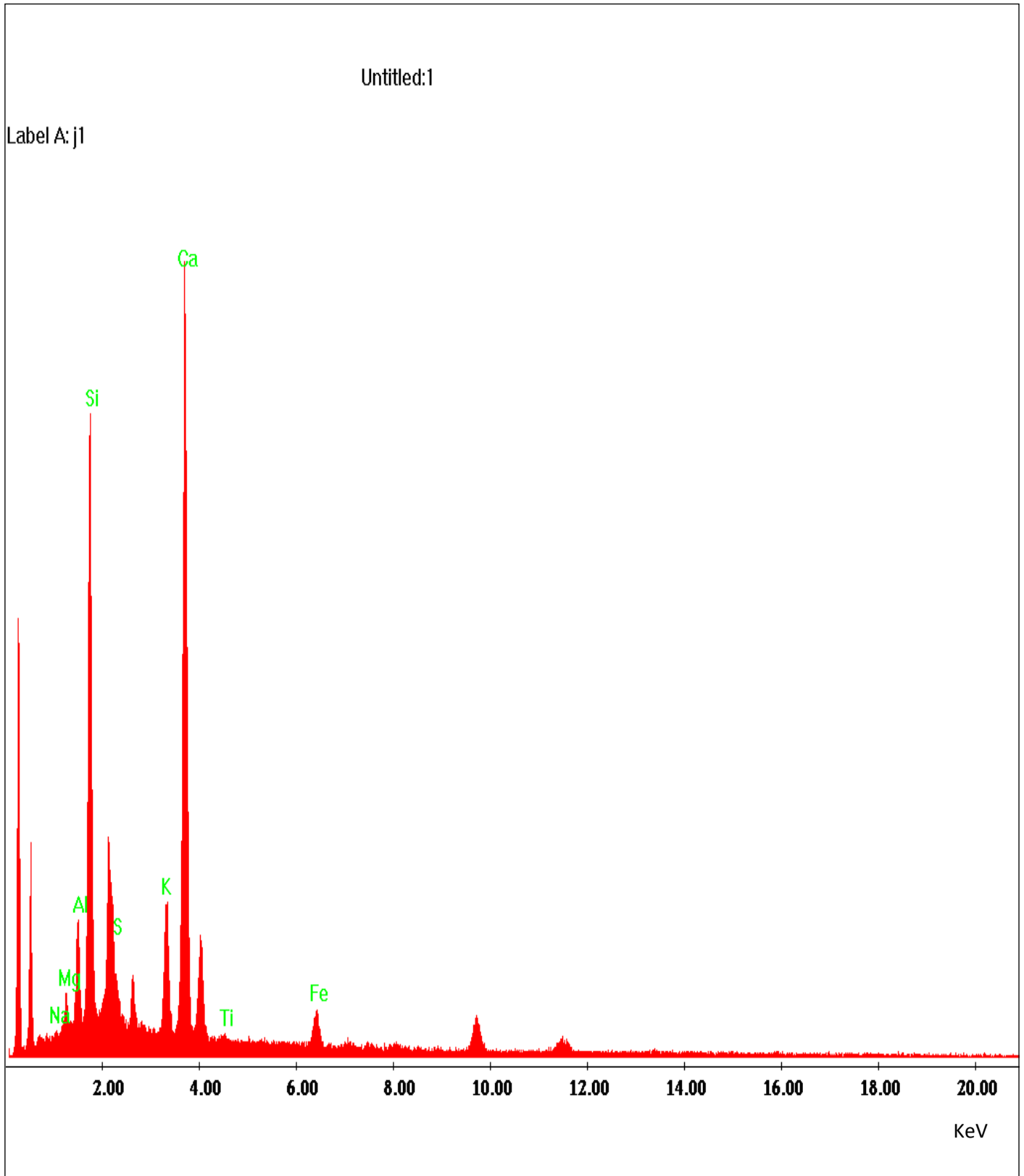
Graph 2. X-ray spectrum of particles for leaf 2

Moreover, this study is hopefully explaining the un-safe situation for the plant species and other kinds of living

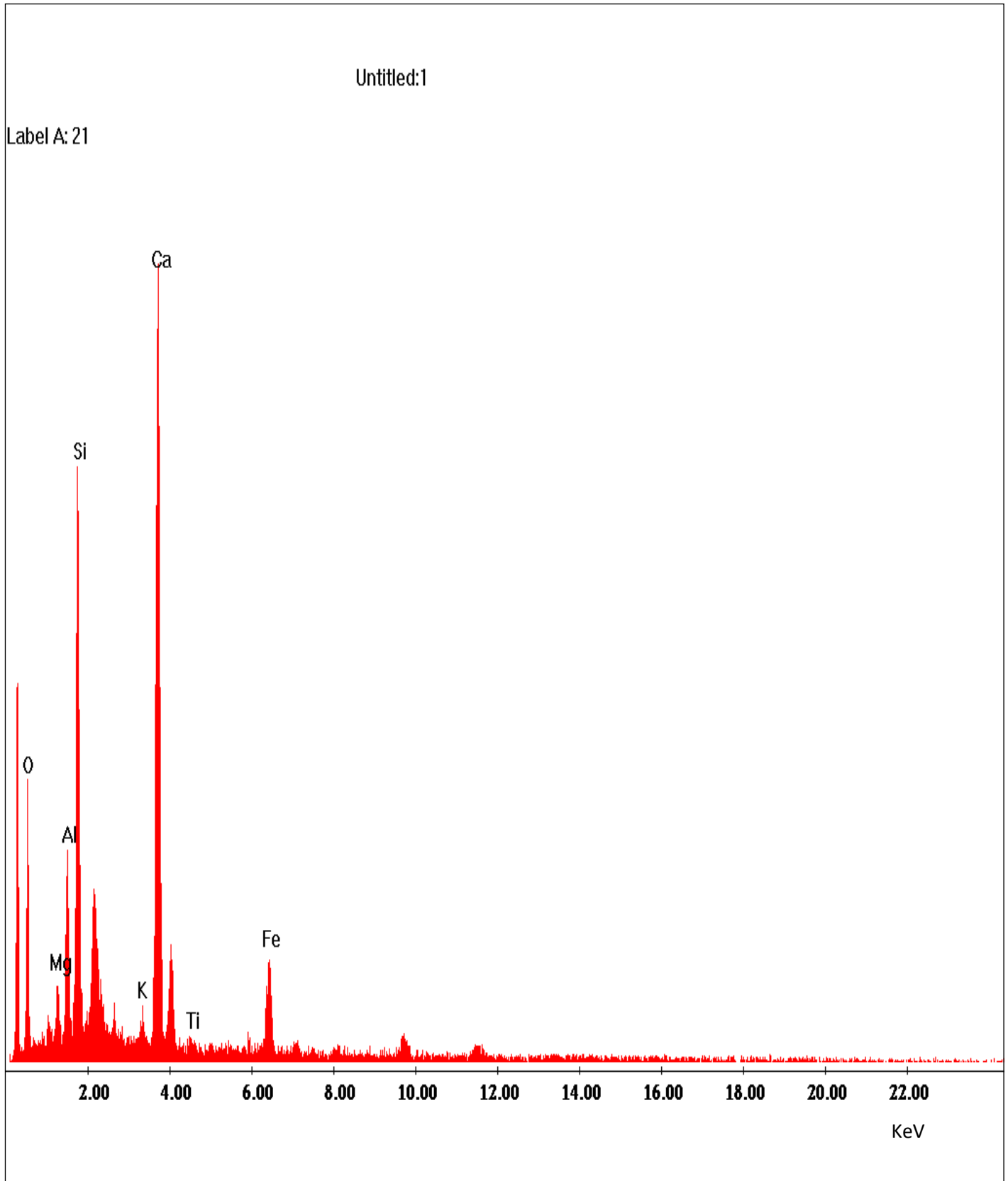
organisms that are eventually affected by this pollution and are exposed to the air pollutants emitted from the



