Determination of heavy metals and Morpho-anatomical characteristics of monocots Aristida mutabilis and Cenchrus ciliaris in dust polluted stone crushing industry of Sargodha, Pakistan

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Abstract

The current investigation was carried out to examine the population dynamics in the vegetation growing in the severe dust pollution caused by the stone crushing industry in Sargodha's Kirana Hills. The floristic composition of the area was completed and study sites were chosen through a thorough survey. Data about dust, soil, and vegetation were gathered from all study sites at all times of the year in order to investigate seasonal variations in the structure of the plant community and the factors causing these variations. All heavy metal concentrations were higher at extreme dust sites, especially in the winter, according to a heavy metal analysis of the dust. Based on soil analysis, different sites and seasons had different soil compositions. Herbs were collected from all research sites in all seasons to investigate seasonal fluctuations in morpho-anatomical, biochemical, and physiological features in the vegetation and heavy metal analysis. All plants' morpho-anatomical features were severely affected in extreme dust sites, particularly in the winter. However, all plants in these sites also showed high levels of sclerification in their leaves, roots, and stems, as well as the presence of large aerenchyma cells in their roots. These modifications help the plants survive in such a harsh and polluted environment. Extreme dust areas significantly reduced the physiological characteristics of all plants, especially during the winter.Reactive oxygen species (H2O2) production was elevated in extreme dust sites according to biochemical parameters. Additionally, enzymatic, non-enzymatic, and osmoprotective antioxidant activity was elevated at extreme dust sites, primarily during the winter. These modifications aid in the survival of local plants in the severely dust-polluted environment. Heavy metal concentration in all studied ecotypes increased at extreme dust areas, particularly in the winter; this may have been caused by phytoaccumulation.

INTRODUCTION

Heavy metals in soil affect plant growth [1]. In modern life, industrialization is growing quickly. When products are processed, industries discharge a variety of airborne particles and heavy metals into the environment that pose major risks to both plants and animals [2].

Presence or introduction of harmful substances in an environment is termed as environmental pollution. Environmental pollution mainly includes air pollution, water pollution and soil pollution [3]. Environmental pollutants affect plants and animals in one way or other. Water pollution decreases photosynthesis which ultimately leads to reduced growth of plants and may cause death of some plants [4]. Soil pollution particularly addition of heavy metals adversely effects the growth of plants [5]. Air pollutants mainly affect the leaves of vegetation leading to poor growth of plants [6]. Particulate matter affects the morpho-anatomical attributes and physiological processes of plants in a negative way [7]. The study was conducted on riparian vegetation in industrial contaminated area of Faisalabad and Most of the morpho-anatomical parameters notably attained a decrease in *E. alba* [8].

Dust pollution is a type of air pollution. Dust is fine, dry powder of minute particles of soil. The amount of dust received by a plant depends on its height, surface geometry, arrangement of leaves and leaf

external features such as cuticle, hairs etc.[9]. Most visible symptoms of damage caused by dust pollution to plants appear on their leaves e.g. chlorosis, necrosis etc. [10]. Dust severely affects plant diversity, cover and growth [11]. Dust blocks the stomata which affects the gaseous exchange of plants that may ultimately effects biochemical reactions i.e. photosynthesis and respiration, leaf morphological attributes may also be disturbed e.g. reduction in leave area take which weakens the plants and they may die and disturb the ecosystem [12, 13]. Affect of dust on plants also depend upon their dust capturing abilities mainly depending on their leaf morphology [14].

Dust carrying toxic substances badly affect all the plant attributes [15]. Dust also reduces the yield of plants [16]. Dust particles blown by wind may cause abrasive injury to leaves which lead to reduced photosynthesis and ultimately reduced plant growth [17]. Dust polluted soil may reduce the growth of vegetation [18]. Dust particles released from different sources e.g. stone crushers affect the stomatal aperture which lead to disturbed gaseous exchange between the leaf and the outer environment finally resulting in reduced photosynthesis and plant growth [19].

Dust disturbs the ecological attributes of vegetation which include density, frequency and ultimately leading to unbalanced ecosystem [20]. Dust storms cause physical injury to the plants in non-forest areas which may lead to destruction of vegetation finally resulting in ecosystem disturbance [21]. Dust deposition on plants lead to reduced leaf area and leaf thickness in majority of plants [22]. Changes in leaf shape and leaf color may also occur in plants growing in extremely dust polluted environment. Leaf area index (LAI) of forest plant species decreases due to deposition of dust the surface [23].

The vegetation cover and crop productivity may decrease due to radioactive interference of dust pollution with plant surface [24]. Stomatal index is reduced when plants are exposed to dust pollution which may prove fatal to the vegetation [25]. Leaf chlorophyll decreases under the abiotic stress caused by dust pollution leading to reduced photosynthesis and growth [26]. Leaf relative water content (LRWC) decreases in the plants growing under dust the pollution [27]. Hydrogen peroxide is a reactive oxygen species (ROS) which may produce as a result of stress caused by dust pollution and damage photosynthetic apparatus [28].

Altered anatomical characters of plant organs growing in dusty stressed conditions are considered as adaptive responses to habitat ecology of these species [29]. Plants often tolerate stresses by developing abilities to withstand them. When the stress crosses the normal tolerance level, plants gather variable quantities of organic osmolytes, osmoprotectants (Gycinebetaine, proline etc.) and antioxidant enzymes (superoxide dismutase, catalase etc.) which help the plants to tolerate stresses and help them in their acclimatization towards maintaining their growth and development [30]. Increase in leaf thickness under dust pollution is also an adaptation to dust pollution in various plants [31]. Plants show high sclerification to tolerate dust pollution [32]. Root aerenchyma formation shown by plant growing in dust polluted environment is also an anatomical modification to surive under dust pollution [33]. Anatomical structural modifications and heavy metals like, Zn, Pb, Cd, and Cu were recorded above the permissible limits of the WHO in riparian vegetation [34].

Formation of large parenchyma cells also helps the plant to cope with dust pollution [35]. Plants may tolerate side effects of dust pollution physiologically by increasing leaf relative water content [36]. Plants may increase activity of enzymatic antioxidants e.g peroxidase (POD), superoxide dismutase (SOD), catalase (CAT) and non-enzymatic antioxidants e.g. Ascorbic acid (AsA) along with production of osmoprotectants e.g proline, glycine beteine to cope with abiotic stress caused by dust pollution [27].

In Pakistan, where natural mountains provide abundant deposits of stone and other minerals, the stone crushing sector is crucial to the nation's economy. Stone is offered in various forms that can be utilized. Poor, illiterate, and unskilled people, primarily from rural areas, receive revenue from it. Education-based businesses like stone crushing offer jobs to skilled workers like engineers and geologists and generate income for business owners involved in the transportation, crushing, and mining of stone [37].

Literature available on this area only tells us about the minerals and variety of rocks exposed in the area [38, 39]. Yet no concentration is given to its vegetation and no information regarding the biological diversity of Kirana Hills is available on electronic or published media.

Therefore, present project was planned to explore the flora of the area, which gave knowledge about the plants present in the area which can be used in different ways to benefit humanity. Second purpose was to explore the morpho-anatomical, biochemical and physiological modifications along with heavy metal analysis in the commonly growing plants in the area, in order to investigate the affects and plant adaptations and their mechanism of survival under severe dust pollution caused by stone crushing industry of Kirana hills.

MATERIALS AND METHODS

Hills

For this purpose extensive survey of the area was conducted during which to study sites were selected. For exploring seasonal variations in the plant community structure and the factors responsible for these variations dust, soil and vegetation data was collect from all study sitesduring all seasons.

Selection of study sites

Based on extensive survey five ecologically diverse study sites were selected (Fig. 1). Site selection was based on differences in their environmental attributes, mainly dust pollution [40].

Site 1

It was situated at Sargodha bypass 18 km away from Sargodha City. It was totally dust free site with no stone crushers.

S**ite 2**: It was situated at Shaheenabad 12 km away from Sargodha City. It was low dust site because it was situated at some distance from active stone crushers.

Site 3

It was situated near Shaheenabad 15 km away from Sargodha City. It was extremely dust polluted site, surrounded by large number of active stone crushing units.

Site 4

It was situated near Pull 111 22 km away from Sargodha City. It was highly dust polluted site, due to high stone crushing activity.

Site 5

It was situated near 46 adda 27 km away from Sargodha City. It was moderately dust polluted site, because it was situated at some distance from stone crushers.

Sampling Seasons

Data for dust, soil and vegetation was recorded in triplicate from all the selected study sites throughout the year at regular interval of three months to include each season. The collection for each season was done in the following months:

Winter: January

Spring: April

Summer: July

Autumn: October

Analysis of soil

Soil samples at 30 cm depth were collected in triplicate from three quadrates from each study site. Following physiochemical characteristics of soil were analyzed (Table 1).

Table 1
Seasonal soil analysis of all study sites from Kirana Hills Sargodha

		October			
	Site 1	Site 2	Site 3	Site 4	Site 5
pH	8	7.85	7.75	7.75	7.75
EC (mS/cm)	3.81	2.135	1.515	1.56	1.575
O.M (%)	0.935	0.93	0.795	0.97	0.85
P (mg/kg)	7.55	7.95	7.85	7.45	7.2
K (mg/kg	202	194.5	196	190	185
Zn (mg/kg)	0.0721	0.0845	0.2563	0.2203	0.1699
Fe (mg/kg)	1.6443	0.0746	4.442	3.914	2.6743
Pb (mg/kg)	0.1607	0.1678	0.1777	0.1106	0.0753
Cd (mg/kg)	0.0265	0.0318	0.0085	0.0122	0.0417
Ni (mg/kg)	0.6312	0.3556	1.1349	0.8661	0.9993
		January			
	Site 1	Site 2	Site 3	Site 4	Site 5
pH	8.15	7.65	7.5	7.5	7.7
EC (mS/cm)	3.395	2.005	1.595	1.56	1.655
O.M (%)	0.94	0.92	0.765	0.96	0.855
P (mg/kg)	7.65	8	8	7.3	7.45
K (mg/kg	201	189.5	195.5	187.5	184.5
Zn (mg/kg)	0.0789	0.0932	0.2575	0.2257	0.1754
Fe (mg/kg)	1.7012	0.927	4.491	3.9875	2.7665
Pb (mg/kg)	0.1652	0.1695	0.1798	0.1127	0.0781
Cd (mg/kg)	0.0276	0.033	0.0097	0.0145	0.0439
Ni (mg/kg)	0.6347	0.3587	1.1373	0.8694	1.0043
		April			
	Site 1	Site 2	Site 3	Site 4	Site 5
pH	8	7.9	7.8	7.85	7.85

		October			
EC (mS/cm)	1.84	1.62	0.81	1.54	1.615
O.M (%)	0.79	0.725	0.715	0.795	0.725
P (mg/kg)	7.2	7	7.05	7.35	7.1
K (mg/kg	188	182	184	189	186
Zn (mg/kg)	0.0646	0.0802	0.2498	0.2167	0.1642
Fe (mg/kg)	1.5962	0.0658	4.543	3.744	2.589
Pb (mg/kg)	0.1579	0.1653	0.1741	0.1088	0.0734
Cd (mg/kg)	0.0241	0.0298	0.0073	0.0096	0.0386
Ni (mg/kg)	0.6042	0.3538	1.1325	0.8637	0.9964
		July			
	Site 1	Site 2	Site 3	Site 4	Site 5
pH	8.1	7.9	7.6	7.85	7.85
EC (mS/cm)	2.945	2.37	1.41	1.695	2.255
O.M (%)	0.95	0.865	0.725	1.005	0.935
P (mg/kg)	7	8.2	8	7.75	7.85
K (mg/kg	181	197	186	189	196
Zn (mg/kg)	0.0618	0.0742	0.2227	0.2004	0.1573
Fe (mg/kg)	1.5943	0.0604	4.2187	3.2761	2.1763
Pb (mg/kg)	0.1555	0.1634	0.1713	0.1063	0.0709
Cd (mg/kg)	0.0227	0.0284	0.0065	0.0089	0.0367
Ni (mg/kg)	0.585	0.3503	1.1293	0.8605	0.9932

pH

For this purpose, [41] method of pH determination was used. Dry soil was taken in a beaker to which distilled water was added. Than with help of pH meter readings of soil pH were recorded.

