



RESEARCH ARTICLE

Hydrochemical Characterization of Some Stands of *Isoetes dixitei* in India

Brij Bhan Yadav⁽¹⁾, Sarvesh Kumar Singh^(2*), Nawal Kishor Dubey⁽²⁾, Santosh Kumar Shukla⁽³⁾ and Gopal Krishna Srivastava⁽¹⁾

1. Department of Botany, University of Allahabad, Allahabad-211002, UP., India.

2. Department of Botany, Banaras Hindu University, Varanasi-221005, UP., India.

3. Department of Botany, KNIPSS, Sultanpur-228118, UP., India.

* Corresponding author. Email: pteridologicaexpress@gmail.com

(Manuscript received 27 March 2014; accepted 18 April 2015)

ABSTRACT: In the present study soil, water and plant samples were collected from seven different natural populations of *Isoetes dixitei* which are found growing on the tablelands of Satara and Kolhapur districts of Maharashtra, India. The collected soil and water samples have been analysed for the colour, texture, available plant nutrients, electrical conductivity, pH and BOD. The soil and water factors along with biotic factors are compared with the number of plants per m², length of plants, number of leaves and roots per plant to understand the morphological diversity and biology within different populations of *I. dixitei*. The results of present analysis reveals that these ecological factors such as organic carbon, iron, phosphorus and potassium in soil and low pH of soil and water have positive relationships with the growth, robustness and diversity of *I. dixitei*. However, copper, zinc and electrical conductivity in the soil have negative significant role in influencing the plant characters. The role of manganese appears to be not significant. The biological factors play major role in controlling the diversity (number of plants per m²) of this taxon.

KEY WORDS: Biotic factor, *Isoetes dixitei*, Maharashtra, plant characters, soil, water.

INTRODUCTION

Lycopsid genus *Isoetes* L. is a cosmopolitan, aquatic, ligulate, heterosporous and consists of about 350 species in the world (Hickey *et al.*, 2003) and 16 species in India (Srivastava *et al.*, 1993; Shukla *et al.*, 2007). Four species of *Isoetes* have been described from Maharashtra state of India. *Isoetes sahyadriensis* Mahabale was the first species that was described by Mahabale (1938) from Panchgani tableland, Maharashtra. Afterwards, Shende (1945) has described *Isoetes dixitei* Shende from the same tableland. After a long gap of 48 years, Srivastava *et al.* (1993) described the third species *Isoetes panchganiensis* Srivastava, Pant and Shukla from the same locality. Subsequently, Shukla *et al.* (2005) described the fourth species *Isoetes divyadarshanii* P. K. Shukla, G. K. Srivast. & S. K. Shukla from Lonawala, Khandala. Later on, *I. dixitei* have been recorded from Amarad, Bhilar, Dandeghar, Khingar, Panhala Fort, Rajapuri, Wilson Point of Satara district, Maharashtra state and Bababuddangiri and Kemangundi of Chikmangalur district, Karnataka state of India (Srivastava *et al.*, 2001; Shukla *et al.*, 2002, 2007).

The impact of ecological factors in controlling the diversity and growth of plant communities are well known. The soil and water are the important sources of all the minerals for the plant growth and survival.

Braun (in Grenier and Godron, 1855) made important contribution towards the ecological study of *Isoetes*. He was the first man to emphasize the relationship of *Isoetes* to water and subdivided the genus into aquatic, amphibious and terrestrial forms. Dorris (1964) and Hall (1971) studied the influence of microhabitat on morphological and population variation of *Isoetes*. Later on, Rury (1987) analyzed the influence of microhabitats to understand the importance of polymorphism encountered within *Isoetes*. Hickey *et al.* (1989) have studied different populations aggregates of *Isoetes storkii* and *I. Isoetes melanopoda* complex growing at different altitude, in Costa Rica and endemic to the granite outcrops in USA respectively to understand the range of variations in morphological characters such as general plant length, leaf length, number of leaves, arrangement of leaf and corm shape etc. Pietsch (1991) made important contribution on the phyto-sociology and ecology of *Isoetes asiatica* Makino in the oligotrophic water bodies of south Sakhalin. Garrett and Kantvilas (1992) have studied the morphology, ecology and distribution of *Isoetes* in Tasmania. Wagai *et al.* (1992) have studied the soil in relation to distribution and growth performance of *I. dixitei* in the Western Ghat of India. They have correlated plant length with the different components of soils. Srivastava and Srivastava (2001) have made an attempt to correlate different soil factors with the