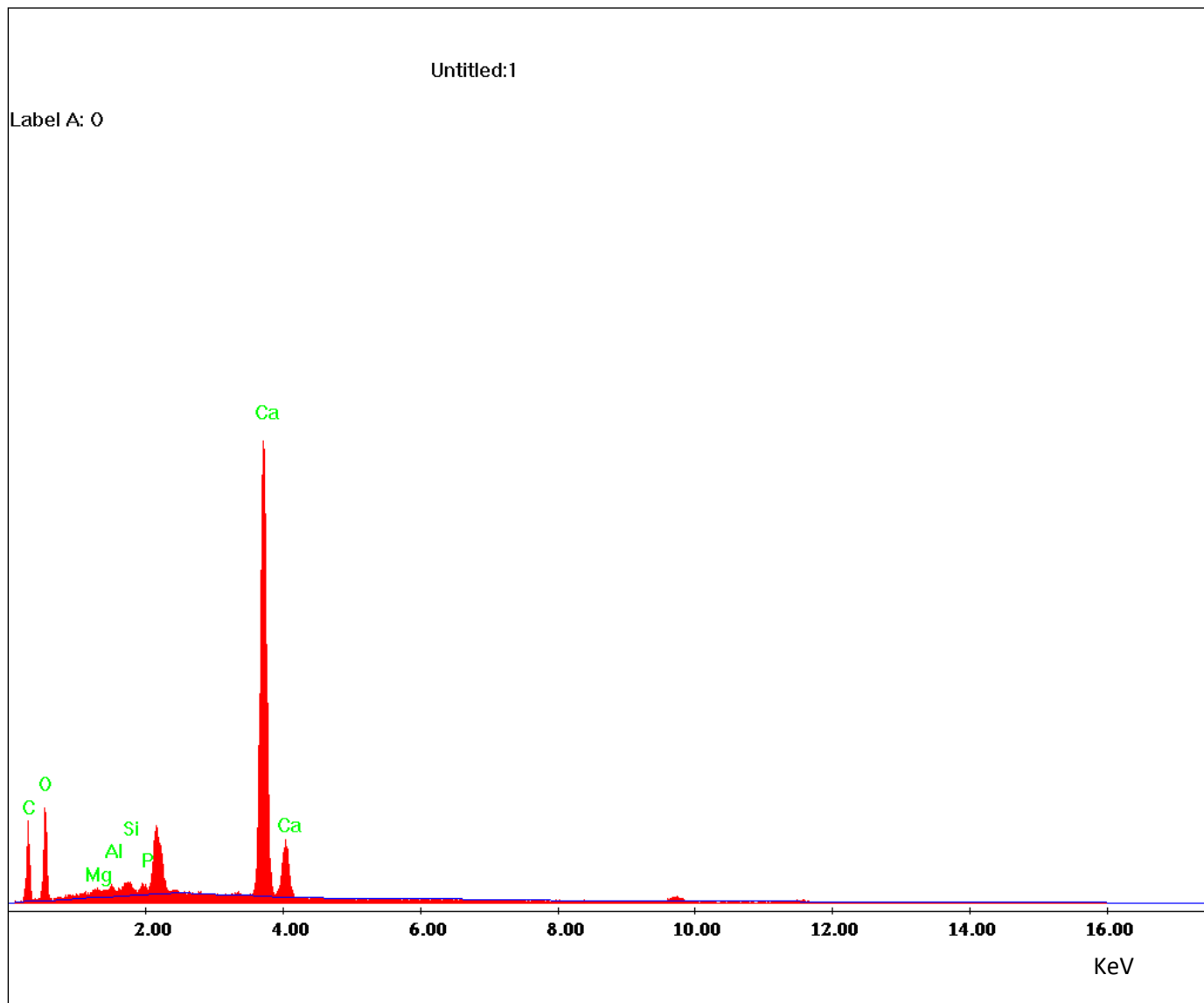
Graph 3. X-ray spectrum of particles for leaf 3.



Graph 4. X-ray spectrum of particles for leaf 4.



Graph 5. X-ray spectrum of particles for leaf 5.



Graph 6. X-ray spectrum of particles for the control sample.

neighboring Al- Rashadeyeh cement factory in south of Jordan, and as result decline and probably degrade wild plant species of the area.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Full Length Research Paper

Distribution and diversity of small mammals In Borena-Sayint National Park, South Wollo, Ethiopia: Implications of habitat specialization

Meseret Chane^{1*} and Solomon Yirga²

¹Department of Biology, College of Natural and Computational Sciences, Dilla University, Dilla, P.O Box 419, Ethiopia.

²Department of Biology, Faculty of Science, Addis Ababa University, Addis Ababa, P.O Box 1176, Ethiopia.

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The study on distribution and diversity of small mammals in Borena-Sayint National Park (BSNP), South Wollo Zone, Ethiopia was conducted from December, 2009 to April, 2010. Representative sample sites were taken from each habitat type and surveyed using random line transect method. Five species of rodents and two species of shrews were identified and recorded from BSNP. Small and large sized Sherman and snap traps were used to trap small mammals and morphometric measurement was taken for the species. From a total of 88 small mammals trapped and caught, *Lophuromys flavopunctatus* had the highest relative abundance (37.7%), followed by *Pelomys harringtoni* and *Otomys typus* with 17% each. *Stenocephalemys albipes*, *Arvicanthis dembeensis* and *Crocidura flavessens* had 12.5, 8 and 5.7% of abundance, respectively. *Crocidura fumosa* had the lowest relative abundance (2.3%).

Key words: Small mammals, diversity, distribution, relative abundance.

INTRODUCTION

Small mammals are categorized based on criteria such as body size and home range size. Those included in the small mammal category are species such as rodents (mice, rats and ground squirrels). Many of these species are difficult to observe in the wild because of their size, their habit of moving only at night or because they live under ground or in other hidden places (NLFC, 2005).

Small mammals are important components of biological diversity (Hashim and Mahgoub, 2007). They are known to have economical, ecological, social and cultural values (Afewerk Bekele and Leirs, 1997; Martin, 2003). They also play an important role in natural

communities and provide the main supply of live-food for many of the predatory mammals, birds and reptiles (Decher and Bahian, 1999; Granjon et al., 2002). They make up a significant percentage of the diet of a variety of carnivores (Ray, 1998; Jorge, 2008).

Small mammals consume invertebrates, vegetation, fruits and seeds, playing extremely important role as dispersal and pollination agents in different habitats. Thus, changes in their abundance and distribution can affect the dynamics of other species as well (Ray, 1998; Solari et al., 2002).