Electrical conductivity

The electrical conductivity was determined by using the methods of [41]. Dry soil was taken in a beaker to which distilled water was added. Suspension was than filtered. Finally filtrate was transferred to a

bottle and soil electrical conductivity was recorded with help of EC meter.

Organic matter

The organic matter was calculated by the standard method described by [42]. 1g soil was taken in a beaker to which 1*N* potassium dichromate solution and concentrated H_2SO_4 was added. Than distilled water and concentrated H_3PO_4 was added. Than 10–5 drops of diphenylamine indicator were added and it was titrated with 0.5*M* ferrous ammonium sulfate solution, until the color changed from violet blue to green. Finally organic matter was calculated.

Available phosphorus

The extractable Phosphorus was determined by Sodium bicarbonate procedure of [43]. 5g soil was taken in a flask and 0.5*M* $NaHCO_3$ solution was added to it. The suspension was filtered. Clear filtrate was added in a flask. The required acid to all the unknown solutions was added and diluted as described in detail by [41]. A standard curve was prepared. The absorbance of blank, standards, and samples on the Spectrophotometer at 882-nm wavelength was taken. The phosphorus concentration in the unknown samples was read from the calibration curve.

Available potassium

A flame photometer was used for the determination of extractable potassium by method described by [41]. 10 g dry soil was taken in a flask. 1*N* NH_4O solution was added in 1:5 ratios. The suspension was then filtered. The available potassium in the samples was measured by taking the emission readings on the Flame photometer at 767-nm wavelength. The potassium concentrations were calculated according to the calibration curve.

Determination of heavy metals in soil

Soil samples were analyzed for heavy metals like zinc (Zn), iron (Fe), lead (Pb), cadmium (Cd) and nickel (Ni). Samples were collected in triplicate from each site. The collected soil samples were then digested by Tri-Acid digestion method as described by [44]. 1 g dry sample was added into digestion flask. 2 ml concentrated HClO4 and 12ml concentrated HF was added. The remaining residue was dissolved in 8ml concentrated HCl and then 20 ml distilled water was added. The volume was brought to 100ml, and stored in polyethylene bottle. Heavy metals were determined by Atomic Absorption Spectrophotometer.

HM ($mgkg^{-1}$) = $mgkg^{-1}$ HM (from calibration curve) * V/Wt

Where HM = Heavy Metals

V = Total volume of the soil extract (ml)

Wt = Weight of air dry soil (g)

Analysis of dust

Measurement of dust

To determine the effects of dust on plants, concentration of dust was measured in each season. For measurement of dust glass jars were placed at all five selected study sites and were checked at regular intervals because glass jars cannot be left unattended for too long. Dust fall was measured on weekly basis, ten times in a season (Table 2). The total dust fall was expressed as g/m^2 by using the following formula [45]:

Table 2
Seasonal dust analysis of all study sites from Kirana Hills dust studied sites

		October			
	Site 1	Site 2	Site 3	Site 4	Site 5
Dust (g/m^2)	1035	2642	9142	7785	4678
Zn (mg/kg)	0.2635	0.2555	0.0387	0.2404	0.2502
Fe (mg/kg)	1.4325	1.0907	0.0843	0.9267	1.0173
Pb (mg/kg)	0.0224	0.0912	0.1567	0.1142	0.1246
Cd (mg/kg)	0.0314	0.0185	0.0346	0.0037	0.0279
Ni (mg/kg)	0.2212	0.8007	0.8303	0.9281	0.7646
		January			
	Site 1	Site 2	Site 3	Site 4	Site 5
Dust (g/m^2)	1285	3107	10071	8535	5500
Zn (mg/kg)	0.2676	0.2579	0.0407	0.2426	0.2537
Fe (mg/kg)	1.436	1.0942	0.0867	0.9294	1.0115
Pb (mg/kg)	0.0246	0.0935	0.1596	0.1171	0.1273
Cd (mg/kg)	0.0336	0.0206	0.0375	0.0089	0.0308
Ni (mg/kg)	0.251	0.8036	0.834	0.9315	0.7695
		April			
	Site 1	Site 2	Site 3	Site 4	Site 5
Dust (g/m^2)	607	1571	6892	5535	3428
Zn (mg/kg)	0.2617	0.2532	0.0349	0.2393	0.2481
Fe (mg/kg)	1.4302	1.0889	0.0824	0.9238	1.0145
Pb (mg/kg)	0.0206	0.0887	0.1539	0.1107	0.1213
Cd (mg/kg)	0.0297	0.0161	0.0319	0.0013	0.0258
Ni (mg/kg)	0.2176	0.7984	0.8273	0.9232	0.7609
		July			
	Site 1	Site 2	Site 3	Site 4	Site 5

	October				
Dust (g/m^2)	750	1892	8107	6750	4142
Zn (mg/kg)	0.2606	0.2518	0.0331	0.2378	0.2466
Fe (mg/kg)	1.4272	1.0865	0.0801	0.9216	1.0123
Pb (mg/kg)	0.0177	0.0866	0.1516	0.1083	0.1181
Cd (mg/kg)	0.0276	0.0138	0.0301	0.0089	0.0227
Ni (mg/kg)	0.2143	0.7959	0.8265	0.9218	0.7585

Dust fall per week (gm^2) = Weight (g) of 7 days dust fall in glass jar/Area of glass jar (m^2)

Total dust fall per season (gm^2) = Sum of dust fall in each week of the season in glass jar/Area of glass jar (m^2)

Determination of Heavy metals in dust

Dust samples were analyzed for heavy metals like zinc (Zn), iron (Fe), lead (Pb), cadmium (Cd) and nickel (Ni) (Table 2). Dust samples were collected in triplicate in glass jars from each site. The collected dust samples were then digested by Tri-Acid digestion method of [44] and heavy metals were determined by Atomic Absorption Spectrophotometer as described above for soil heavy metal analysis.

Exploration of morpho-anatomical modifications in common plants of Kirana Hills to identify the adaptation to such harsh dust polluted environment of the area

Selection of plants for study

Two easily available commonly growing monocots present on all study sites throughout the year were selected for the current study. Two monocots (*Aristida mutabilis* and *Cenchrus ciliaris*) were collected in triplicate from all study sites in all the seasons (Fig. 1). Morpho-anatomical attributes of these plants were analyzed for comparative study. Affect of dust pollution on morpho-anatomical attributes of selected plants was analyzed and their mechanism of their survival was explored.

Anatomical Parameters

Anatomical study of stem and root was done by taking triplicate samples. For stem anatomical study, 1cm piece from central node of largest stem was taken and for root anatomical study one cm base of the thickest root was taken. All samples were preserved in 70% ethyl alcohol solution. Then free hand section cutting and staining was done by using the method of [46]. After staining sections were treated with xylene solution to remove the debris and placed on the slides where Canada balsam was already pasted. Finally the sections were covered with cover slip and viewed under compound microscope and photography was done.

Exploration of physiological and biochemical modifications along with heavy metal analysis of common plants at Kirana Hills to identify their adaptation to such a harsh dust polluted environment of the area

Same two species selected for morpho-anatomical analysis were also analyzed for physiological and biochemical attributes to investigate the effects of dust pollution on them and to identify their mechanism of their survival adaptations in harsh and dust polluted environment of the area. Samples were taken as triplicate.

Physiological Parameters

Biochemical Parameters

Peroxidase (POD) activity (Enzymatic antioxidant)

Enzyme extraction:

Fresh leaf sample (0.5g) was grinded in 5ml of 50m*M* cooled potassium phosphate buffer (pH 7.8). Grinded material was then filtered. Than homogenate was centrifuged at 15000g for 20 minutes at 4°C and supernatant was used for the enzyme assay.

Peroxidase (POD) activity:

[47] guaiacol oxidation method was used to for this purpose. The final volume of the reaction mixture for POD (3ml) contained 50m*M* phosphate buffer (pH 7.0), 20m*M* guaiacol, 40m*M* H_2O_2, and 0.1ml enzyme extract. Changes in absorbance of the reaction solution at 470 nm were read with the interval of 20s. One unit POD activity was defined as the change of 0.01 absorbance unit per min per mg of protein.

Leaf ascorbic acid (AsA) contents [Non enzymatic antioxidant]

It was estimated by the method of [48]. For this purpose fresh leaf material (0.25g) was homogenized in 10ml solution of 6% TCA. 4ml of the extract was used further to which 2ml of 2% dinitrophenyl hydrazine solution (in acidic medium) was added. Then one drop of 10% thiourea solution (prepared in 70% ethanol) was added. In water bath the reaction mixture was boiled for 20 minutes then it was cooled at room temperature. Afterwards 5 ml of 80% H_2SO_4 (v/v) was added to the reaction mixture at 0°C. The absorbance was read at 530nm. The quantity of AsA content in the extracted leaf samples was determined from a standard curve which was prepared by use of different AsA standards.

Hydrogen peroxide (H_2O_2) concentration [Reactive oxygen species (ROS)]

Hydrogen peroxide content was determined by following the method of [49]. Leaf samples were homogenized in ice bath with 1ml of 0.1% (w/v) trichloroacetic acid (TCA). Then homogenate was

centrifuged at 12000*g* for 15 minutes. 0.1ml of the supernatant was then treated with 0.1ml of 10mM potassium phosphate buffer (pH 7.0) and 1M potassium iodide. Absorbance of mixture was read at 390nm using spectrophotometer. H_2O_2 content was calculated by comparing with a standard calibration curve prepared by using different concentrations of H_2O_2.