growth of four different species of *Isoetes*. Smolders *et al.* (2002) studied the interaction between the isoetids and the sediment. They are found that these interactions are important for the maintenance of the typical characteristics of isoetid dominated sites and disruptions may lead to serious threats to isoetid vegetation. Voge (2003) studied the environmentally related demography on *Isoetes lacustris* L. in Europe. She has observed that the variety of morphological and reproductive characteristics within a population suggests an individual life history performance according to plant vitality and environment. Wang *et al.* (2005) have described the ecology and conservation of endangered quillwort, i.e., *Isoetes sinensis* Palmer in China. Ctvrtlikova *et al.* (2012) have studied the effect of temperature on the phenology of germination of *I. echinospora* Durieu. They have observed that germination of spores is inhibited by low temperature and with increasing temperature microspores germinate a bit earlier and more successful than macrospores. Further, Abeli *et al.* (2012) have made a case study on *I. malinverniana* Ces. & De Not. using a predictive model for the preliminary site evaluation for the reintroduction and to limit expensive chemical analysis. They found that the conductivity of water and pH are the most effective predictors of the suitability of sites for the reintroduction of *Isoetes malinverniana*. Ctvrtlikova *et al.* (2013) have tracked the change in *Isoetes* reproductive ecology responding to changes in the lake water temperature and chemistry. Troia and Azzella (2013) reported a new species of *Isoetes sabatina* Troia & Azzella which are found to grow in the eutrophic and warm hard water, on the other hand, other European aquatic *Isoetes* species normally occur in oligotrophic and cold soft water. Recently, Abeli *et al.* (2014) have studied the hydrochemical characterization of threatened endemic quillwort (*I. malinverniana*) of northern Italy. They found that the growing habitat of this species are characterized by presence of oxidized sediment with a relatively low availability of phosphors, high pH, very low availability of carbon dioxide in the sediment pore and nitrate enrichment. They have also noted that increase in phosphorus availability in the water layers may cause serious threat for the conservation of this endemic species of *Isoetes* due to easily growth of epiphytic algae in the habitat.

Observations have shown that *I. dixitei* exhibit slight variations in a number of its morphological characters such as length of plants, number of leaves and roots per plant, size of sporangia, size and ornamentation of megaspores *etc.* Genetical or ecological factors may be the important factors which are responsible for such morphological variations in taxa. Jung *et al.* (2013) have studied the genetic diversity and population structure of six populations of

I. dixitei from Maharashtra, India. They have found that the plants of these six populations exhibit genetic diversity and assigned them into two groups. However, there are an inadequate knowledge and also difficulties in the interpreting some of these variations in the morphological characters exhibited by different populations of this taxa. Here we have selected seven populations of *I. dixitei* and aim to investigate the interactions and relationships amongst different components of soil, water, biotic factors and plant characters such as number of plants per m², length of plants, number of leaves per plant and number of roots per plant. This may be helpful in understanding the reason of its morphological variations and intimate association with their habitats.

MATERIALS AND METHODS

I. dixitei is an endemic aquatic quillwort of the Western ghat of India and growing in two habitats, i.e., aquatic emerged and aquatic submerged. The materials for the present study were collected from different localities of Satara and Kolhapur districts of Maharashtra state, India (Table 1). Habitat, land use and number of plants/m² were observed in the field. Fresh plant materials were fixed in FAA for observation in the laboratory. Herbarium vouchers were prepared from each locality and deposited in Department of Botany, University of Allahabad, UP., India and Botanical Survey of India, Central Circle Allahabad (BSA).

Soil (250 g) and water (500 ml) samples were collected every year in the same season from all the populations of *I. dixitei* from immediately around the plants. The data of habitat and land-use were based on observations of many years. However, those are of soil and water analyses were based on the samples collected from 2008 to 2010. Each year randomly 5 quadrates (1×1m²) per population were fixed to count the number of plants per m². To examine length of plants, number of leaves and roots per plant randomly 15 plants per population every year were selected from herbaria, as well as, fixed materials.

The soil samples collected from all these populations of *I. dixitei* were analysed for the following parameters: 1. Soil colour was compared with the Munsell colour chart and the values were recorded for each sample under both wet and dry conditions (Piper, 1950). 2. Soil texture for each sample were determined by the feel and rubbing. The texture class of known soil samples were taken for comparison of the present study and determined according to the International Pipette Method using STD (Piper, 1950). 3. Organic carbon (OC) of each soil sample was determined by digestion within K₂Cr₂O₇ solution, conc. H₂SO₄ and the titration was done with FeSO₄ solution as described by Jackson.

Table 1. Different localities of *I. dixitei* and textural classification of soil.

Localities	Altitude (m)	Soil colour	Soil texture		
			Soil textural class	Feel of moist soil	String formation from wet soil
Panchgani tableland, Satara, MH	1312	Brown	Clay	Smooth	No string formation
Dandeghar tableland, Satara, MH	1334	Very dusky red	Silty loam	Very smooth	Short string formation
Panhala Fort, Kolhapur, MH	1268	Dusky red gray	Clay	Slightly gritty	No string formation
Rajapuri tableland, Satara, MH	1276	Dark brown	Silty loam	Smooth	Tendency to string formation
Wilson Point tableland, Satara, MH	1432	Very dusky red	Silty loam	Smooth	Short string formation
Khingar tableland, Satara, MH	1290	Brownish red	Silty loam	Very Smooth	Short string formation
Amarad tableland, Satara, MH	1302	Dusky gray	Clay	Very Smooth	No string formation

(1973). 4. Copper, Iron, Manganese and Zinc of each soil sample was analysed in a single extraction with DTPA by using AAS and respective standard curve (Lindsay and Norvell, 1978). 5. Five grams soil from each soil samples and titration with the 0.002N H_2SO_4 to extract phosphorus. 6. Five grams soil from each soil sample was taken with 25 ml of neutral Ammonium Acetate for 5 min. and then filtered using filter paper. The concentration of Potassium was determined by using Flame photometer after necessary setting and calibration. 7. Five grams soil were taken from each samples to measure pH by using pH meter (1:2, soil: water) and 8. Electrical conductivity (EC) ($\mu S/cm$) of each soil sample was measured by using AC salt bridge and conductivity cell having electrodes coated with Platinum.