Small mammals are considered to be good

*Corresponding author. E-mail: meseret.chane2009@gmail.com.

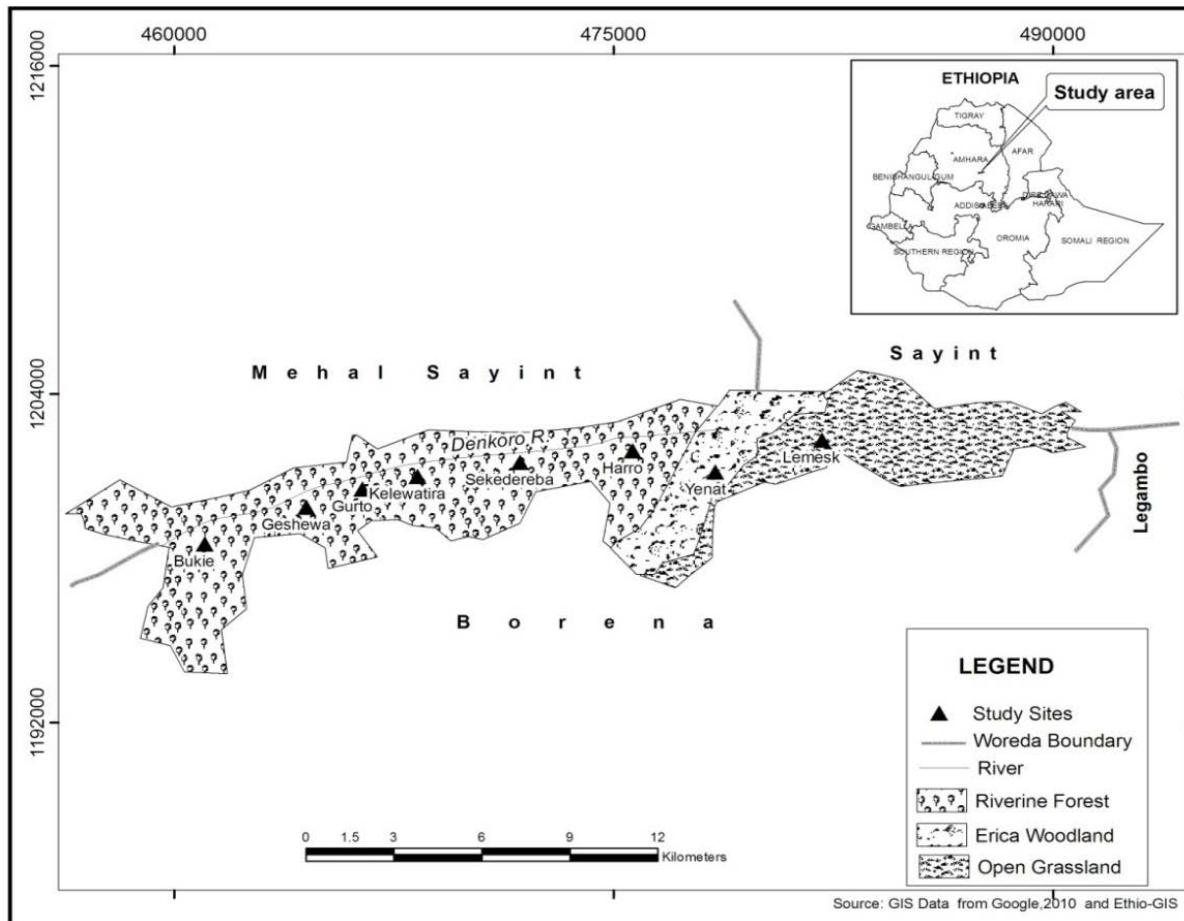


Figure 1. Location map of the study area, Borena-Sayint National Park, South Wollo, Ethiopia, 2009.

bio-indicators of habitats because of their short lifespan, rapid population dynamics and low level of pressure on their populations as a result of hunting in comparison with large mammals (shrews are never hunted because of the strong, unpleasant smell of their flank glands). They are also good bio-indicators because of the diversity, in tropical Africa, in terms of species and habitat preference (Barriere et al., 2006). Therefore, this study aims at describing the distribution and diversity of small mammals in BSNP.

MATERIALS AND METHODS

Geographic location

Borena-Sayint National Park is found in South Wollo Zone (Amhara Regional State) and lies between $10^{\circ}50'45.4''$ - $10^{\circ}53'58.3''$ latitude and $38^{\circ}40'28.4''$ $38^{\circ}54'49''$ longitude (Figure 1). The park is located in the north eastern part of Ethiopia about 600 km by road from Addis Ababa, 205 km from Dessie and 16 km from Mekane Selam, the capital of Borena Woreda. The park is situated among three Woredas namely Borena to the south, Sayint to the north and Mehal Sayint (a newly established Woreda) to the east. Borena Woreda on south (with its seven Kebeles) and southwest (with its two Kebeles), Sayint on the north (with one Kebele) and Mehal

Sayint on the north (with its two Kebeles) and on the west with one Kebele. Legambo Woreda is located bordering the two Woredas Borena and Sayint. The largest portion of the park is found in Borena Woreda.

Duration of the study

Small mammals' trapping was conducted from December 2009 to April 2010 in BSNP. During this period, random transect lines were established randomly and the locations were marked using Global Positioning System (GPS.) Study area was classified into three major vegetation zones based on vegetation types and altitude. A total of 40 days of fieldwork was done. Random study sites were taken to trap small mammals and both snap traps and live traps were used. Specimens were collected and identified.

Study area sub division

Preliminary study was conducted in the first field work. This showed that the study area was heterogeneous in vegetation type and topography and classified into three Vegetation Zones. These include Vegetation Zone 1/Riverine forest (RF), Vegetation Zone 2/Erica woodland (EWL) and Vegetation Zone 3/Open grassland (OGL). Classification of the study area was based on the map of Denkoro Chaka sketched by Park Development and Protection Authority in December, 2006. Each vegetation zone has distinguish-

ing features in vegetation type and topography. Censes zones were established in all three vegetations.

Small mammals trapping

Recent studies define small mammals as those of less than 200 g body mass, but the threshold is still debatable (Juokaitis and Baranauskas, 2001; Hashim and Mahgoub, 2007). Therefore, in this study, mammal with body weight less than 200 g was taken as small.

Both live and snap trapping were used in randomly selected transects at different habitats of the study area to represent all the vegetation types. The length of line transect varied from 400 to 500 m. A total of twenty small and large sized (13 x 13 x 38 and 7.5 x 9 x 22 cm) Sherman and five snap traps were placed 20 m apart along the transect. Traps were set for three consecutive days along transect of each habitat types so as to cover different habitat types.

Trapping was conducted from December 15, 2009 to January 5, 2010 during the first data collection period and from March 19, 2010 to April 9, 2010 during the second data collection period. The total trap nights during the survey period were 720.

Each trap was baited with peanut butter and covered with foliage and hays to camouflage and avoid excess heat during day time. This also protected the local people from being attracted by these shiny and glittering objects from far. The traps were checked twice a day, early in the morning hours (07:00-08:00) and late in the afternoon hours (17:00-18:00). The trapped specimens were removed from the trap and kept in polyethylene bags. Live trapped animals were weighted, sex identified, and released at the place of capture after being marked.