Free Proline Determination (Osmoprotectant)

Method of [50] was adopted for free proline estimation. For this purpose fresh leaf sample (0.5g) was homogenized in 10ml of 3% sulfo-salicylic acid. Homogenate was then filtered. 2ml of the filtrate was then mixed with 2ml acid ninhydrin (1.25g ninhydrin in 30ml glacial acetic acid), 20ml of 6*M*orthophosphoric acid) and 2ml of glacial acetic acid in a test tube. The material was incubated at 100^{o}C for 1 hour and then cooled in an ice bath. Then 4ml of toluene was added to the solution and mixed strongly. Finally the mixture was warmed at room temperature. Absorbance was read at 520nm using spectrophotometer. Toluene was used as blank. Free proline concentration was determined from a standard curve and calculated on fresh weight basis by following formula:

µmole proline g^{-1} fresh weight = (µg proline ml^{-1} x ml of toluene/115.5)/(g of sample)

Determination of Heavy Metals in Root and Shoot

The root and shoot samples were analyzed for determination of heavy metals like i.e (Zn), Iron (Fe), Lead (Pb), Cadmium (Cd) and Nickel (Ni) by following the method of [41]. First 0.5g grounded plant material was taken in a digestion flask. 5ml concentrated HNO3 was added to it and swirled carefully. A funnel was placed on the digestion flask and left for about 6–8 hours. After pre-digestion, 10ml di-acid mixture was added and swirled carefully. Now the digestion flask was placed on hot plate and thetemperature was slowly increased about 180–200°C until the dense white fumes were evolved and transparent white content was left. Than digestion flasks was cooled down at room temperature. The content was then filtered. Volume was brought to 50ml. The concentration of heavy metals was determined on Atomic Absorption Spectrophotometer.

Trace elements (ppm) = ppm trace element (from calibration curve) * V/Wt

Where:

V = Total volume of the plant digest (ml)

Wt = Weight of dry plant (g)

Statistical analysis

The data obtained after dust and soil analysis along with ecological, morpho-anatomical, physiological, biochemical and heavy metal analysis of vegetation was subjected to Canonical Correspondence Analysis (CCA) using conoco software version 4.5. Moreover the data was also subject to two-way ANOVA using MS Excel data analysis tool.

RESULTS

Bar graphs showing morpho-anatomical attributes of *Aristida mutabilis*

Bar graphs showing stem morpho-anatomical attributes of *Aristida mutabilis*highlighted at spatial scale all stem parameters (stem epidermal cell area, stem metaxylem cell area, stem parenchyma cell area, stem radius and vascular bundle thickness) decreased at extremely dust polluted Site 3 except stem sclerenchyma thickness which highly increased at Site 3. All other stem anatomical parameters highly increased at dust free Site 1 except stem sclerenchyma thickness which highly decreased at Site 1. At temporal scale, content of all other stem anatomical parameters decreased during peak winter season in January except stem sclerenchyma thickness which highly increased in January. All other stem anatomical parameters highly increased during spring season in April except stem sclerenchyma thickness which highly decreased in April (Fig. 2).

Bar graphs showing root morpho-anatomical attributes of*Aristida mutabilis*highlighted at spatial scale all root anatomical parameters (root radius, root epidermal cell area, root metaxylem cell area, root endodermis thickness, root parenchyma cell area, root radius and root vascular bundle thickness)decreased at extremely dust polluted Site 3 except root aerenchyma area and root sclerenchyma thickness which highly increased at Site 3. Values of all other root anatomical parameters were recorded highest at dust free Site 1 except root aerenchyma area and root sclerenchyma thickness which highly decreased at Site 1. At temporal scale values of all other root anatomical parameters were recorded highest during spring season in April except root aerenchyma area and root sclerenchyma thickness which highly decreased in April. All other root anatomical parameters decreased during peak winter season in January except root aerenchyma area and root sclerenchyma thickness which highly increased in January (Fig. 3).

Bar graphs showing morpho-anatomical attribute of *Cenchrus ciliaris*

Bar graphs showing stem morpho-anatomical attributes of*Cenchrus ciliaris* highlighted at spatial scale all stem parameters (stem epidermal cell area, stem metaxylem cell area, stem parenchyma cell area, stem radius and vascular bundle thickness) decreased at extremely dust polluted Site 3 except stem sclerenchyma thickness which highly increased at Site 3. All other stem anatomical parameters highly increased at dust free Site 1 except stem sclerenchyma thickness which highly decreased at Site 1. At temporal scale, content of all other steman atomical parameters decreased during peak winter season in January except stem sclerenchyma thickness which highly increased in January. All other stem anatomical parameters highly increased during spring season in April except stem sclerenchyma thickness which highly decreased in April (Fig. 4).

Bar graphs showing root morpho-anatomical attributes of *Cenchrus ciliaris* highlighted at spatial scale all root anatomical parameters (root radius, root epidermal cell area, root metaxylem cell area, root endodermis thickness, root parenchyma cell area, root radius and root vascular bundle thickness)decreased at extremely dust polluted Site 3 except root aerenchyma area and root sclerenchyma thickness which highly increased at Site 3. Values of all other root anatomical parameters were recorded highest at dust free Site 1 except root aerenchyma area and root sclerenchyma thickness which highly decreased at Site 1. At temporal scale values of all other root anatomical parameters were recorded highest during spring season in April except root aerenchyma area and root sclerenchyma thickness which highly decreased in April. All other root anatomical parameters decreased during peak winter season in January except root aerenchyma area and root sclerenchyma thickness which highly increased in January (Fig. 5).

Analysis of variance (ANOVA) showing physiological and biochemical attributes along with heavy metals in Aristida mutabilis

The analysis of variance showing physiological and biochemical attributes along with heavy metals in*Aristida mutabilis* at spatial and temporal scale highlighted specific variation in physiological and biochemical attributes along with heavy metals.

Analysis of variance showing heavy metals in *Aristida mutabilis* highlighted that at spatial scale among all sites variation in concentration of all heavy metals (cadmium, iron, nickel, lead and zinc) in root and stem was very highly significant. At temporal scale in all seasons variation among most all heavy metals in root and stem was also very highly significant except for root lead concentration which showed not very highly significant but significant variation (Table 3).

Table 3
Summary of ANOVA (*F* ratios) for physiological and biochemical attributes along with heavy metals in *Aristida mutabilis*

Attributes	Sites	Seasons
Chl a	335.5355***	22.48908***
Chl b	287.1867***	13.1151***
T Chl	386.9222***	21.54151***
Car	1124.21***	84.9953***
LRWC	257.3988***	47.68554***
H2O2	69.41729***	32.23852***
POD	130.5429***	22.66299***
AsA	664.2497***	58.00036***
Pro	102.2265***	16.88305***
RCd	601.8361***	168.1696***
RFe	348.6445***	12.15107***
RNi	142.0493***	10.40582***
RPb	27.49775***	5.278577*
RZn	670.1526***	53.80707***
SCd	712.1393***	143.4026***
SFe	410.4087***	34.69568***
SNi	442.2433***	52.334***
SPb	162.2801***	88.62355***
SZn	638.6883***	45.09738***
df: Sites 4, Season 3, Error 12, * = significant variation, **= highly significant variation		
***= very highly significant variation, ns = non significant variation		

Analysis of variance (ANOVA) showing physiological and biochemical attributes along with heavy metals in Cenchrus ciliaris

The analysis of variance showing physiological and biochemical attributes along with heavy metals in*Cenchrus ciliaris* at spatial and temporal scale highlighted specific variation in physiological and biochemical attributes along with heavy metals.

Analysis of variance showing heavy metals in *Cenchru sciliaris* highlighted that at spatial scale among all sites variation in concentration of all heavy metals (cadmium, iron, nickel, lead and zinc) in root and stem was very highly significant. At temporal scale in all seasons variation among all heavy metals in root and stem was also very highly significant (Table 4).

Table 4
Summary of ANOVA (*F* ratios) for physiological and biochemical attributes along with heavy metals in *Cenchrus ciliaris*

Attributes	Sites	Seasons
Chl a	160.1018***	33.28133***
Chl b	136.6145***	23.42273***
T Chl	163.8429***	30.98414***
Car	1.245473^{ns}	1.425274^{ns}
LRWC	1077.118***	146.2258***
H2O2	53.04129***	57.45345***
POD	518.6307***	77.14864***
AsA	1255.338***	188.4743***
Pro	85.32687***	8.361611***
RCd	121.1484***	44.51893***
RFe	98.6424***	24.30333***
RNi	115.2453***	9.630365***
RPb	97.93036***	18.45392***
RZn	428.1042***	42.6601***
SCd	212.7748***	24.79704***
SFe	355.0168***	56.39719***
SNi	517.819***	43.24205***
SPb	34.20318***	42.93707***
SZn	32.47714***	32.47714***
df: Sites 4, Season 3, Error 12, * = significant variation, **= highly significant variation		
***= very highly significant variation, ns = non significant variation		

CCA triplots showing physiological and biochemical attributes along with heavy metals in Aristidamutabilis

During January CCA triplots showing physiological and biochemical attributes along with heavy metals in *Aristida mutabilis* presented that in soil parameters EC, phosphorus and organic matter were associated with Site 3 while pH and potassium were associated with Site 1 and Site 2. In dust heavy metals nickel, lead and iron were associated with Site 3 and Site 4 while iron and zinc were not associated with any site. Dust concentration was associated with Site 3 and Site 4. Leaf chlorophyll *a*, leaf chlorophyll *b*, and leaf relative water content were associated with Site 1 which peroxidase (POD), ascorbic acid (AsA), proline and hydrogen peroxide were associated with Site 3 and Site 4. In root and shoot heavy metals zinc and iron were not associated with any particular site while lead, nickel and cadmium were associated with Site 3 and Site 4.

During April CCA triplots showing physiological and biochemical attributes along with heavy metals in*Aristida mutabilis* presented that in soil parameters EC and pH were associated with Site 4 while organic matter, phosphorus and potassium were associated with Site 5. In dust heavy metals cadmium, nickel and lead were associated with Site 3 and Site 4 while iron and zinc were not associated with any site. Dust concentration was associated with Site 3 and Site 4. Leaf chlorophyll *a*, leaf chlorophyll *b*, and leaf relative water content were associated with Site 1 which peroxidase (POD), ascorbic acid (AsA), proline and hydrogen peroxide were associated with Site 3 and Site 4. In root and shoot heavy metals zinc and iron were not associated with any particular site while cadmium, lead and nickel were associated with Site 3 and Site 4.

During July CCA triplots showing physiological and biochemical attributes along with heavy metals in*Aristida mutabilis* presented that in soil parameters EC was associated with Site 4, phosphorus and organic matter were associated with Site 3, pH was not associated with any Site while potassium was associated with Site 5. In dust heavy metals cadmium, nickel and lead were associated with Site 3 and Site 4 while iron and zinc were not associated with any site. Dust concentration was highly associated with Site 3 and Site 4. Leaf chlorophyll *a*, leaf chlorophyll *b*, and leaf relative water content were associated with Site 1 which peroxidase (POD), ascorbic acid (AsA), proline and hydrogen peroxide were associated with Site 3 and Site 4. In root and shoot heavy metals cadmium, nickel and lead were associated with Site 3 and Site 4 while iron and zinc were not associated with any site.

Soil attributes showed variation in each season. Heavy metals in dust were associated with October and January. Dust concentration was highly associated with October and January. Leaf chlorophyll *a* and chlorophyll *b*, and leaf relative water content were associated with April and July while peroxidase (POD), ascorbic acid (AsA), proline and hydrogen peroxide were associated with October and January. In root and shoot all heavy metals were associated with October and January.

The monocot*Aristida mutabilis* at Site 1 presentedthat insoil variables organic matter, phosphorus and potassium were associated with January; EC was associated with October while pH was associated with July. All heavy metals in dust were associated with January. Dust concentration was also associated with January. Leaf chlorophyll *a* and chlorophyll *b*, and leaf relative water content were associated with April and July while peroxidase (POD), ascorbic acid (AsA), proline and hydrogen peroxide were

associated with October and January. In root and shoot all heavy metals were associated with October and January.