The water samples were collected from all these populations of *I. dixitei* which were analysed for both Hydrogen ion concentration (pH) and Biological oxygen demand (BOD). Water (20 ml) from each sample was taken to measure pH using pH meter. First of all, initial dissolved oxygen (DO) of the sample was taken by using Wrinkler's titration method (APHA, AWWA and WEF, 2005). After that the sample was kept in BOD incubator for 5 days at 20°C. This sample was again investigated to find final DO by same method. The BOD of the sample was calculated by using following formula:

$$BOD = DO_{Initial} - DO_{Final}$$

Where,

"BOD" is biological oxygen demand and
"DO" is dissolved oxygen

All experiment was repeated thrice and data are the mean $\pm$ standard deviation (SD) subjected to one way ANOVA. Mean were separated by Tukey's multiple range test when was significant ($p < 0.05$). The analysis of data was performed with SPSS 11 (2001). Correlations between variables were carried out using the non-parametric Spearman's rank method, because the variables were assumed to be not normally distributed and Spearman rank correlation is conservative.

RESULTS

The seven populations of *I. dixitei* from different localities including their altitudes, soil colour, soil classes and soil texture are presented in Table 1. Table 2 presents the comparative data on the ecological factors and plant characters (within studied year's) where as Table 3 shows habitats, soil and water analyses, number of plants per m^2 , length of plants, number of leaves and roots per plant of different populations of *I. dixitei*. Table 4 presents the significance level among habitat properties and between habitat and plant characters.

All these populations of *I. dixitei* are located within 30 km from Panchgani tableland except Panhala Fort, located about 437 km away. The altitudes of all these localities do not show much variation (1268-1432 m) from each other (Table 1). The plants of *I. dixitei* are found growing along the margins of permanent ponds, seasonal pools and small ditches. The plants are found growing as aquatic emerged or aquatic submerged. The lands of these localities are used for tourism, grazing and sewage discharge or remain unused (Table 3).

The soil colour is brown in Panchgani and Rajapuri tablelands, very dusky red in Dandeghar tableland and Wilson Point. It is dusky red gray in Panhala Fort, brownish red in Khingar and dusky gray in Amarad tablelands (Table 1).

Soil could be grouped into two classes viz., clay and silty loam (Table 1). Moist soils of Panchgani, Rajapuri tablelands and Wilson point feel to be smooth when it is rubbed between fingers whilst those of Dandeghar, Khingar and Amarad tablelands are found to be very smooth. The moist soil of Panhala fort feels to be slightly gritty. Very firm balls are formed from moist soils of all populations but it is slightly hard or hard or very hard on drying. Wet soils of all populations are stick to both fingers and short strings are formed or no strings formation or has tendency to string formation (Table 1).

Mineral contents, pH as well as electrical conductivity of the soil are found to be more or less uniform in each year's across all the populations (Table 2). Iron, manganese, organic carbon, phosphorous and potassium are found to be highest in Panchgani tableland population whereas they are lowest in Amarad tableland population. Similarly, pH and electrical conductivity are highest in Panchgani

**Table 2. Comparative analysis on the ecological factors between studied years of *I. dixitei* populations.**

		Analysis years		
		2008	2009	2010
Soil analysis	Organic carbon (%)	21.87±3.33 ^a	20.71±3.30 ^a	20.71±2.22 ^a
	Iron (kg/ha)	111.00±6.36 ^a	109.71±5.32 ^a	110.00±6.06 ^a
	Manganese (kg/ha)	87.15±6.15 ^a	85.57±6.93 ^a	85.43±5.77 ^a
	Potassium (kg/ha)	214.29±34.27 ^a	204.15±30.26 ^a	211.43±33.00 ^a
	Phosphorous (kg/ha)	84.29±10.21 ^a	86.86±9.25 ^a	85.29±8.48 ^a
	Zinc (kg/ha)	28.86±11.18 ^a	32.15±11.31 ^a	30.43±10.51 ^a
	Copper (kg/ha)	31.29±10.28 ^a	31.43±11.32 ^a	30.86±11.80 ^a
	pH	5.67±0.62 ^a	6.13±0.60 ^a	6.17±0.53 ^a
Water analysis	Electrical conductivity (μS/cm)	2.51±0.53 ^a	2.71±0.64 ^a	2.61±0.71 ^a
	pH	5.71±0.49 ^a	5.95±0.43 ^a	5.90±0.58 ^a
Plant characters	BOD (mg/l)	5.93±1.37 ^a	5.78±0.91 ^a	5.49±1.80 ^a
	No. of plants/m ²	24.43±13.88 ^a	29.29±14.71 ^a	26.15±13.35 ^a
	Length of plant (cm)	16.86±4.76 ^a	15.29±4.54 ^a	15.79±4.17 ^a
	No. of leaves/plant	15.71±3.20 ^a	16.86±2.92 ^a	16.00±4.44 ^a
	No. of roots/plant	57.43±4.99 ^a	52.15±11.94 ^a	49.00±8.39 ^a

Values are mean (n=7) ± SD of all studied populations. Means followed by the same letter within a row are not significantly different from each other according to Turkey's test (ANOVA) at 0.05 probability level.