Snap trapped specimens were used for standard morphological measurement such as head to body length (HB), tail length (TL), hind leg length (HL), front leg length (FL) and bodyweight (W). Some specimens were skinned and dried and identified at species level in Zoological Natural History Museum (ZNHM) of Addis Ababa University, Ethiopia.

Data analysis

Species diversity of small mammals were calculated using the Shannon-Weaver index of diversity, $H' = -\sum P_i \ln P_i$ where P_i is the proportion of the i^{th} species in the habitat (Shannon and Weaver, 1949). H' is influenced both by number of species as well as by the evenness with which mammals are distributed with those species.

Equal H' values may thus be obtained if one habitat contains fewer and evenly distributed species of mammals. The evenness of mammalian species was calculated as $J = H'/H'_{\text{max}}$; where, $H'_{\text{max}} = \ln(s)$ and s is the number of species.

This measure varies between 1 (complete evenness) and 0 (complete unevenness). Chi-square (χ^2) was used to compare differences in abundance of mammal species between habitats and the overall significant difference in abundance of mammal species in the study area. SPSS computer Programme was used for Chi-square analysis to test the association of medium and large mammal species and their habitats (Flower and Cohen, 1990).

Simpson similarity index (SI) was also computed to assess the similarity among and between three habitats with reference to the composition of species.

$$SI = \frac{3C}{I+II+III}$$

Where: SI= Simpson's similarity index, C = the number of common species to all three habitats, I= the number of species in habitat one, II = the number of species in habitat two, III = the number of species in habitat three.

RESULTS

Distribution of small mammals and their relative abundance in different habitats

Seven species of small mammals, *Arvicanthis dembeensis* (Grass rat), *Lophuromys flavopunctatus* (Harsh furred rat), *Pelomys harringtoni*, *Stenocephalemys albipes* (White footed Stenocephalemys), *Otomys typus* (Typical veli rat), *Crocidura fumosa* (Smoky white toothed shrew) and *Crocidura flavescens* (Great red musk shrew) were trapped (Table 1). From a total of seven species recorded in the study area, open grassland contained six species, while erica woodland and riverine forest contained five and four species, respectively. A total of 88 individuals belonging to family Muridae (five species) and Soricidea (two species) were encountered. Of the seven species, *L. flavopunctatus* was with 33 individuals (a total of 37.7%). This was followed by *P. harringtoni* and *O. typus* with 17% each. *S. albipes*, *A. dembeensis* and *C. flavescens* had 12.5, 8 and 5.7%, respectively. *C. fumosa* had the lowest frequency (2.3%) as shown in Figure 2.

L. flavopunctatus and *O. typus* were the most widely dispersed species, occurring in all three habitat types but *L. flavopunctatus* had relatively high numbers as compared to other species in all habitat types. *A. dembeensis*, *P. harringtoni*, *S. albipes* and *C. flavescens* were present in two of the three habitats. *C. fumosa* was found only in riverine forest habitat.

Morphometric measurements of small mammals in the study area were taken (Table 2). The difference in abundance of small mammals is given in Table 3.

Diversity indices for small mammals

Diversity index (H') and evenness (J) of small mammals varied among different habitats (Table 4). The highest diversity index was recorded in open grassland habitat, but the highest evenness was recorded in *Erica* woodland habitat. Riverine forest had the lowest diversity index and evenness.

The Simpson similarity index (SI) for small mammals showed that the similarity of species composition of small mammals among three habitats of the study area was 0.4 (Table 5). This means that 40% of the species were common for all three habitats.

The overall difference in abundance of small mammals among the three habitats of the study area was significant at ($\chi^2=6.72$, $df=2$, $p<0.05$).

DISCUSSION

This study has recorded seven small mammal species. Five of them were rodents and two of them were insecti-

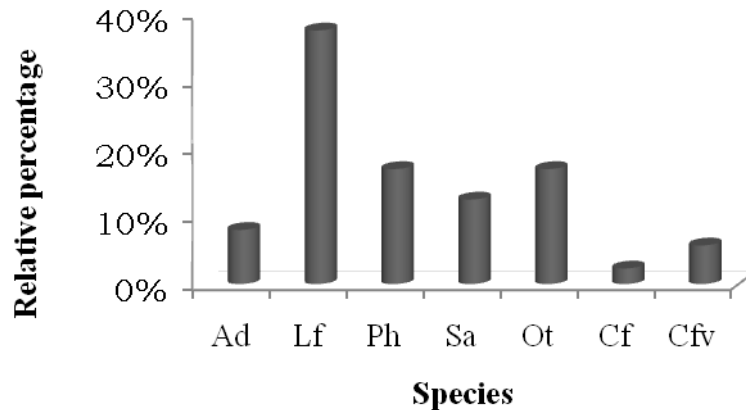


Figure 2. Relative abundance of small mammal species of the study area. Ad = *A.dembeensis*, Lf = *L. flavopunctatus*, Ph = *P. harringtoni*, Sa = *S. albipes*, Ot = *O. typus*, Cf = *C. fumosa*, Cfv = *C. flavescens*.

Table 1. Small mammals caught and their relative abundance (in brackets) at three habitats of the study area.

Species	Number of caught at each habitat type				Occurrence (%)
	RF	EWL	OPG	Total	
<i>Arvicanthis dembeensis</i>	3 (3.4)	-	4 (4.6)	7	8
<i>Lophuromys flavopunctatus</i>	9 (10.2)	12 (13.6)	12 (13.6)	33	37.5
<i>Pelomys harringtoni</i>	-	8 (9)	7 (8)	15	17
<i>Stenocephalemys albipes</i>	-	8 (9)	3 (3.4)	11	12.5
<i>Otomys typus</i>	4 (4.6)	5 (5.7)	6 (6.8)	15	17
<i>Crocidura fumosa</i>	2 (2.3)	-	-	2	2.3
<i>Crocidura flavescens</i>	-	3 (3.4)	2 (2.3)	5	5.7
Total	18 (20.4)	36 (41)	34 (38.6)	88	100

RF= Riverine forest, EWL = *Erica* woodland, OPG = open grassland; N.B. Blank (-) indicates the absence of species in the habitat.

Table 2. Morphometric measurements of small mammals in the study area.

Species	HBL (cm)	TL (cm)	HLL (cm)	FLL (cm)	BW (g)	Habit
<i>A. dembeensis</i>	8	10	3.8	2.9	78	Diurnal
<i>L. flavopunctatus</i>	9.5	5.5	4.5	3	48	Nocturnal/Diurnal
<i>P. harringtoni</i>	12	11.5	4.6	3	74	Nocturnal/Diurnal
<i>S. albipes</i>	12	11.5	4.9	3.2	85	Nocturnal
<i>O. typus</i>	8	17	4.6	2.5	64	Diurnal
<i>C. fumosa</i>	7	5	2.5	2.1	15	Nocturnal
<i>C. flavescens</i>	6	4.5	1.6	1.4	11	Nocturnal

HBL = Head body length, TL = tail length, HLL = hind leg length; FLL = front leg length, BW = body weight.

vores. This may not represent all the species present in the study area, but it gives update accounts of some of the small mammal species present in the study sites. The species composition and abundance in natural habitats

(ground water forest and riverine forest) were very poor. This might be due to the homogeneous vegetation that is dominated by few species of trees. In addition, the underground habitat is open or has less cover resulting

Table 3. Comparison abundance of small mammal between habitats of the study area.