CCA triplot showing physiological and biochemical attributes along with heavy metals in *Aristida mutabilis* at Site 2 presented that in soil variables potassium, organic matter and phosphorus were associated with October, EC was associated with July and pH was associated with January. All heavy metals in dust were associated with January. Dust concentration was also associated with January. Leaf chlorophyll *a* and chlorophyll *b*, and leaf relative water content were associated with April and July while peroxidase (POD), ascorbic acid (AsA), proline and hydrogen peroxide were associated with October and January. In root and shoot all heavy metals were associated with October and January.

CCA triplot showing physiological and biochemical attributes along with heavy metals in*Aristida mutabilis* at Site 3 presented that all soil variables except pH was associated with April, EC and potassium were associated with October and January while organic matter and phosphorus were not associated with any season. All heavy metals in dust were associated with January. Dust concentration was also associated with January. Leaf chlorophyll *a* and chlorophyll *b*, and leaf relative water content were associated with April and July while peroxidase (POD), ascorbic acid (AsA), proline and hydrogen peroxide were associated with October and January. In root and shoot all heavy metals were associated with October and January.

CCA triplot showing physiological and biochemical attributes along with heavy metals in*Aristida mutabilis* at Site 4 presented that insoil variables phosphorus, potassium and EC were associated with July, pH was associated with April and organic matter was associated with October. All heavy metals in dust were associated with January except cadmium which was associated with October. Dust concentration was associated with January. Leaf chlorophyll *a* and chlorophyll *b*, and leaf relative water content were associated with April and July while peroxidase (POD), ascorbic acid (AsA), proline and hydrogen peroxide were associated with October and January. In root and shoot all heavy metals were associated with October and January.

CCA triplotshowing physiological and biochemical attributes along with heavy metals in*Aristida mutabilis* at Site 5 presented that insoil variables pH, organic matter and potassium were associated with July while EC and phosphorus were associated with October. All heavy metals in dust were associated with January except iron which was associated with July. Dust concentration was associated with January. Leaf chlorophyll *a* and chlorophyll *b*, and leaf relative water content were associated with April while peroxidase (POD), ascorbic acid (AsA), proline and hydrogen peroxide were associated with January. In root and shoot all heavy metals were associated with January (Fig. 6).

CCA triplots showing physiological and biochemical attributes along with heavy metals in*Aristida mutabilis* at spatial scale highlighted specific distribution of environmental and supplementary variables. Soil attributes and heavy metals in dust showed variation at each site. Dust concentration was highly associated with Site 3 and Site 4. Leaf chlorophyll *a*, leaf chlorophyll *b*, and leaf relative water content were associated with Site 1 while peroxidase (POD), ascorbic acid (AsA), proline and hydrogen peroxide

were associated with Site 3 and Site 4. In root and shoot heavy metals zinc and iron were not associated with any particular Site while lead, nickel and cadmium were associated with Site 3 and Site 4.

During October CCA triplots showing physiological and biochemical attributes along with heavy metals in *Aristida mutabilis* presented that in soil parameters EC, phosphorus and organic matter were associated with Site 3 while pH and potassium were associated with Site 1 and Site 2. In dust heavy metals nickel, lead and iron were associated with Site 3 and Site 4 while iron and zinc were not associated with any site. Dust concentration was associated with Site 3 and Site 4. Leaf chlorophyll *a*, leaf chlorophyll *b*, and leaf relative water content were associated with Site 1 which peroxidase (POD), ascorbic acid (AsA), proline and hydrogen peroxide were associated with Site 3 and Site 4. In root and shoot heavy metals zinc and iron were not associated with any particular site while lead, nickel and cadmium were associated with Site 3 and Site 4 (Fig. 7).

CCA triplots showing physiological and biochemical attributes along with heavy metals in Cenchrus ciliaris

CCA triplots showing physiological and biochemical attributes along with heavy metals in *Cenchrus ciliaris* at spatial scale highlighted specific distribution of environmental and supplementary variables. Soil attributes and heavy metals in dust showed variation at each site. Dust concentration was highly associated with Site 3 and Site 4. In plant physiological parameters leaf chlorophyll *a*, leaf chlorophyll *b* and leaf relative water content were associated with Site 1 while in biochemical attributes peroxidase (POD), ascorbic acid (AsA), proline and hydrogen peroxide were associated with Site 3 and Site 4. In root and shoot heavy metals zinc and iron were not associated with any particular site while lead, nickel and cadmium were associated with Site 3 and Site 4.

During October CCA triplots showing physiological and biochemical attributes along with heavy metals in *Cenchru sciliaris* presented that in soil parameters EC was associated with Site 3 and Site 4, phosphorus and organic matter were associated with Site 3 while pH and potassium were associated with Site 2. In dust heavy metals nickel and lead were associated with Site 3 and Site 4 while cadmium, iron and zinc were not associated with any site. Dust concentration was associated with Site 3 and Site 4. In plant physiological parameters leaf chlorophyll *a*, leaf chlorophyll *b*, and leaf relative water content were associated with Site 1 which in biochemical attributes peroxidase (POD), ascorbic acid (AsA), proline and hydrogen peroxide were associated with Site 3 and Site 4. In root and shoot heavy metals cadmium, zinc and iron were not associated with any particular Site while lead and nickel were associated with Site 3 and Site 4.

During January CCA triplots showing physiological and biochemical attributes along with heavy metals in *Cenchrus ciliaris* presented that in soil parameters EC was associated with Site 3 and Site 4, phosphorus and organic matter were associated with Site 3 while pH and potassium were associated with Site 2. In dust heavy metals nickel and lead were associated with Site 3 and Site 4 while iron and zinc were not associated with any site. Dust concentration was associated with Site 3 and Site 4. In plant physiological parameters leaf chlorophyll *a*, leaf chlorophyll *b* and leaf relative water content were

associated with Site 1 while in biochemical attributes peroxidase (POD), ascorbic acid (AsA), proline and hydrogen peroxide were associated with Site 3 and Site 4. In root and shoot heavy metals cadmium, zinc and iron were not associated with any particular site while lead and nickel were associated with Site 3 and Site 4.

During April CCA triplots showing physiological and biochemical attributes along with heavy metals in *Cenchrus ciliaris* presented that in soil parameters EC was associated with Site 3 and Site 4, pH was associated with Site 4 while organic matter, phosphorus and potassium were associated with Site 5. In dust heavy metals nickel and lead were associated with Site 3 and Site 4 while cadmium, iron and zinc were not associated with any site. Dust concentration was associated with Site 3 and Site 4. plant physiological parameters leaf chlorophyll *a*, leaf chlorophyll *b* and leaf relative water content were associated with Site 1 while in biochemical attributes peroxidase (POD), ascorbic acid (AsA), proline and hydrogen peroxide were associated with Site 3 and Site 4. In root and shoot heavy metals cadmium, zinc and iron were not associated with any particular Site while lead and nickel were associated with Site 3 and Site 4.

During July CCA triplots showing physiological and biochemical attributes along with heavy metals in*Cenchrus ciliaris* presented that in soil parameters EC, phosphorus and organic matter were associated with Site 3, pH was associated with Site 1 while potassium was associated with Site 4. In dust heavy metals cadmium, nickel and lead were associated with Site 3 and Site 4 while iron and zinc were not associated with any site. Dust concentration was highly associated with Site 3 and Site 4. plant physiological parameters leaf chlorophyll *a*, leaf chlorophyll b and leaf relative water content were associated with Site 1 while in biochemical attributes peroxidase (POD), ascorbic acid (AsA), proline and hydrogen peroxide were associated with Site 3 and Site 4. In root and shoot heavy metals cadmium, nickel and lead were associated with Site 3 and Site 4 while iron and zinc were not associated with any site.

CCA triplots showing physiological and biochemical attributes along with heavy metals in*Cenchrus ciliaris* at temporal scale highlighted specific distribution of environmental and supplementary variables. Soil attributes showed variation in each season. Heavy metals in dust were associated with October and January. Dust concentration was highly associated with October and January. Leaf chlorophyll *a* and chlorophyll *b*, and leaf relative water content were associated with April and July while peroxidase (POD), ascorbic acid (AsA), proline and hydrogen peroxide were associated with October and January. In root and shoot all heavy metals were associated with October and January (Fig. 8).

CCA triplot showing physiological and biochemical attributes along with heavy metals in *Cenchrus ciliaris* at Site 1 presented that all soil variables except pH were associated with October and January while pH was associated with July. All heavy metals in dust were associated with January. Dust concentration was also associated with October and January. Leaf chlorophyll *a* and chlorophyll *b*, and leaf relative water content were associated with April while peroxidase (POD), ascorbic acid (AsA),

proline and hydrogen peroxide were associated with October and January. In root and shoot all heavy metals were associated with October and January.

CCA triplotshowing physiological and biochemical attributes along with heavy metals in *Cenchrus ciliaris* at Site 2 presented that all soil variables except EC were associated with October and January while EC was associated with July. All heavy metals in dust were associated with October and January. Dust concentration was also associated with October and January. Leaf chlorophyll *a* and chlorophyll *b*, and leaf relative water content were associated with April and July while peroxidase (POD), ascorbic acid (AsA), proline and hydrogen peroxide were associated with October and January. In root and shoot all heavy metals were associated with October and January.

CCA triplot showing physiological and biochemical attributes along with heavy metals in *Cenchrus ciliaris* at Site 3 presented that in soil variables except pH was associated with April, EC and potassium were associated with January while phosphorus and organic matter were not associated with any season. All heavy metals in dust were associated with January. Dust concentration was also associated with January. Leaf chlorophyll *a* and chlorophyll *b*, and leaf relative water content were associated with April and July while peroxidase (POD), ascorbic acid (AsA), proline and hydrogen peroxide were associated with October and January. In root and shoot all heavy metals were associated with October and January.

CCA triplot showing physiological and biochemical attributes along with heavy metals in *Cenchrus ciliaris* at Site 4 presented that insoil variables phosphorus, potassium and EC were associated with July, pH was associated with April and organic matter was associated with October. All heavy metals in dust were associated with January except cadmium which was associated with October. Dust concentration was also associated with October and January. Leaf chlorophyll *a* and chlorophyll *b*, and leaf relative water content were associated with April while peroxidase (POD), ascorbic acid (AsA), prolineand hydrogen peroxide were associated with January. In root and shoot all heavy metals were associated with October and January.

CCA triplot showing physiological and biochemical attributes along with heavy metals in*Cenchru sciliaris* at Site 5 presented that insoil variables pH was associated with April, phosphorus, organic matter and potassium were associated with July and EC was associated with October. All heavy metals in dust were associated with October and January. Dust concentration was associated with January. Leaf chlorophyll *a* and chlorophyll *b*, and leaf relative water content were associated with April while peroxidase (POD), ascorbic acid (AsA), proline and hydrogen peroxide were associated with October and January. In root and shoot all heavy metals were associated with October and January (Fig. 9).