Table 3. Comparative relationships between ecological factors and different populations of *I. dixitei*.

Habitat		Aquatic emerged				Aquatic submerged		
Population		Panchgani	Dandeghar	Panhala Fort	Rajapuri	Wilson Point	Khingar	Amarad
Land use		Frequently tourism	Not used	Tourism	Grazing, Sewage	Frequently tourism	Not used	Not used
Soil analysis	Organic carbon (%)	24.33±1.52 ^a	24.00±1.00 ^a	22.00±1.00 ^{ab}	21.67±1.15 ^{ab}	20.33±0.58 ^b	19.33±0.58 ^b	16.00±1.00 ^c
	Iron (kg/ha)	117.67±1.16 ^a	115.67±1.53 ^{ab}	113.67±1.53 ^{bc}	111.00±1.74 ^c	107.00±2.00 ^d	104.00±0.00 ^{de}	102.67±0.58 ^e
	Manganese (kg/ha)	84.67±3.52 ^{bc}	91.33±2.09 ^a	81.33±2.09 ^{cd}	90.00±1.00 ^{ab}	94.00±2.00 ^a	94.00±2.00 ^a	84.33±1.53 ^{bc}
	Potassium (kg/ha)	256.67±5.78 ^a	231.67±10.41 ^{ab}	221.67±10.41 ^{bc}	213.33±11.55 ^{bcd}	200.00±10.00 ^{cd}	188.67±6.11 ^d	157.67±8.63 ^e
	Phosphorous (kg/ha)	96.33±3.06 ^a	94.00±2.00 ^{ab}	91.33±1.53 ^{ab}	86.00±2.00 ^{bc}	82.33±2.52 ^{cd}	73.67±4.05 ^e	74.67±4.16 ^{de}
	Zinc (kg/ha)	18.67±0.58 ^d	21.67±1.53 ^{cd}	24.33±2.09 ^{cd}	26.67±2.52 ^{bc}	32.00±6.09 ^b	42.67±0.58 ^a	47.33±0.58 ^a
	Copper (kg/ha)	16.67±1.16 ^e	24.00±1.00 ^d	25.67±0.58 ^d	29.33±0.58 ^c	31.00±1.00 ^c	43.00±0.00 ^b	48.67±1.53 ^a
	pH	6.77±0.16 ^a	6.60±0.20 ^{ab}	5.87±0.48 ^{abc}	6.03±0.48 ^{abc}	5.80±0.44 ^{abc}	5.70±0.37 ^{bc}	5.17±0.26 ^c
	Electrical conductivity (μS/cm)	2.13±0.21 ^b	2.40±0.30 ^b	2.43±0.12 ^b	2.03±0.21 ^b	2.40±0.30 ^b	3.43±0.33 ^a	3.47±0.31 ^a
Water analysis	pH	6.47±0.21 ^a	6.30±0.10 ^{ab}	6.07±0.16 ^{abc}	5.67±0.26 ^c	5.87±0.16 ^{bc}	5.53±0.31 ^{cd}	5.07±0.12 ^d
	BOD (mg/l)	6.77±0.38 ^a	5.57±0.42 ^a	6.47±0.36 ^a	6.90±0.20 ^a	6.57±0.26 ^{ab}	4.27±0.59 ^{bc}	3.53±1.13 ^c
Plant characters	No. of plants/m ²	10.67±3.06 ^d	48.67±3.06 ^a	18.67±1.53 ^{cd}	20.00±2.00 ^c	16.33±2.09 ^{cd}	40.00±4.00 ^b	32.00±4.00 ^b
	Length of plants	24.13±1.21 ^a	18.13±1.21 ^b	17.03±1.05 ^b	15.83±0.77 ^{bc}	14.00±1.00 ^{dc}	12.03±0.06 ^{de}	10.67±0.77 ^e
	No. of leaves/plant	21.00±1.00 ^a	19.67±1.53 ^a	17.67±1.53 ^{ab}	15.67±1.53 ^{bc}	14.33±1.16 ^{bcd}	13.33±1.16 ^{cd}	11.67±1.53 ^d
	No. of roots/plant	64.00±8.00 ^a	58.00±5.29 ^a	53.67±5.86 ^{ab}	54.67±5.03 ^{ab}	53.00±2.65 ^{ab}	47.67±7.37 ^{ab}	39.00±8.19 ^b

Values are mean (n=3) ±SD. Means followed by the same letter within a row are not significantly different from each other according to Turkey's test (ANOVA) at 0.05 probability level.

tableland population and lowest in Amarad tableland population (Table 2, Fig. 1A,1B). However, copper and zinc contents of the soils are found to be highest in Amarad tableland population and lowest in Panchgani tableland population (Table 3, Fig. 1A).