Species	Habitats		
	RF vs. EWL	RF vs. OGL	EWL vs. OGL
<i>A.dembeensis</i>	3	0.14	4*
<i>L.flavopunctatus</i>	0.43	0.43	0
<i>P.harringtoni</i>	8***	7**	0.067
<i>S.albipes</i>	8***	3	2.27
<i>O. typus</i>	0.11	0.4	0.09
<i>C.fumosa</i>	2	2	2
<i>C.flavescens</i>	3	2	0.2

Figures in the table represent χ^2 values. RF= Riverine forest, EWL = Erica woodland, OGL = open grassland. *significance at $p<0.05$; **= $p<0.01$; ***= $p<0.005$; $df=1$.

Table 4. Diversity indices (H') and evenness (J) for small mammal species in different habitat types of the study area.

Habitat	Number of Species	Abundance	H'	J
Riverine forest	4	18	1.224	0.883
Erica woodland	5	36	1.515	0.942
Open grassland	6	34	1.633	0.911

Table 5. Simpsons' similarity index (SI) for small mammals caught among the three habitats. Formula for SI for three habitats, $SI = 3C/I+II+III$.

Species in habitat I	Species in habitat II	Species in habitat III	Species common to habitat I, II and III	Similarity index $SI=3C/I+II+III$
Ad	Lf	Ad	Lf	SI= 0.4
Lf	Ph	Lf	Ot	
Ot	Sa	Ph		
Cf	Ot	Sa		
	Cfv	Ot		
		Cfv		
$\Sigma S=4$	$\Sigma S=5$	$\Sigma S=6$	$\Sigma S=2$	

Habitat I = Riverine forest, Habitat II = Erica woodland, Habitat III = Open grassland. Ad = *Arvicanthis dembeensis*, Lf = *Lophuromys flavopunctatus*, Ph = *Pelomys harringtoni*, Ot = *Otomys typus*, Sa = *Stenocephalemys albipes*, Cf = *Crocidura fumosa*, Cfv = *Crocidura flavescens*.

in shortage of cover, food and diversity of microhabitats (Demeke et al., 2007). Likewise, in this study, the lowest composition and abundance of small mammals was recorded in riverine forest. On the other hand, high small mammal diversity was recorded in Erica woodland and open grassland. This might be due to the difference in vegetation cover, foliage and availability of food in these habitat types (Mgatha, 2002).

According to Morris (1987), distribution of small mammals over an area is not uniform and species are more

abundant in some habitats than others. This is due to the abundance and distribution of small mammals depending mainly on the nature and density of vegetation for food and shelter (Happold, 1974). In the present study area, the distribution of rodents and insectivores were not uniform. Some species were widely distributed and others were restricted only to two or one habitat. For instance, *L. flavopunctatus* and *O. typus* were recorded in all three habitats. *A. dembeensis* was recorded from riverine forest and open grassland, whereas *P.*

harringtoni, *S. albipes* and *C. flavessens* were recorded from *Erica* woodland and open grassland. On the other hand, *C. fumosa* was recorded only from riverine forest.

L. flavopunctatus is one of the most widespread and numerous rodents in the moister areas of East Africa, inhabiting a range of different habitats with a preference for montane habitats (Clausnitzer et al., 2003). Misonne (1969) stated that this species occurred from lowland forests at about 500 m asl to afro-alpine, reaching well above 4200 m and extending into ericaceous habitats and montane moorlands. Yalden (1988) reported that this species was also the most abundant, ranging from near the lower tree line at 1550 m, right up through the forested zones and on to the Afro-alpine moorland at 3900 m in the Bale Mountains. Likewise, this species was the most abundant and widely distributed in all three habitats of the present study area. It occurred in riverine forest, *Erica* woodland and open grassland habitats and accounted for 37.5% of the total catches.

P. harringtoni was recorded in *Erica* woodland and open grassland habitats at an altitude of more than 3200 m asl in the present study and this second most abundant species accounted for 17% of the total catches. Yalden et al. (1976a) indicated that this species was widespread on Ethiopian plateau, on both sides of the Rift Valley, at altitudes of 1800-2800 m asl. Serekebirhan (2006) also recorded this species from Wonji sugarcane plantation area and indicated that it was one of the Ethiopian endemic rodent species. This shift in habitat by the species in the present study area might be due to the scarcity of food and cover at lower altitude habitat in the present study area.

According to Delany and Happold (1979), *O. typus* had been recorded from different mountains in Kenya, Uganda, Zambia and Tanzania in addition to Ethiopia.

This species somewhat resembles *Dasymys incomtus* but differ in its shorter tail and agouti-brown fur. The species has a distribution of altitudinal range 1800-4000 m asl (Yalden et al., 1976a). In the present study, this species was recorded from riverine forest, *Erica* woodland and open grassland. It was the second most abundant species comprising 17% of the total catches.

A. dembeensis is common between sea level and 2200 m asl and considered to be a lowland species (Afework, 1996). Capula et al. (1997) reported that this species is the third endemic species of the genus in Ethiopia. Yalden et al. (1976a) also indicated that the species is widely distributed in Ethiopia including Awash National Park, Holeta, Akordat, Arbaminch, Koffole, Koka, Shore of Lake Zeway, Shore of Lake Tana and Awash River basin and feeds mainly on leaves, seeds and shoot of grass. This species is reported as agricultural pest in Ethiopia (Afework et al., 1993). Dawud (2008) also recorded this species from Mazie National Park. The present study showed that this species was recorded in riverine forest and open grassland and accounted for 8% of the total catches. The presence of this species in these

two habitats might be due to closeness to agricultural fields.

S. albipes is an endemic rodent of Ethiopia species inhabiting varied habitats from forest to scrubs in altitudes between 1500 - 3300 m asl (Afework and Corti, 1997). According to Yalden et al. (1976a), this species has been recorded from various parts of Ethiopia including Bahir-Dar, Lake Zeway, Alemaya, Debre Markos, Demebecha, Mendi, Nijabara, Goba, Kebré Mengist and Bako. However, in the present study, this species was recorded in *Erica* woodland and open grassland at an altitude of 3100 m asl and never in riverine forest. This might be due to the feeding behavior of the species as it feeds mainly on grass, leaves and animal matters. The species was the third most abundant and comprised 12.5% of the total number of rodent species in the present study area. *C. flavescens* is a very large shrew with flat brain case and likely to be confused with *Suncus murinus*. It is, however, a very variable species in both size and color, and there are some suggestions that both characters are influenced by altitude. This shrew is one of the most common and widespread in Ethiopia, where it ranges from approximately 1000-3000 m asl. It was recorded in different parts of Ethiopia including Addis Ababa, Chilalo Mountains, Debre Markos and west shore of Lake Tana. It was thought to be a typical forest species (Yalden et al., 1976a). Yalden (1988) also observed the species in Bale Mountains National Park, below the tree line, and in association with clearings and within the forest. However, in the present study, it was not trapped in the riverine forest. Instead, it was trapped in *Erica* woodland and open grassland habitats. This species was not abundant in the present study area. It was accounted for only 5.7% from the total catches.