Analysis of variance (ANOVA) showing morpho-anatomical attributes of *Aristida mutabilis*

Analysis of variance showing stem morpho-anatomical attributes of *Aristida mutabilis* highlighted at spatial scale among all sites variation in all stem parameters (stem epidermal cell area, stem metaxylem

cell area, stem parenchyma cell area, stem radius, stem sclerenchyma thickness and stem vascular bundle thickness) was very highly significant. At temporal scale in all season's variation among all stem parameters was also very highly significant except for stem epidermal cell area which showed not very highly significant but significant variation (Table 5).The structural modifications in stem parenchyma cell area, stem radius variation are very high (Fig. 10).

Table 5
Summary of ANOVA (*F* ratios) for morpho-anatomical attributes of *Aristida mutabilis*

Attributes	Sites	Seasons
RA	45.32238***	5.829141*
RE	13.2381***	4.849206*
RM	13.81517***	6.078989**
RN	19.8***	5*
RP	62.33224***	16.55646***
RR	2462.231***	144.2051***
RS	19.83871***	30.19355***
RV	57.35294***	12.32353***
SE	33.94118***	3.254902*
SM	46.9726***	19.39726***
SP	41.59644***	10.9658***
SR	302.2857***	24.5119***
SS	13.30189***	21.84906***
SV	79.26979***	31.90082***
df: Sites 4, Season 3, Error 12, * = significant variation, **= highly significant variation		
***= very highly significant variation, ns = non significant variation		

Analysis of variance showing root morpho-anatomical attributes of *Aristida mutabilis* highlighted at spatial scale among all sites variation in all root parameters (root aerenchyma area, root epidermal cell area, root metaxylem cell area, root endodermis thickness, root parenchyma cell area, root radius, root sclerenchyma thickness and root vascular bundle thickness) was very highly significant. At temporal scale in all season's variation among root aerenchyma cell area, root epidermal cell area and root endodermis thickness was significant, it was highly significant in root metaxylem cell area and very highly significant in root parenchyma cell area, root radius, root sclerenchyma thickness and root vascular bundle thickness (Table 5).

Analysis of variance (ANOVA) showing morpho-anatomical attributes of

Analysis of variance (ANOVA) showing morpho-anatomical attributes of *Cenchrus ciliaris*

Analysis of variance showing stem morpho-anatomical attributes of *Cenchrus ciliaris* highlighted at spatial scale among all sites variation in all stem parameters (stem epidermal cell area, stem metaxylem cell area, stem parenchyma cell area, stem radius, stem sclerenchyma thickness and stem vascular bundle thickness) was very highly significant. At temporal scale in all season's variation among all stem parameters was also very highly significant except for stem epidermal cell area which showed not very highly significant but significant variation (Table 6).

Table 6
Summary of ANOVA (*F* ratios) for morpho-anatomical attributes of *Cenchrus ciliaris*

Attributes	Sites	Seasons
RA	90.24539***	16.29006***
RE	27.74043***	9.191489**
RM	462.8571***	112.0714***
RN	9.666667***	2.666667^{ns}
RP	240.7549***	22.21772***
RR	1277.741***	27.37931***
RS	42.33333***	64.88889***
RV	398.7391***	39.91304***
SE	15***	6**
SM	63.24354***	11.27675***
SP	151.0484***	15.54687***
SR	272.1429***	25.33673***
SS	36.78947***	11.26316***
SV	43.89714***	15.64189***
df: Sites 4, Season 3, Error 12, * = significant variation, **= highly significant variation		
***= very highly significant variation, ns = non significant variation		

Analysis of variance showing root morpho-anatomical attributes of *Cenchrus ciliaris* highlighted at spatial scale among all sites variation in all root parameters (root aerenchyma area, root epidermal cell area, root metaxylem cell area, root endodermis thickness, root parenchyma cell area, root radius, root

sclerenchyma thickness and root vascular bundle thickness) was very highly significant. At temporal scale in all seasons variation among most root parameters was aerenchyma very highly significant except for root epidermal cell area and root endodermis thickness. Root epidermal cell area showed highly significant while root endodermis thickness did not showed any significant variation The structural modification in root arenchyma cell area, root endodermis, root parenchyma cell area variation are very high (Fig. 11).

DISCUSSION

Soil and dust analysis

For Soil and dust analysis samples were collected from all five study sites of Kirana Hills in all four seasons. Soil pH, EC, organic matter, phosphorus, potassium and presence of heavy metals (zinc, iron, lead, cadmium and nickel)were calculated. Dust concentration heavy metals concentration (Zinc, Iron, Lead, Cadmium and Nickel) in dust samples were also calculated.

At spatial scale soil maximum pH value 8.15 which is moderately basic was recorded at dust free Site 1 followed by high dust site and moderate dust site respectively while minimum pH value 7.65 which is slightly basic pH according to [51] was recorded at low dust site followed by extreme dusty site. Soil maximum EC was recorded at dust free site followed by low dust site and moderate dust site while minimum soil EC was recorded at extreme dust site followed by high dust site, higher EC represents higher salt concentration in the soil and vice versa according to [52] in their study on soil electrical conductivity. Soil maximum organic matter was recorded at dust free site followed by low dust site and moderate dust site respectively, while minimum organic matter was recorded at extreme dust site followed by high dust site. Higher soil organic matter may be due to vegetation growth and soil microorganisms activity according to [53] while lower soil organic matter may be due addition of nutrients as revealed by [54].

Soil maximum phosphorus and potassium were recorded at low dust site and dust free site respectively while minimum phosphorus and potassium were recorded at moderate followed by and extreme dust site respectively. Higher or lower phosphorus and potassium may be due to increased or decreased bacterial activity mentioned by [55], increased or decreased soil moisture as studied by [56], due to plant invasion as revealed by [57], fungal growth as supported by the study of [58] and addition of fertilizers according to [59]. Soil maximum heavy metals (zinc, iron, lead, cadmium and nickel) concentration was recorded at extreme dust site and high dust site while minimum heavy metals concentration was recorded at dust free site followed by low and moderate dust sites, Moreover iron concentration was highest and cadmium concentration was lowest among all sites. Higher or lower heavy metals concentration may be due to industrial effluents (stone crushing industry) and roadside dust according to [60].

On temporal scale almost all soil parameters were higher in concentration in winter and autumn and their concentration was relatively lower in spring and summer. It may be due to the changes in soil moisture, amount of rainfall and temperature which are minimum in winter and maximum in summer as revealed by [61] or it may be due to amount of dust-fall which is maximum in winter and minimum in summer, dust carry heavy metals and deposit them on soil according to the study of [62] on dust pollution.

Anatomical analysis of vegetation

Data of two selected monocots was collected from all five study sites of Kirana Hills in all four seasons to investigate the effect of dust pollution of stone crushing industry onmorpho-anatomical attributes of selected plants collected from each study site.

Plant survive in harsh dust polluted conditions by making some morpho-anatomical changes in their structures i.e. changes in sclerification, extensive parenchyma cells according to [35] in their study on morpho-anatomical adaptations in halophytes, xerophytes and certain sedges.

in his study on impact of dust pollution on plant morpho-anatomical attributes, higher heavy metals concentration in soil and air because of higher dust pollution of stone crushing industry which effect plant growth as studied by [5, 63], lower concentration of organic matter which negatively effects plant morphology as mentioned by [64] in their study on importance of organic matter for plant growth, lower phosphorus and potassium content which limit growth of plant morpho-anatomical parameters as described by [57] in their work on effect of micro and macro nutrient concentrations on growth of plant morphological features, basic pH value which effects growth of plant morpho-anatomical features according to [65] in their work on soil pH, high EC which represents high salinity which reduce the growth of plant morpho-anatomical attributes [66].

At spatial scale most of the stem anatomical attributes of selected plants i.e. stem radius, epidermal cell area, parenchyma cell area, vascular bundle thickness and metaxylem cell area were highest at dust free site followed by low dust site and intermediate at moderate dust site. Higher values of stem attributes may be due to the absence of stone crushers on these sites as supported by the study of [63] on impact of dust pollution on stem anatomical attributes, lower heavy metals concentration in soil and air because of no industrial effluents on these sites [60], higher concentration of soil organic matter which according to supports the stem anatomical growth features, higher phosphorus and potassium content also enhance stem anatomical features as studied by [59] during their study on plant nutrients, neutral pH value increase stem anatomical growth as mentioned in the findings of [51] in his study on soil pH, and higher soil EC increase microflora as revealed by the findings of [67].

All the above stem attributes were lower at extreme dust site and high dust site. This decrease in the values of stem anatomical parameters may be due to the high dust pollution caused by active stone crushes at these sites as supported by the results of the study by [63] on dust pollution, higher heavy metals concentration in soil and air because of higher dust pollution of stone crushing industry also effect stem anatomical growth as described by [5, 63] during their study on heavy metals, lower

concentration of soil organic matter decrease stem anatomical growth as revealed by [64], lower phosphorus and potassium content negatively impacts stem anatomical attributes as suggested by [57].

Stem sclerification increased at extreme and high dust polluted sites, it may be an adaptive response of local plants to the abiotic stress induced by dust pollution of stone crushing industry as supported by the studies of [68, 32] on cytological changes in plants in response to air pollution.

At spatial scale values of root anatomical attributes of selected plants i.e. root radius, epidermal cell area, endodermis thickness, parenchyma cell area, vascular bundle thickness and metaxylem cell area were the highest at dust free site followed by low dust site and intermediate at moderate dust site. Higher values of root anatomical attributes may be due to the absence of stone crushers at these sites, on impact of stone crushers dust on root anatomical growth, lower heavy metals concentration in soil and air because of no industrial effluents so little effect of heavy metals on root anatomical features of selected plants as supported by the study of [60] on heavy metals impacts on plant growth, higher concentration of soil organic matter which enhance root anatomical growth as revealed by [69].

All the above root parameters were lower at extreme dust site and high dust site. This decrease in these leaf parameters may be due to high dust pollution caused by active stone crushes at these sites as revealed by [63] that high dust concentration decreases root growth, higher heavy metals concentration in soil and air because of higher dust pollution of stone crushing industry which effect root growth as described by [5, 63], lower concentration of organic matter also limit root anatomical growth as mentioned by [64] in their work on relation of organic matter with plants, lower phosphorus and potassium content also halt root growth as indicated by the study of [57] on plant nutrients, basic pH value also effects root anatomical growth as studied by [65], high EC which represents high salinity which reduce growth of root anatomical attributes as supported by the work of [66] on relation between soil EC and plant growth.

Root sclerification increased at extreme and high dust polluted sites, it may be adaptive response of local plants to the abiotic stress induced by dust pollution of stone crushing industry in accordance with the study of [68, 32] on plant adaptations to dust pollution. Root aerenchyma development was also noticed in all selected plant species at extreme and high dust polluted sites, it may be because root aerenchyma development is considered as an adaption to survive in air pollution and other abiotic stress conditions as revealed by the study of [70, 33] on air pollution tolerance in plants.