Water pH of all populations is slightly variable in time (comparing different years) but it is more variable

in space (comparing different years). Highest water pH is recorded at Panchgani tableland whereas lowest at Amarad tableland (Table 3, Fig. 1B). Similarly, BOD of water in all populations is variable in each year and from each other. The highest BOD is recorded at Rajapuri tableland whereas the lowest BOD at Amarad tableland (Table 3, Fig. 1B).

**Table 4. Spearman's rank correlation analysis among habitat properties and between habitat properties and plant characters**

r	Organic carbon	Fe	Mn	K	P	Zn	Cu	Soil pH	EC	Water pH	BOD
Iron(Fe)	.948**										
Manganese(Mn)	.279 ^{ns}	.214 ^{ns}									
Potassium(K)	.924**	.962**	.198 ^{ns}								
Phosphorous(P)	.899**	.915**	.220 ^{ns}	.886**							
Zinc(Zn)	-.967**	-.970**	-.207 ^{ns}	-.970 ^{ns}	-.918**						
Copper (Cu)	-.923**	-.968**	-.165 ^{ns}	-.961**	-.935**	.966**					
Soil pH	.732**	.798**	.184 ^{ns}	.764**	.783**	-.746**	-.833**				
Electrical conductivity (EC)	-.625**	-.664**	-.479*	-.662**	-.579**	.673**	.634**	-.452*			
Water pH	.790**	.874**	.172 ^{ns}	.814**	.854**	-.837**	-.908**	.859**	-.483*		
BOD	.588**	.566**	.411 ^{ns}	.616**	.496*	-.597**	-.547*	.333 ^{ns}	-.739**	.344 ^{ns}	
No. of plants	-.235 ^{ns}	-.315 ^{ns}	-.116 ^{ns}	-.378 ^{ns}	-.289 ^{ns}	.369 ^{ns}	.328 ^{ns}	-.117 ^{ns}	.495*	-.229 ^{ns}	-.719**
Length of plants	.958**	.985**	.199 ^{ns}	.982**	.910**	-.991**	-.967**	.752**	-.673**	.832**	.619**
No. of leaves/plant	.857**	.915**	.142 ^{ns}	.837**	.871**	-.872**	-.927**	.808**	-.589**	.934**	.454*
No. of roots/plant	.883**	.781**	.282 ^{ns}	.795**	.689**	-.840**	-.746**	.552**	-.522*	.588**	.584**

ns = not significant; * $P < 0.05$; ** $P < 0.01$.

The number of plants/m², length of plants, number of leaves and roots per plant of all populations are variable in each year in every population. The highest number of plants per m² is counted in Dandeghar tableland, and Khingar and Amarad tablelands come next to it whereas the lowest number of plants per m² is in Panchgani tableland in each year. However, length of plants, number of leaves and roots per plant are measured the highest in Panchgani tableland whereas the lowest in Amarad tableland in each year (Table 3, Fig. 1C).

Correlation between habitat properties and plant characters, tested with Spearman's rank correlations (data not shown), revealed that all correlations between plant length, number of leaves and number of roots (on root side) and habitat characters (on other side) are always significant at $p = 0.01$ (only root/EC and leaves/BOD were significant at $p = 0.05$), with only exception regarding manganese that was always not-significant. The correlations regarding the character number of plant/m² was always not-significant (Table 4).

DISCUSSION

Soil, water, biotic factors and plants are the important components of an ecosystem in which they are closely related with each other and interact to bring circulation, transformation and accumulation of matter and energy. The diversity, luxuriance and dwarfness of taxa in any ecosystem is constant or variable from one habitat to other which may be affected by few or combination of many factors. The soil and water factors such as changes in the organic matter, available mineral nutrients, pH, BOD, water retention capacity and ions exchange capacity may influence the biological populations in any area. Biotic factors like grazing, sewage discharge, ore mining, constructions and tourism etc. also play an important role in the growth, diversity and succession of taxa in any population or

habitat. An examination of the biotic factors and physicochemical properties of soil and water supporting *I. dixitei* clearly showed variation in the growth and diversity amongst different populations.

In the present study we have found that the plants are growing as aquatic emerged and aquatic submerged conditions in clay or silty loam soils. Braun (in Grenier and Godron, 1855) has previously subdivided the genus in three ecological classes: aquatic, amphibious and terrestrial plants. Wild (1993) noted that "clay soil characterized by higher absolute particle surface, water holding capacity, availability of nutrients with pH being only moderately acidic". Etherington (1976) reported that clay was the main source of many plant nutrients and has certain exchange capacity. Our observations show that robustness, luxuriance and dwarfness of *I. dixitei* plants are somewhat equally affected by both clay and silty loam soil (Table 1, 3, 4).