C. fumosa is essentially a montane shrew with thick fur usually showing little contrast between the grey brown dorsum and silvery-grey venter. It has been recorded in Ethiopia at an altitude of 1750-3900 m and is also known from mountains in Kenya, Uganda and Malawi (Yalden et al., 1976a). This species was trapped only in the riverine habitats of the study area. The species was poorly distributed and the least abundant of small mammals species that comprised 2.3% of the total number.

Conclusion

The present study identifies and documented seven small mammalian species of BSNP and gave base line information about their presence. The distribution and abundance of small mammals' species in the park varied because of vegetation types and altitudinal differences.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Full Length Research Paper

Planning the priority protected areas of *Chosenia arbutifolia* due to climate change in Northeastern China

Jinghua Yu^{1*}, Jizhong Wan¹, Chunjing Wang¹, Shijie Han² and Qinggui Wang³

¹State Engineering Laboratory of Bio-Resource Eco-Utilization (Heilongjiang), Northeast Forestry University, Haerbin, Heilongjiang Province, China.

²Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang, Liaoning Province, China.

³College of agricultural Resource and Environment, Heilongjiang University, Haerbin, Heilongjiang Province, China.

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Chosenia arbutifolia was used as a case study for establishing a priority protected area (PPA) due to climate change in Northeastern China. A detailed investigation of 2884 plots in Northeastern China and model current and future potential suitable habitat distributions of *C. arbutifolia* was done to plan PPAs due to climate change. A general computational method was tested for the PPAs prediction using different classical models. First, the current and future suitable habitats of *C. arbutifolia* was estimated using species distribution model (Maxent). Second, PPAs was identified using the results of Maxent and systematic conservation planning software (zonation). In doing so, we were able to identify key protection areas. Our results suggest that PPAs may be able to integrate the current and future suitable habitats of *C. arbutifolia* into a single practical zone for sustainable development. Existing nature reserves in these areas were found to be much smaller than the PPAs we identified, suggesting the need for expansion of these zones. Finally, some recommended protection areas based on our PPAs were highlight, which can provide areas of both *in situ* and *ex situ* conservation for *C. arbutifolia*. Our results have broad implications for conservation biology and the redefinition of protective zones for at-risk species.

Key words: *Chosenia arbutifolia*, Northeastern China, Maxent, zonation, climate change.

INTRODUCTION

Protecting suitable habitats of endangered species, from environmental threats over large geographical spaces

and over long periods of time is a significant challenge for environmentalists (Langpap and Kerkvliet, 2012). Climate

*Corresponding author. E-mail: wan1276@126.com. Tel: 150040 +86 18686881276.

Abbreviations: PPA, Priority protected area; SDM, species distribution model; GIS, geographic information system; GCM, global climate model; ROC, receiver operating characteristic; AUC, the area under the ROC curve; GSPC, Global Strategy for Plant Conservation.

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Table 1. Environmental variables used.

Code	Environmental variables	Unit
Bio1	Annual mean temperature	°C
Bio4	Temperature seasonality	C of V
Bio5	Max. temperature of the warmest month	°C
Bio8	Mean temperature of the wettest quarter	°C
Bio12	Annual precipitation	mm
Bio15	Precipitation seasonality	C of V

Environmental variables were used as environmental layers for predicting the current and future species geographical distribution by Maxent simulation; C of V means coefficient of variation.

change has had a profound effect on species habitats, whereby species either migrate, adapt, or become extinction as the climate changes, all the while making endangered species harder to protect. Preserving species habitats in the face of climate change is both imperative and urgent (Muñoz et al., 2013).

The Northeastern China contains some of the richest forest resources in the earth's North Temperate Zone (Fang et al., 2013). *Chosenia arbutifolia* is a plant native to China, Korea, Russian and Japan. Due to loss and degradation of this habitat, the population of *C. arbutifolia* has declined severely, is listed as vulnerable by the International Union for Conservation of Nature and Natural Resources (IUCN; Slaght et al., 2013).

In this study, we first determined the current and future suitable habitat distributions of *C. arbutifolia* using species distribution model (SDM; Maxent; Phillips et al., 2006). Second, we generated maps of PPAs based on the results of Maxent using systematic conservation planning software (zonation) and geographic information system (GIS; Moilanen et al., 2011). Finally, some suggestions on application of PPAs were proposed. Overall, this study will help form the knowledge base needed to develop a conservation plan for *C. arbutifolia* concerning suitable habitats.

MATERIALS AND METHODS

Study area

The study area included the entire northeastern region of China, which is located in the northeast corner of mainland China. The region includes the Heilongjiang, Jilin and Liaoning provinces, along with parts of Inner Mongolia, covering a total area of 9.329 x 10⁵ km² (38°40' N 53°30' N, 115°05' E-35°02' E (Fang et al., 2013).

Species data and environmental variables

C. arbutifolia is a deciduous tree that belongs to the genus *Chosenia* and the family *Salicaceae*. This species is the main endangered tree found in northeast China. *In situ* investigations of species occurrence for both plants were conducted across Northeast

China. We used ArcGIS 9.2 as the meshing tool and divided the map of northeast China into a number of 25 x 25 km² grids. Each grid that was located in the mountains and forests of northeast China was systematically surveyed. Investigation plots (occurrence localities) of 30 x 30 m² were selected in each study grid, and 3-7 plots were established according to the vegetation condition of the survey area. When possible, plots were located in the centre region of the grids, but a plot's distance from the edge of the grid was never less than 15% of the side length of the grid. A GPS was used to determine plot location. Records of *C. arbutifolia* occurrence localities in each plot were quantified using the grid method, and the presence or absence of this species was recorded for 36 quadrats of 5 x 5 m² (Ohmann and Gregory, 2002). A total of 2884 plots were investigated across the northeast region of China from 2008 to 2012 and 30 occurrence localities of *C. arbutifolia* were recorded.

A 0.5 arc-minute (0.86 x 0.86 km² at the equator) was used for current and future data for the environmental layer input of SDM. Nineteen current bioclimatic variables, downloaded from the WorldClim database (www.worldclim.org), were modeled. We used the bioclimatic variables whose Pearson correlation coefficients with other variables were between 0.8 and -0.8. The influence of these variables was assessed on the current potential distribution of *C. arbutifolia* as coded in Table 1. The Special Report on Emission Scenarios (SRES) of the IPCC (Fourth Assessment) was considered to project changes in temperature and precipitation for various regions, simulated using the Global Climate Model (GCM). Future bioclimatic variables were assessed using HCCPR_HADCM3 analog data, downloaded from the International Centre for Tropical Agriculture (<http://ccafs-climate.org>), and used to predict how climate change would alter the occurrence of *C. arbutifolia* in the 2080s. We used the A2 and B2 emission scenarios for the future environmental layer input of SDM. A2 is different from B2 in which A2 has larger cumulative concentrations or emissions of carbon dioxide than B2. Thus, each scenario will cause different climate changes due to variant anthropogenic emissions of greenhouse gases and other pollutants. Therefore, A2 and B2 were used as high and low emission scenarios, respectively (Robinet et al., 2013). The conservation map of northeast China was obtained from the World Database on Protected Areas (<http://www.wdpa.org>).