At temporal scale all root and stem morpho-anatomical attributes increased in spring followed by summer except stem and root sclerification along with root arenchyma formation which decreased in spring and summer. On seasonal impact of dust pollution on plants, amount of rainfall which is maximum in summer, temperature which is quite ideal for most of the plants growth in spring i.e. 25–30°C as revealed by the study of [71] on impact of seasonal variation in soil moisture on plant growth, lower heavy metals concentration in dust during spring and summer which reduces their impact on plant growth as supported by the study of [60](, soil 7–8 range of pH value which supports plant growth according to the work of [51] on soil pH, low soil EC value so less saline soil which is ideal for most of

the plant species growth as described by the study of [66] on soil electrical conductivity and high soil moisture in summer followed by spring also supports plant growth as revealed in the findings of [72] in their study on vegetation dynamics due to variations in seasonal rainfall.

All root and stem morpho-anatomical attributes decreased in winter and autumn except stem and root sclerification along with root arenchyma formation which increased in winter and autumn. It may be due to the higher dust concentration in winter and autumn which negatively effects vegetation growth as revealed by [73] in their study on relation between dust pollution and plant growth, amount of rainfall which is minimum in winter followed by autumn which decreases the vegetation growth and distribution, temperature which is not ideal for most of the plants growth in January i.e. 5–20°C, higher heavy metals concentration in dust which negatively influence the growth of plants as indicated by [5].

All selected plants survived in all seasons especially in cold winters with high suspended dust, it may be due to their morpho-anatomical modifications i.e. presence of large parenchyma cells and root and stem sclerification as revealed by the findings of [32] and root aerenchyma formation as described by [70] in their study on plant morpho-anatomical adaptations in response to dust pollution.

Physiological, biochemical and heavy metal analysis of vegetation

Data of two selected monocots was collected from all five study sites of Kirana Hills in all four seasons to investigate the effect of dust pollution of stone crushing industry on plants physiological and biochemical attributes.

In present study at spatial scale physiological parameters of all selected plants i.e. leaf chlorophyll *a*, chlorophyll *b* and carotenoids, leaf relative water showed significant variation in response to dust pollution. All these parameters were highest in concentration at dust free site followed by low dust site and intermediate at moderate dust site. Higher values of physiological attributes may be because of no dust pollution caused by stone crushing industry due to the absence of stone crushers on these sites as revealed by [20, 63] that dust pollution severely effect plant physiological attributes, lower heavy metals concentration in soil and air because of no industrial effluents so reduced effect of heavy metals on physiology of selected plants as described by [60].

All the above physiological attributes were lower at high dust site and extreme dust site. This decrease in these physiological parameters may be due to high dust pollution caused by active stone crushers at these sites as supported by the study of [63] that dust pollution strongly effects plant physiological attributes, higher heavy metals concentration in soil and air because of higher dust pollution of stone crushing industry which increase the impact of heavy metals on plant physiology as revealed by [5, 63], lower concentration of soil organic matter decrease plant physiological parameters as indicated by the study of [64].

Biochemical attributesof selected plants i.e. peroxidase (POD) activity, ascorbic acid content (AsA), hydrogen peroxide (ROS) and proline content showed significant variation in response to dust pollution. All these parameters were decreased at dust free site followed by low dust site and moderate dust site respectively.

All the above biochemical attributes were increased at extreme dust site and high dust site. The increase in these biochemical parameters may be due to high dust pollution caused by active stone crushes at these sites which cause abiotic stress and reactive oxygen species (ROS) are produced in response to which plants produce enzymatic and non-enzymatic antioxidants along with osmoprotactants to survive in such harsh environment as revealed by [63] in their study on impact of dust pollution on plants, higher heavy metals concentration in soil and air because of higher dust pollution of stone crushing industry results in metal toxicity in response to which plants produce of antioxidants and osmoprotects.

Heavy metals (Cd, Fe, Ni, Zn, Pb) in high concentrations can be toxic to plants and can decrease plant growth and yield as revealed by [74] in their study on heavy metal toxicity.

Heavy metal concentrationi.e.Cd, Fe, Ni, Zn, Pbin root and shoot of selected plants varied in response to dust pollution. All heavy metals were lowest in concentration at dust free site followed by low dust site and moderate dust site, lower concentration of heavy metals may be due to the absence of stone crushers on these sites as revealed by [20, 63] in their study that dust pollution from stone crushing industry carry heavy metals, or may be due to lower heavy metals concentration in soil because of no industrial effluents at these sites as in accordance with study of [60] on heavy metals.

Concentration of all above heavy metals in root and shoot of all selected plants were highest at extreme dust site followed by high dust site. This increase in heavy metal concentration may be due to high dust pollution caused by active stone crushes at these Sites as revealed by [63] that dust pollution emitted during crushing process carry heavy metals, or may be due to higher heavy metals concentration in soil because of higher dust pollution of stone crushing industry as supported by the study of [5, 63] on dust pollution and heavy metals.

All selected plants survived in all seasons especially in cold winters with high suspended dust, it’s may be due to their physiological and biochemical modification i.e. formation of enzymatic and non-enzymatic anti-oxidants as revealed by [75] along with increase in osmoprotecrants as elaborated by [76] in their study on plants abiotic stress resistance mechanisms.

CONCLUSIONS AND RECOMMENDATIONS

From the result of present study, it was concluded that:

- Kirana Hills is highly neglected area with important treasures of plant diversity consisting mostly of herbs and shrubs which have fodder and medicinal importance.

- The growth of local plants is highly affected due to the high dust pollution of active stone crushers of the area which affect their morpho-anatomy, physiology and biochemistry.
- The native plants of the area are surviving by making morpho-anatomical, physiological and biochemical modifications to cope with extreme dust polluted environment.

Declarations

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Competing Interest

All authors declare that there are no competing interests.

Voucher specimens

Plant specimens were identified by Dr. Iftikhar Ahmad associate professor of Botany, University of Sargodha, Sargodha, Pakistan and Dr. Mansoor Hameed, Professor at University of Agriculture Faisalabad. Voucher specimens are available Department of Botany, University of Sargodha (S2020/3341-3344 (Muhammad Ali, contact: +923036200205). Prepared slides (Number M2020/78-145) are deposited at Department of Botany University of Sargodha, Sargodha Pakistan.

Consent for Publication

Not Applicable

Availability of data and materials

Data is provided within the manuscript or supplementary information files

Authors' contributions

Muhammad Asim Sultan and Toqeer Abbas conducted the experiments and analyzed the data Iftikhar Ahmad and Anis Ali Shah worked on graph and tables and all authors revised the manuscript.