The result of the present study indicates that whenever organic carbon, iron, potassium and phosphorous contents in the soil increase then length of plants, number of leaves per plant and number of roots per plant consequently show a marked increase. Whenever the amounts of these nutrients in the soil decrease the plants of *I. dixitei* consequently show a decrease in the above mentioned characters (Table 3, Fig. 1A,C). These correlations, as said above, are significant (Table 4). Wagai *et al.* (1997) have reported that a positive correlation exists between some elements (such as Fe and K) and plant length of *I. dixitei*. Srivastava and Srivastava (2001) have suggested a negative significant relationship between iron content of soil and plant length of *I. mahadevensis* Srivastava, Pant and Shukla. It appears that some nutrients are specific to the plant length, growth and robustness of specific taxa. Abeli *et al.* (2014) have noted a negative correlation between the availability of phosphorus in the water surface and the *I. malinverniana*, i.e., increase in phosphorus availability in the water layers may cause serious threat for the

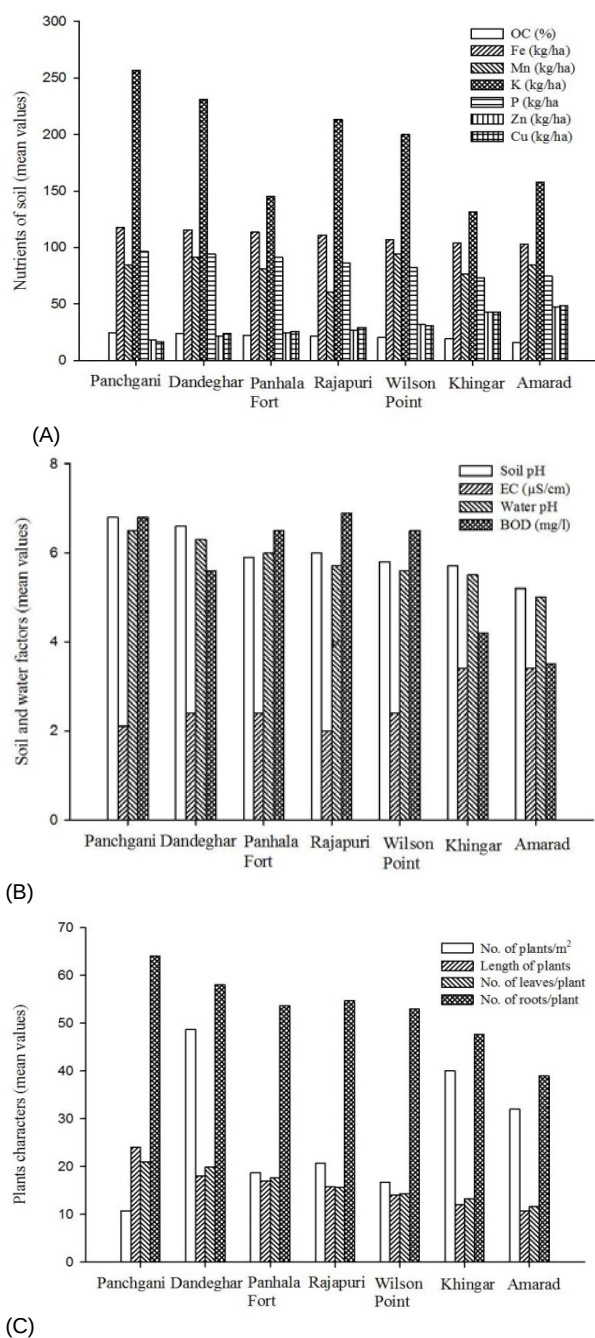


Fig. 1. Bar graph showing (A) nutrients contents of soil of different population of *I. dixitei*. (B) soil and water factors of different population of *I. dixitei*. (C) plant characters of different population of *I. dixitei*.

conservation of this endemic species of *Isoetes* due to easily growth of algae in the habitat.

The preceding analysis, however, showed that there is a certain sensitivity of plant characters such as plant length, number of leaves and roots per plant to the amount of copper and zinc and electrical conductivity of the soil. The results of present analysis indicate that

when the amounts of copper and zinc content and electrical conductivity of the soil are increased these plant characters are found to a marked decrease. If the amount of copper and zinc contents and electrical conductivity of the soil are decreased consequently there is an increase in the plant characters (Table 3, Fig. 1). Wagai *et al.* (1997) have observed a similar negative correlation between these elements (Cu and Zn) and plant length of *I. dixitei*. Srivastava and Srivastava (2001) have suggested a negative effect of copper to the plant length of different species of *Isoetes*, however, zinc play a positive role in increasing the plant length except *I. coromandelina* L. f. where it is insignificant.

The plants of *I. dixitei* are found growing in pH ranges 6.8-5.2 (soil) and 6.5-5.1 (water) in different localities (Table 3). The pH is an indication of hydrogen ion concentration and expresses the quantity of an acidic/alkali condition of soil and water dependent upon its dissociation and its total amount present in soil and water (Peech, 1941). The present investigation reveals that the lower scale of acidity of soil and water promoted the growth and robustness of *I. dixitei*. Wagai *et al.* (1997) and Srivastava and Srivastava (2001) have also observed a positive relationship between low acidity of soil and the plant length of *Isoetes* species. It clearly indicates that the populations of different species of *Isoetes* survive well in habitats which have low acidic pH value of surrounding environment.