Modeling suitable habitats of *C. arbutifolia*

We modeled the current and future potential habitat distributions of *C. arbutifolia* using Maxent (ver.3.3.3; <http://www.cs.princeton.edu/~schapire/maxent/>). For the potential habitat map cells reported by Maxent, cell values of 1 are considered the most suitable, whereas the closer a cell's value is to 0 the less suitable that cell is. For the occurrence of *C. arbutifolia*, 75% of observed localities were used for model training and 25% of them were used for model testing.

The auto features were used. Default settings were used for all other parameters. Receiver operating characteristic (ROC) curves regard each value of the prediction result as a possible judging threshold, and the corresponding sensitivity and specificity were then obtained by calculation. The precision of the model was evaluated calculating the area under the ROC Curve (AUC). We regarded probability values that were equal or greater than a threshold value of 0.5 to indicate the potential suitable habitat of a species (Phillips et al., 2006).

The determination of priority protection areas

First, we used zonation conservation planning software

(<http://cbig.it.helsinki.fi/software/>) to develop plans to protect *C. arbutifolia* due to the effects of climate change. Zonation is usually used as a spatial conservation framework to prioritize large-scale conservation projects that involve many species and model the map with the most meaningful proportions of the most valuable areas. The highest priorities for *C. arbutifolia* conservation were confirmed by identifying the top-ranking cells after computation. We minimized the geographic distance between the current and future suitable habitat distributions of *C. arbutifolia* and considered the influence of climate change on the future suitable habitats when selecting potential sites for reserves. The resulting maps were generated using current and future potential habitat distributions, assessed by the Maxent value for each pixel. With the original core-area cell removal rule, we set spatial priorities and calculated the marginal loss of each cell, which we could then use to determine if a conservation goal had been reached according to a given protection proportion of distributions for *C. arbutifolia* with the high priority ranking (Moilanen et al., 2011).

We set out to protect 75% of the ecological region (scores of Maxent ≥ 0.5) according to the target defined by the Global Strategy for Plant Conservation (GSPC; <http://www.cbd.int/gspc/>). For the practical significance, we therefore regarded the areas with suitable habitats (scores of Maxent ≥ 0.5) as the ecological region of this study under consideration and used 75% of the ecological region, namely, the given protection proportion of habitat distributions for *C. arbutifolia* with the high priority, as PPAs of *C. arbutifolia*.

RESULTS AND DISCUSSION

Our field surveys spanned four years from 2008 to 2012 and the *C. arbutifolia* populations were all rare and were mainly distributed in study areas consisting of the Changbai Mountains, Longgang Mountains, Xiaoxing'an Mountains, Daxing'an Mountains and Wanda Mountains. This information provided a means for predicting the suitable habitat associated with *C. arbutifolia*. The use of IPCC climate change scenarios provided plausible projections of the climate change that will occur by the year 2080 for the A2 and B2 scenarios (HCCPR_HADCM3).

First, we applied Maxent to model the current and future potential suitable habitats of *C. arbutifolia*, which were then mapped using ArcGIS 10.0. The model validation tests were highly reliable (AUC_{train and test} >0.9); the predicted suitable habitats was therefore considered the fit of Maxent is highly reliable (Townsend et al., 2007). Most of the current potential suitable habitats of *C. arbutifolia* examined were found in the eastern and northeastern of the study region while the future would move towards north, particularly B2. Thus, we know the current and future potential suitable habitats showed significantly different locations from each other, but there were also substantial regions of overlapping distributions (Figure 1).

Second, we found that PPAs were all distributed in the eastern and northeastern areas of the study region we examined. Several nature reserves with large PPAs are located in the northeastern parts of Northeastern China from Figure 2. Some nature reserves have small PPAs

because they may have different conservation targets, such as the protection of animals with small natural distributions or ranges unsuitable for *C. arbutifolia*; however, the conservation role of *C. arbutifolia* should not be ignored because they could cover a certain size of PPAs. Our objective was to examine 75% of current suitable habitats of *C. arbutifolia*, namely, PPAs, in order to minimize the current and future potential suitable habitat distribution. Thus, we can use these limited areas to protect species habitats as much as possible for protection strategies to realize sustainable development. Even taking all of this into account, it is important to remember that protection measures should not be taken hastily, and instead need to be steadfast and data-driven in order to realize short-term goals as well as long-term planning (Hubbell and Foster, 1992). For example, while some nature reserves, located in the eastern and northeastern areas of the region we examined were supported by PPAs and made great contributions to *C. arbutifolia* conservation, this was clearly not enough for comparison because *C. arbutifolia* are still at risk. It is noteworthy that Xingkaihu, Tiexi, Yueyahu, Sanjiang and Honghe could cover a large PPA to solve the establishment of suitable habitat conservation in the northeastern area. These nature reserves may be able to provide a reasonable protection zone, and scientists may be able to add conservation areas inside or outside them (Figure 2) (Dobson et al., 1997).

Third, we suggest the use of PPAs for the conservation of *C. arbutifolia*. We proposed a number of solutions, including both *in situ* and *ex situ* conservation, to address the problem of effective *C. arbutifolia* protection. In regards to *C. arbutifolia* we specifically examined, to increase the protective effort in each region, the creation of local protection zones for *C. arbutifolia* is ideal. Strategically, the conservation of the natural environment (*in situ* conservation) would be most effective (Hamilton, 1994). Even though *C. arbutifolia* cannot survive in some of these areas due to unsuitable habitats, it is important to allow them to shift from their natural habitats into alternative suitable growth environments for culturing; in other words, *ex situ* conservation (Cohen et al., 1991). Hence, PPAs would provide areas of both *in situ* and *ex situ* conservation for *C. arbutifolia* we identified in these regions. To retain *C. arbutifolia*, more conservation areas will need to be gradually established, according to the importance of PPAs and the cost of nature reserve development and subsequent conservation measures have a long way to go (Figure 2).

Conclusions

Climate change has the potential to severely threaten suitable habitats of *C. arbutifolia* in northeastern China. In this study, we used a combination of species distribution