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Figures

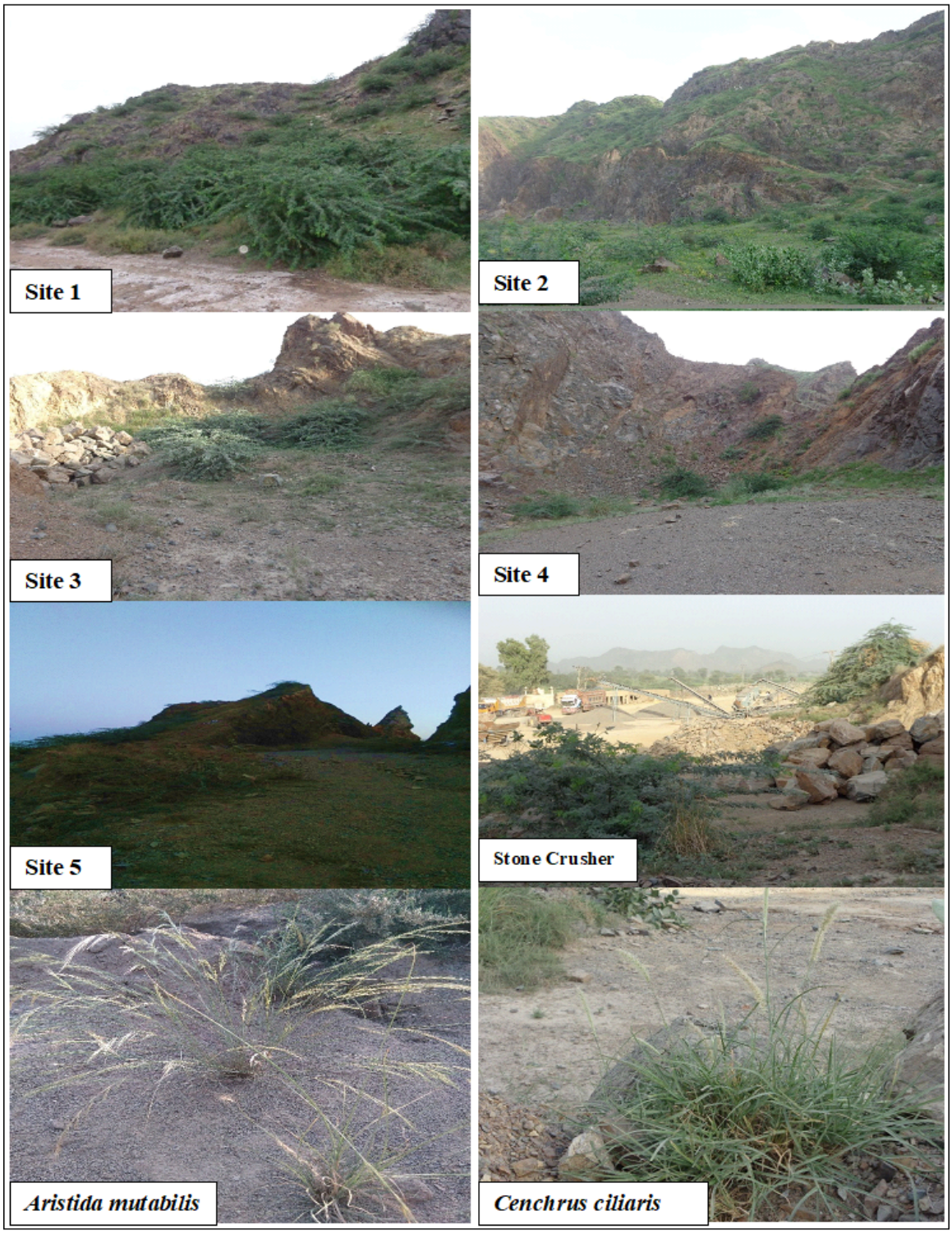

Figure 1

Selected study sites and selected plants under study at Kirana Hills Sargodha

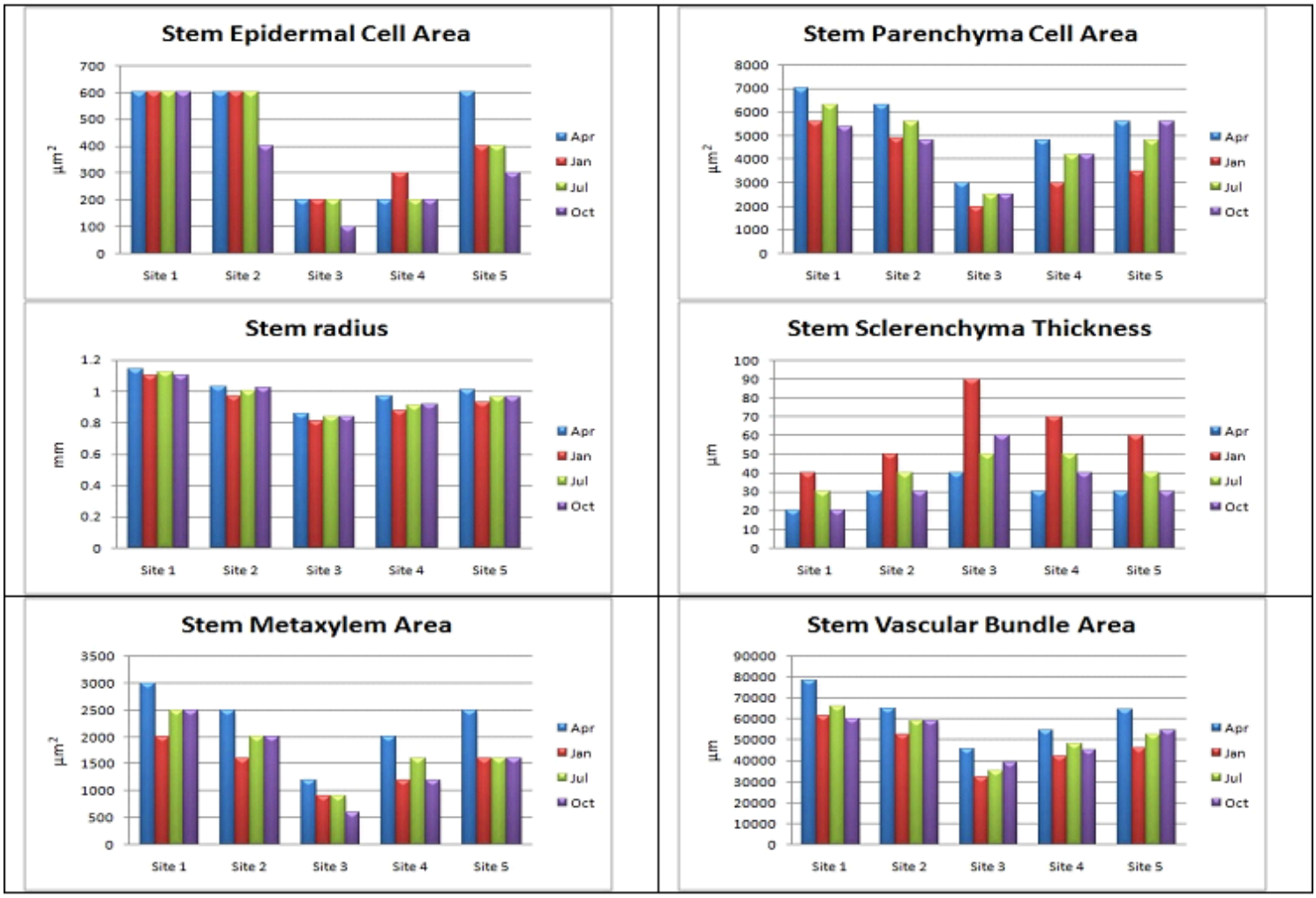

Figure 2

Seasonal variations in stem morpho-anatomical attributes of *Aristida mutabilis* at each site

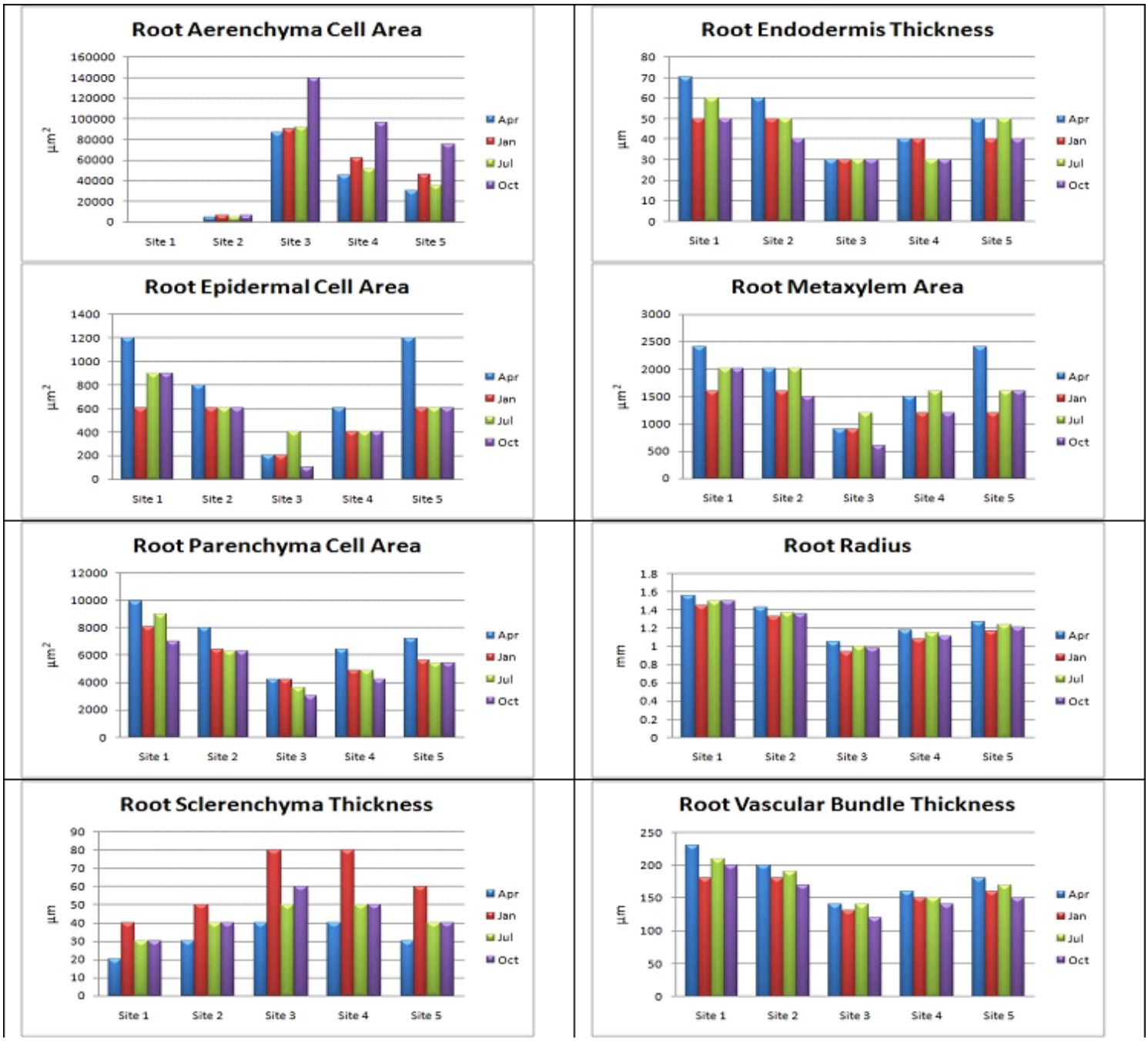

Figure 3

Seasonal variations in root morpho-anatomical attributes of *Aristida mutabilis* at each site

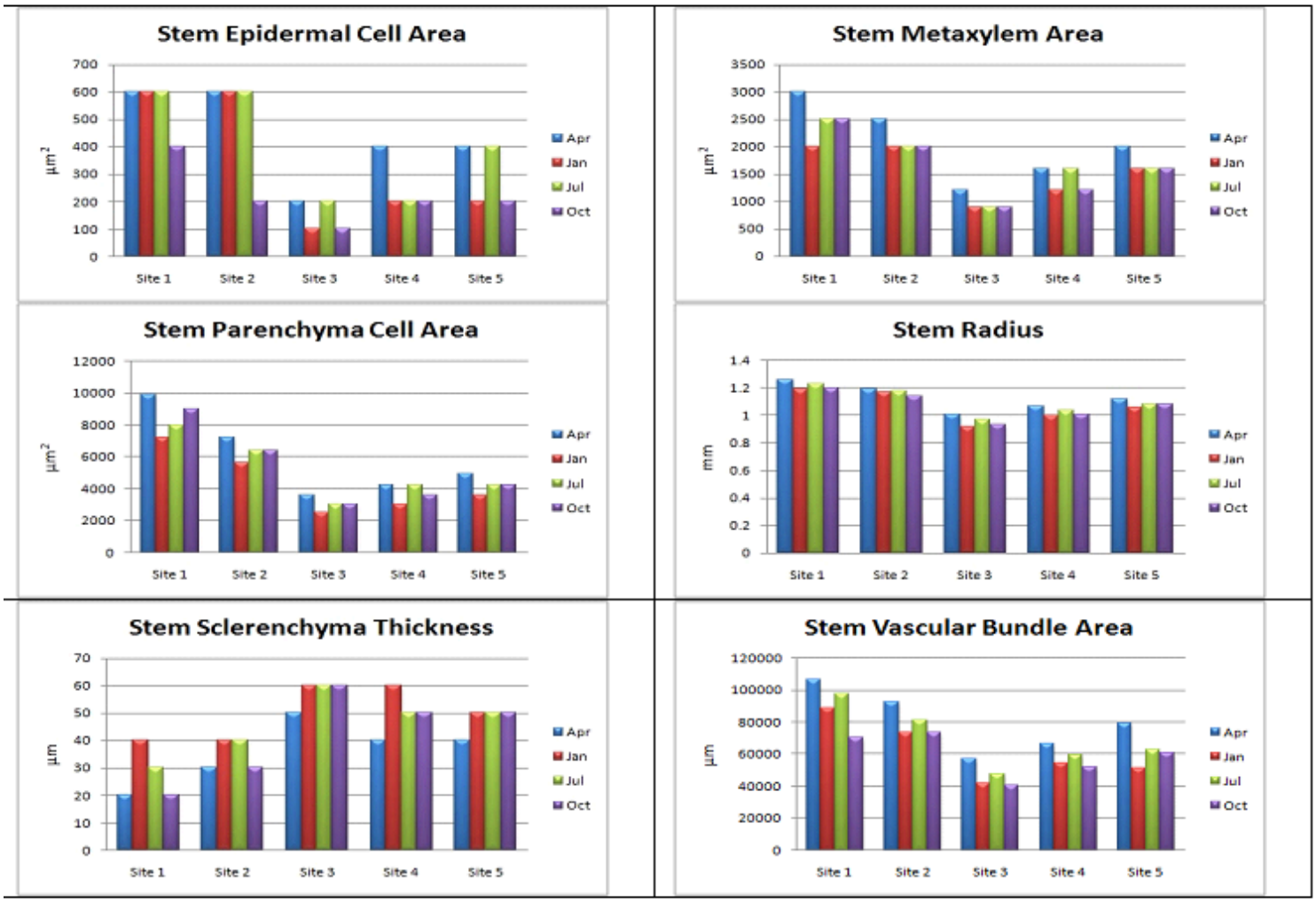

Figure 4

Seasonal variations in stem morpho-anatomical attributes of *Cenchrus ciliaris* at each site