The present investigation has also revealed that the amount of manganese contents in the soil is constant or variable in different populations. Its effect on the growth and robustness of *I. dixitei* plants appear to be not significant. However, Srivastava and Srivastava (2001) have documented a positive and significant correlation between heights of *I. panchganiensis* in relation to manganese content in the soil. But its role in the case of *I. coromandelina* is insignificant (Srivastava and Srivastava, 2001). It appears that the role of manganese is species specific.

Plants of any community/habitat are directly or indirectly affected due to the type of land-use by the biotic factors especially humans and animals. The highest BOD and low number of plants per m^2 were observed in Rajapuri tableland, Panchgani tableland, Wilson point and Panhala fort in comparison to Dandeghar, Khingar and Amarad tablelands populations. It clearly indicates that water and diversity (number of plant per m^2) of *I. dixitei* are affected by the land-use. (Table 3, 4). It may be due to the tourism, grazing and sewage discharge, consequently eutrophication of water body which in turn affects the germination of spores and fertilization. Thus the less number of plants are found in the population. Pietsch (1991) have studied the phyto-sociology and ecology of



I. asiatica and stated that the eutrophication of the water promotes the development of competitive, high-growing aquatic and bag plants which are rich in biomass. Roelofs (1996) have also discussed that plants that grow as aquatic emerged and submerged conditions are the mostly affected by human induced eutrophication. Smolders *et al.* (2002) described that isoetid species are well adapted to the oligotrophic condition and they are found flourish at the low environmental N, P and C availability. The present results show that humans have played a significant role in the recent decline of *I. dixitei* populations in the Western Ghats of India directly by destroying natural habitats and indirectly by introducing domestic herbivores.

In summary, the morphological diversity and biology of *I. dixitei*, as expressed by the length of plants, number of leaves and roots per plant and number of plants per m², is positively influenced by several factors such as high level of organic carbon, iron, phosphorus and potassium in the soil and low values of pH of soil and water. On the contrary, the retarded growth and frailness of *I. dixitei* plants may be attributed to the high levels of copper and zinc (and electrical conductivity) in soil. The results of the present analysis reveal that these ecological factors influence directly or indirectly the growth, robustness and diversity of *I. dixitei*. The BOD and biological factors may also be playing an important role in controlling the diversity of *I. dixitei*.

ACKNOWLEDGMENTS

We thank to Prof. D K Chauhan, Department of Botany, University of Allahabad, Allahabad and Head, Department of Botany, Banaras Hindu University, Varanasi for providing laboratory facilities. We are grateful to Dr. Angelo Toria, Department of Botany and ecology vegetable, University of Degly Studies, Palermo, Italy for reading and made some important change in the manuscript. Thanks also due to Akash Kedia, Department of Botany, Banaras Hindu University, Varanasi for the help in reading the statistical data and bar-graph preparation. We are thankful to the General Manager, Indian Farmers Fertilizers Company (IFFCO) and Dr. M Mohammad, Department of Chemistry, Jamuna Christian College, Allahabad for their help in analysis of soil and water samples respectively. The authors express their most sincere thanks to the anonymous reviewers for their critical remarks and suggestions, which are helpful in the improvement of the paper especially statistical parts of the manuscript. Corresponding author is thankful to University Grant Commission for providing D S Kothari Post Doctoral fellowship.