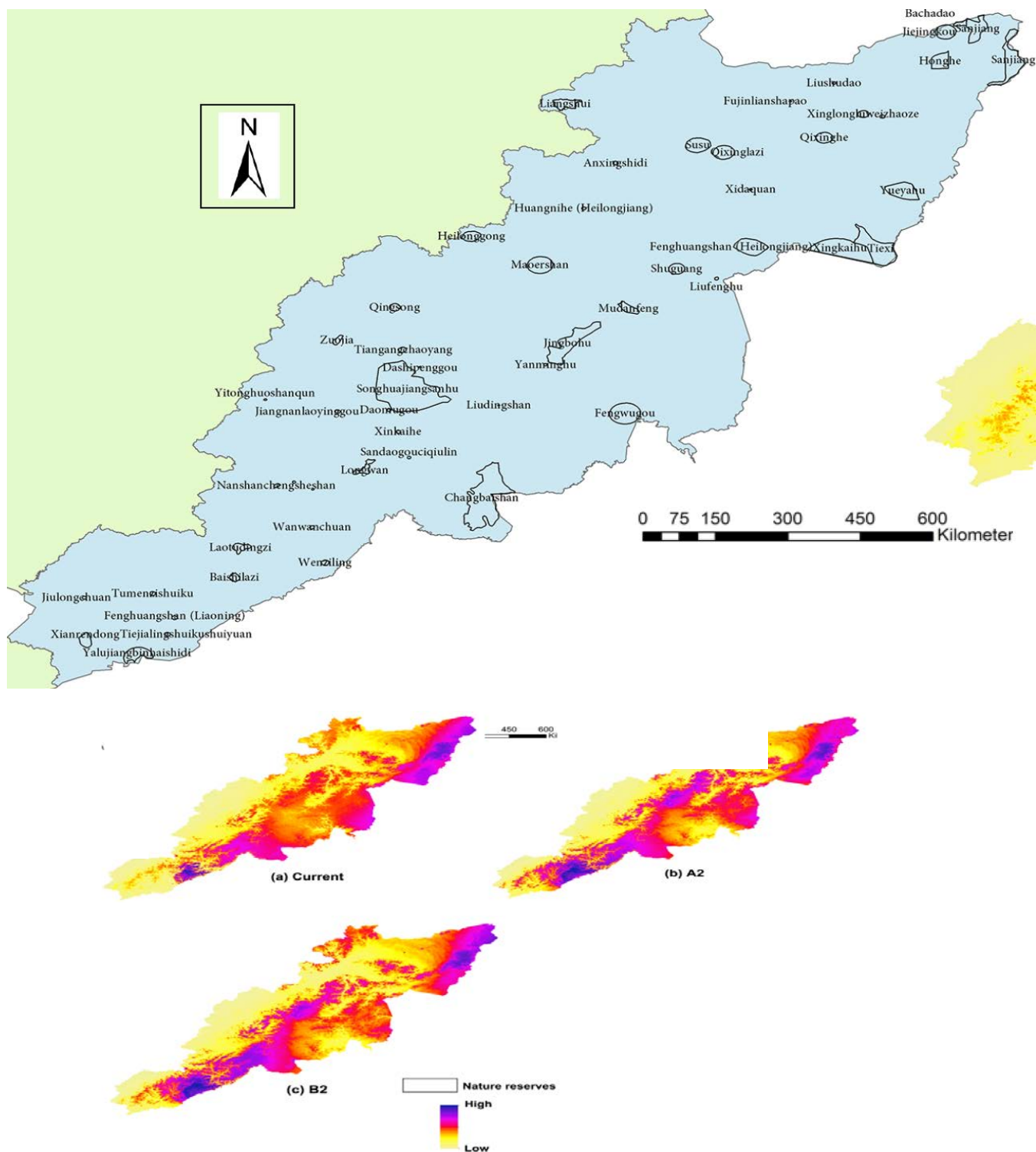


Figure 1. Current and future potential habitat distributions of *C. arbutifolia* through modeling. The color distribution from shallow to deep represents increasing habitat suitability of the species. **(a)** Current represented current potential habitat distribution of *C. arbutifolia*; **(b)** A2 represented future potential habitat distribution of *C. arbutifolia* in A2 emission scenarios; **(c)** B2 represented future potential habitat distribution of *C. arbutifolia* in B2 emission scenarios.

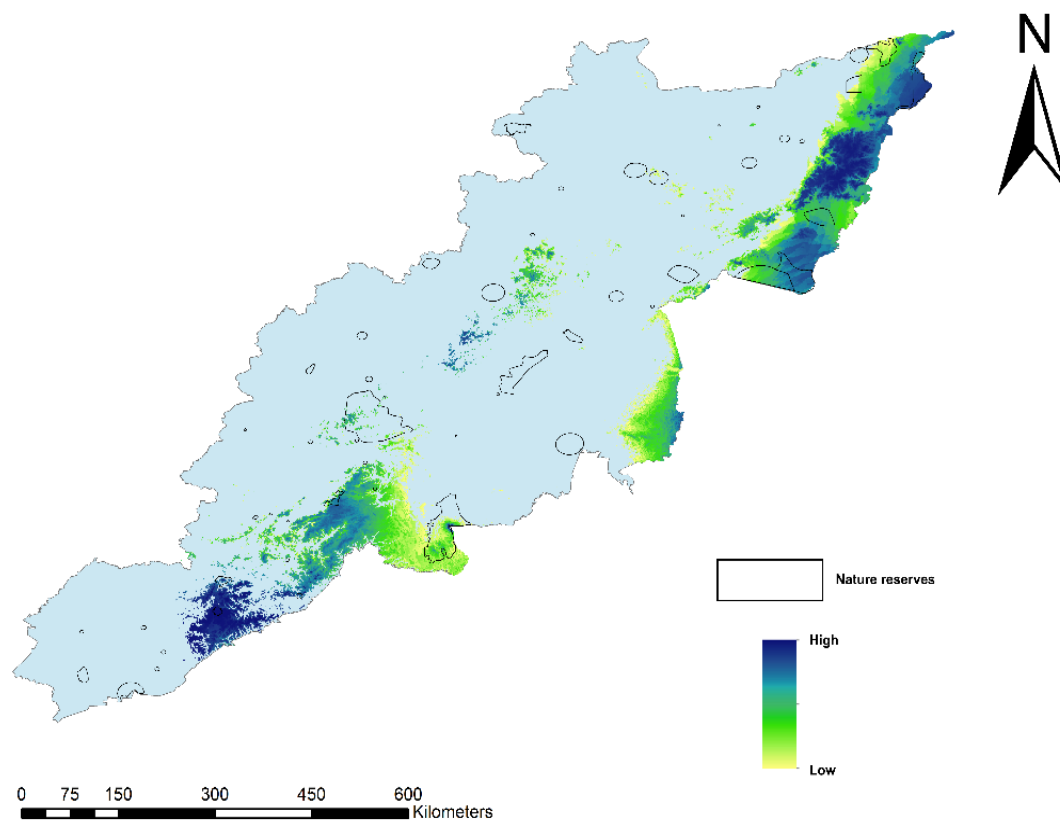


Figure 2. The maps of priority protection areas for *C. arbutifolia*. The color from shallow to deep represents increasing of modeled values of spatial conservation for evaluation of PPAs, namely, higher priority protected levels.

models (Maxent) and systematic conservation planning software (Zonation) to generate PPAs of *C. arbutifolia* in Northeastern China for the present day and in the future. The field surveys performed for this study spanned through 4 years and identified *C. arbutifolia* present in northeastern China. Current versus future potential suitable habitats of *C. arbutifolia* differed; hence, foresight is required when delineating environmental areas of protection and associated conservation actions. Therefore, due to the enormous difficulty of constructing viable reserves over a 100 year period, additional research on such as PPAs due to climate change is required to improve the current protection of *C. arbutifolia*. Based on our extensive findings, we proposed new suggestions for the protection and management of *C. arbutifolia*; application of this study will not only facilitate the sustainable development of *C. arbutifolia* populations through PPAs, but will at the same time reduce the economic costs of conservation by combining the PPAs of other endangered species. PPAs could provide areas of both *in situ* and *ex situ* conservation for *C. arbutifolia* in relation to climate change.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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