LITERATURE CITED

- Abeli, T., E. Barni, C. Siniscalco, C. Amosso and G. Rossi. 2012. A cost-effective model for preliminary site evaluation for the reintroduction of a threatened quillwort. *Aquatic Conserv: Mar. Freshw. Ecosyst.* **22**: 66-73.
- Abeli, T., S. Orsenigo, N. M. G. Ardenghi, E. C. H. E. T. Lucassen and A. J. P. Smolders. 2014. Hydrochemical characterization of a stands of the threatened endemic *Isoetes malinverniana*. *Am. Fern J.* **103**(4): 241-244.
- APHA, AWWA and WEF. 2005. Standard method for the examination of water and waste waters. American Public Health Association, Washington DC.
- Braun, A. 1855. *Isoetes*. In: Grenier M. and M. Godron (eds.), *Flora de France*. **3**(1) Bailliere, Paris.
- Ctvrtilikova, M., P. Znachor, J. Nedoma and J. Vrba. 2012. Effects of temperature on the phenology of germination of *Isoetes echionspora*. *Preslia* **84**: 141-153.
- Ctvrtilikova, M., P. Znachor, J. Nedoma, J. Vrba, J. Kopacek and J. Hejzlar. 2013. Tracking changes in *Isoetes* reproductive ecology responding to change in lake water temperature and chemistry. *Geophy. Res. Abst. EGU General Assembly, Vienna, Austria* doi: 15:EGU2013-9709.
- Dorris, B. J. 1964. Population variability and response to ionizing radiation in *Isoetes melanospora*. Thesis, Emory University, Georgia.
- Etherington, J. R. 1976. *Environment and plant ecology*. Jhon Willey and Sons Ltd, London.
- Garrett, M. and G. Kantvilas. 1992. Morphology, ecology and distribution of *Isoetes* L. in Tasmania. *Proc. Royal Soc. Tasmania* **126**: 115-122.
- Hall, J. B. 1971. Observations on *Isoetes* in Ghana. *Bot. J. Linn. Soc. London* **64**: 117-139.
- Hickey, R. J., C. Macluf and W. C. Taylor. 2003. A re-evaluation of *Isoetes savatieri* in Argentina and Chile. *Am. Fern J.* **93**: 126-136.
- Hickey, R. J., I. G. Sheldon and W. H. Ishbaugh. 1989. Evidences for post translational modification of triose phosphate isomerase (TPI) in *Isoetes*. *Am. J. Bot.* **76**: 215-221.
- Jackson, M. L. 1973. *Soil chemical analysis*. Prentice Hall of India Pvt. Ltd, New Delhi.
- Jung, J., S. K. Singh, H.C. Pande, G. K. Srivastava and H. K. Choi. 2014. Genetic diversity and population structure of Indian *Isoetes dixitei* Shende based on amplified fragment length polymorphisms and intron sequences of *LEAFY*. *Aquat. Bot.* **113**: 1-7.
- Lindsay, W. L. and W. A. Norvel. 1978. Department of DTPA test for zinc, iron, manganese and copper. *Soil Sci. Soc. Am. J.* **42**: 421-428.
- Mahabale, T. S. 1938. On a new species of *Isoetes* in India. *Curr. Sci.* **7**: 62-63.
- Peech, M. 1941. Availability of ions in light sandy soils as affected by soil reaction. *Soil Sci.* **51**: 473-481.
- Pietsch, W. 1991. On the phytosociology and ecology of *Isoetes asiatica* Makino in oligotrophic water bodies of south Sakhalin. *Vegetatio* **97**: 99-115.
- Piper, C. S. 1950. *Soil and plant analysis*. University of Adelaide, Adelaide.
- Roelofs, J. G. M. 1996. Restoration of eutrophid shallow softwater lakes based upon cabon and phosphorus limitation. *Netherland J. Aquat. Ecol.* **30**: 197-202.



- Rury, P. M.** 1978. A new and unique mat forming Merlins grass (*Isoetes*) from Georgia. *Am. Fern J.* **68**: 99–107.
- Shende, D. V.** 1945. A new species of *Isoetes* from the Bombay Presidency. *J. Univ. Bombay B* **14**: 50–52.
- Shukla, P. K., G. K. Srivastava and S. K. Shukla.** 2002. The quillworts (*Isoetes*) of India: distribution, endemism and species radiation. *Biodiv. Conserv.* **11**: 959–973.
- Shukla, P. K., G. K. Srivastava, S. K. Shukla and P. K. Rajagopal.** 2005. Two new species of the genus *Isoetes* L. (Isoetaceae: Lycopsidea) from India. *Taxon* **54**: 109–116.
- Shukla, P. K., G. K. Srivastava, S. K. Shukla and S. K. Singh.** 2007. Cyto-morphological based basal and sub-basal evolutionary relationship among *Isoetes* L. species with special reference to Indian taxa. *Proc. Natl. Acad. Sci. India Sect. B Biol. Sci.* **77**: 88–104.
- Smolders, A. J. P., E. C. H. E.T. Lucassen and J. G. M. Roelofs.** 2002. The isoetid environment: biogeochemistry and threats. *Aquat. Bot.* **73**: 325–350.
- Srivastava, G. K., D. D. Pant and P. K. Shukla.** 1993. The genus *Isoetes* L. in India. *Am. Fern J.* **83**: 105–119.
- Srivastava, G. K., M. Srivastava and S. O. Wagai.** 2001. Quillworts of sahyadris hills (India). *Indian Fern J.* **18**: 89–101.
- Srivastava, M. and G. K. Srivastava.** 2001. Edaphic diversity and growth of some Indian *Isoetes* L. species. *Taiwania* **46**: 145–157.
- Troia, A. and M. M. Azzella.** 2013. *Isoetes sabatina* (Isoetaceae, Lycopodiophyta), a new aquatic species from central Italy. *Plant Biosyst.* **147**: 1052–1058.
- Voge, M.** 2003. Environmentally related demography: Field studies on *Isoetes lacustris* L. (lycophyta, Isoetaceae) in Europe. In: Chandra, S. and M. Srivastava (eds.), *Pteridology in the new millennium*. Kluwer Academic Publisers, Neatherlands. pp. 233–260.
- Wagai, S. O., G. K. Srivastava and M. Srivastava.** 1997. Soil in relation to distribution and growth performance of *Isoetes dixitei* Shende in the western ghats of India. *Trop. Ecol.* **38**: 317–322.
- Wang, J. Y., R. W. Gituru and Q. F. Wang.** 2005. Ecology and conservation of the endangered quillwort *Isoetes sinensis* in China. *J. Natu. Hist.* **39**(48): 4069–4079.
- Wild, A.** 1993. *Soil and the environment- an introduction*. Cambridge University Press, Cambridge.