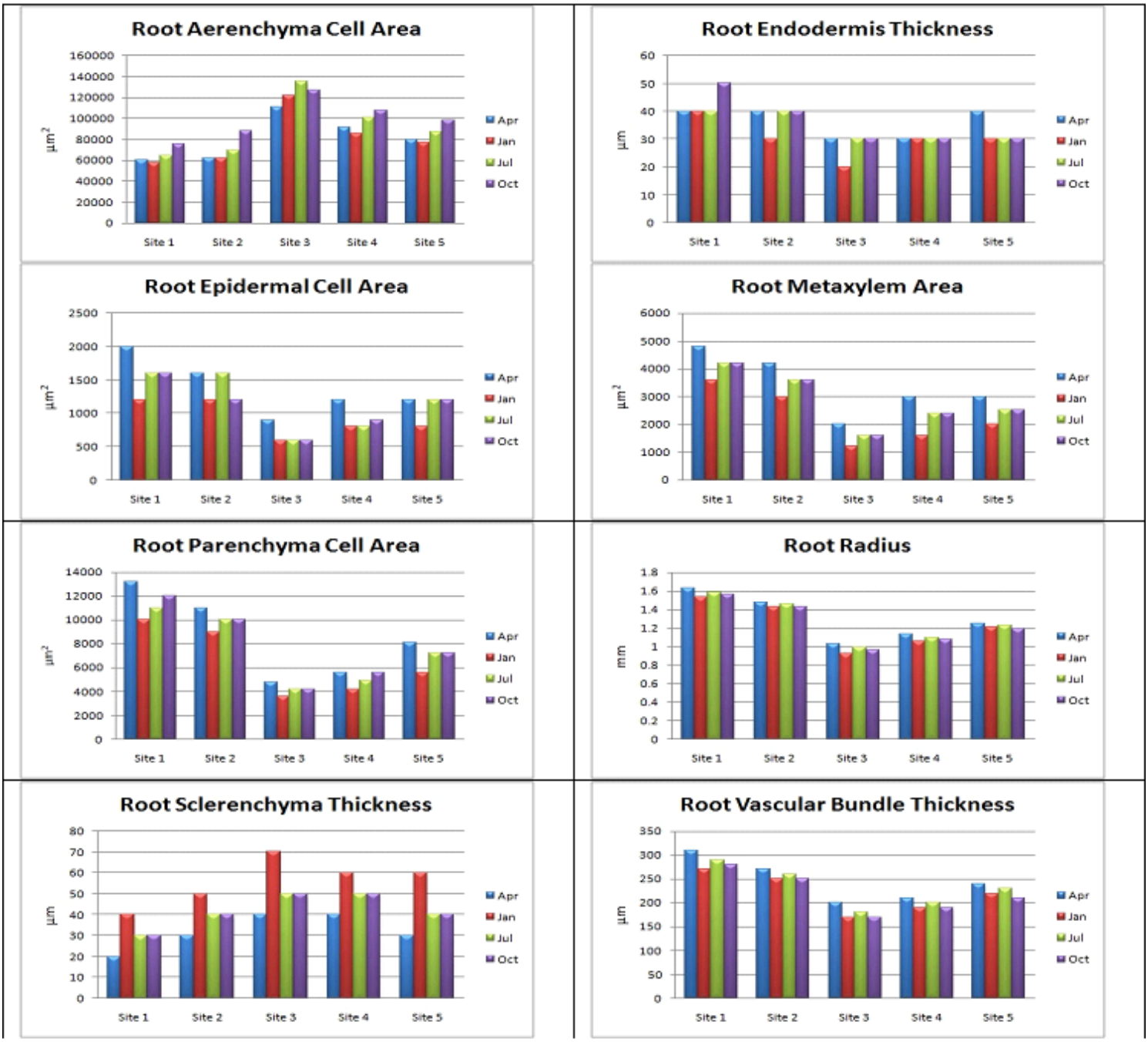

Figure 5

Graph showing Seasonal variations in root morpho-anatomical attributes of *Cenchrus ciliaris* at each site

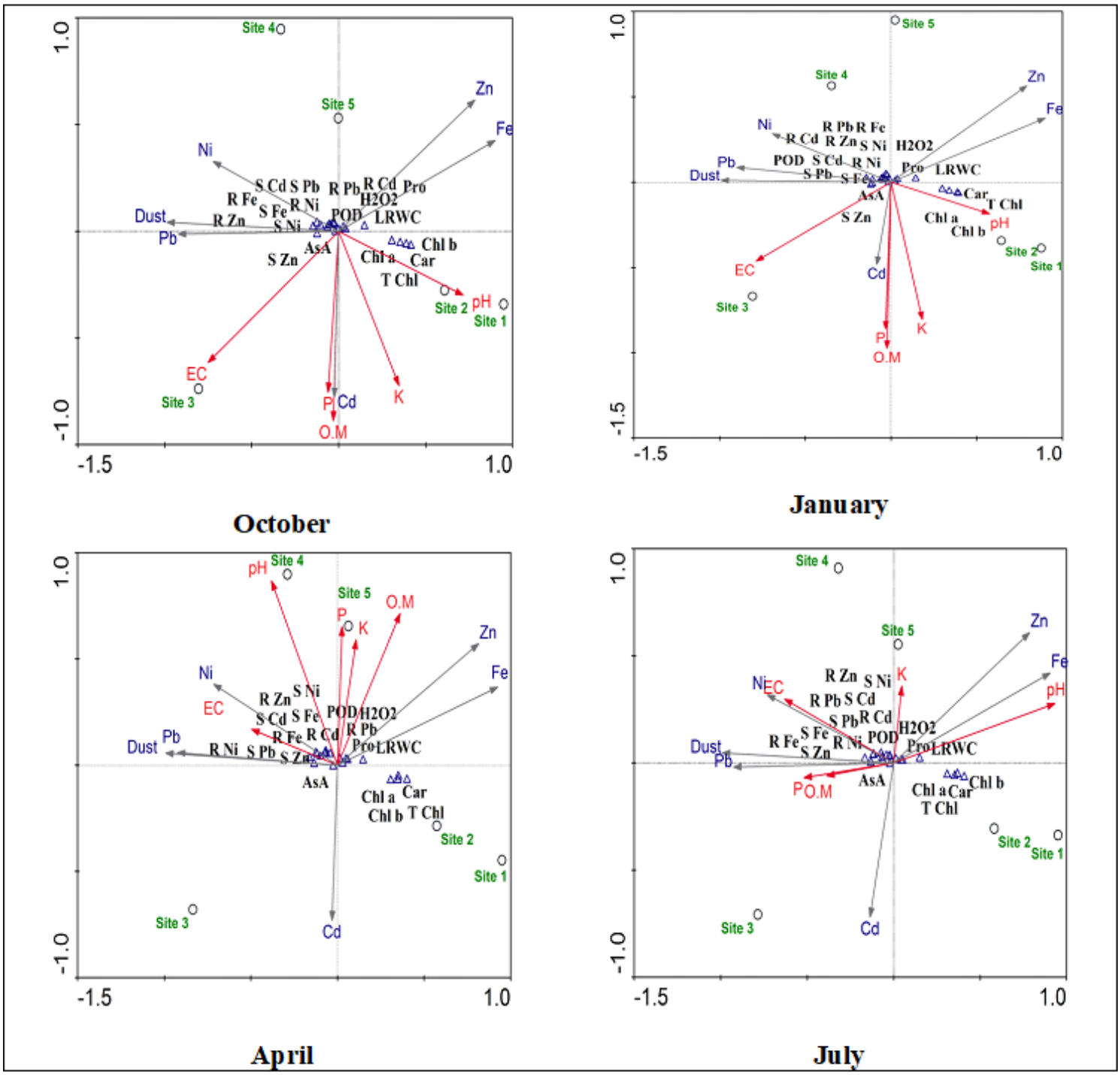

Figure 6

CCA tripolts showing physiological and biochemical attributes along with heavy metals in of *Aristida mutabilis* on spatial scale

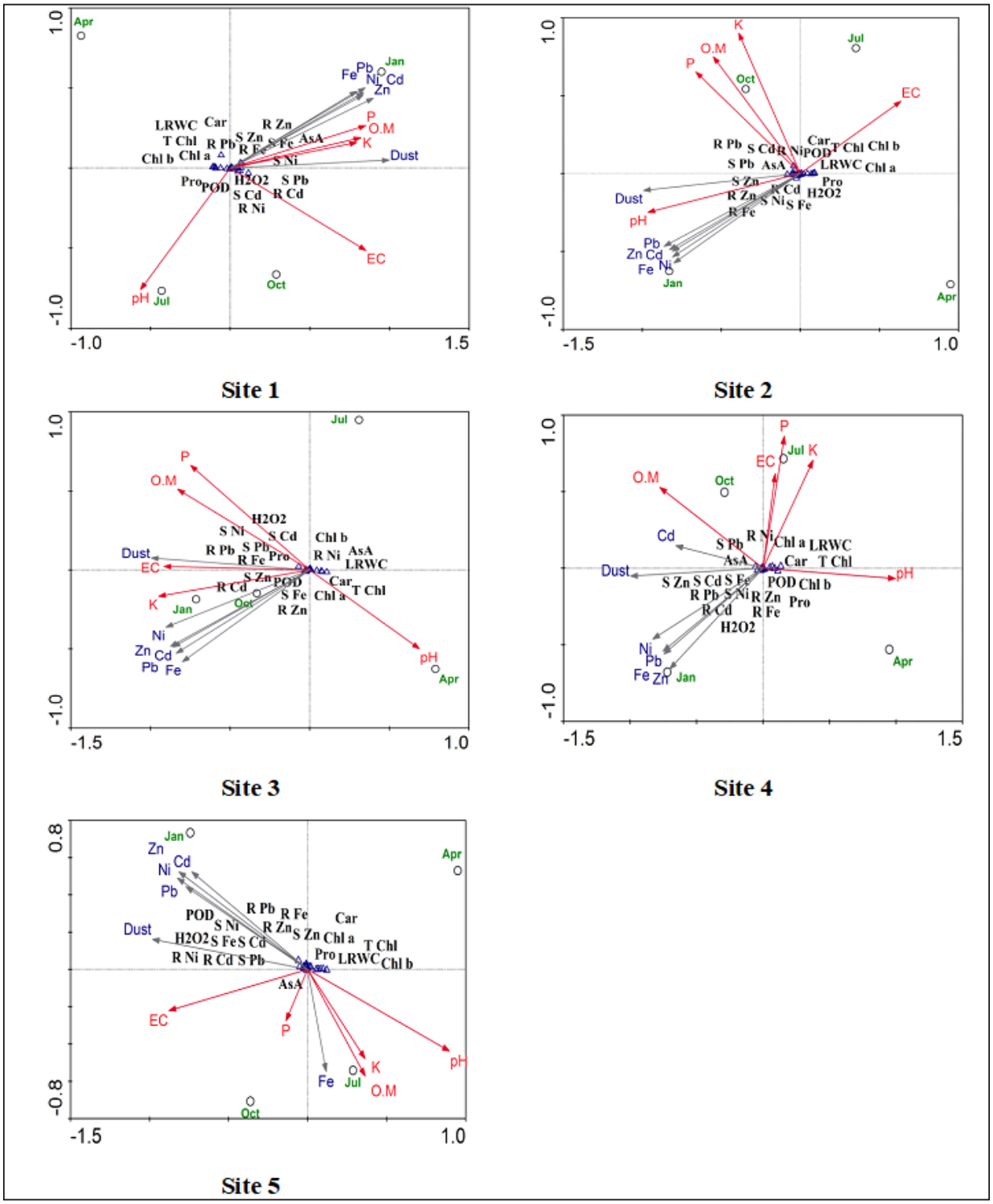

Figure 7

CCA tripolts showing physiological and biochemical attributes along with heavy metals in *Aristida mutabilis* on temporal scale

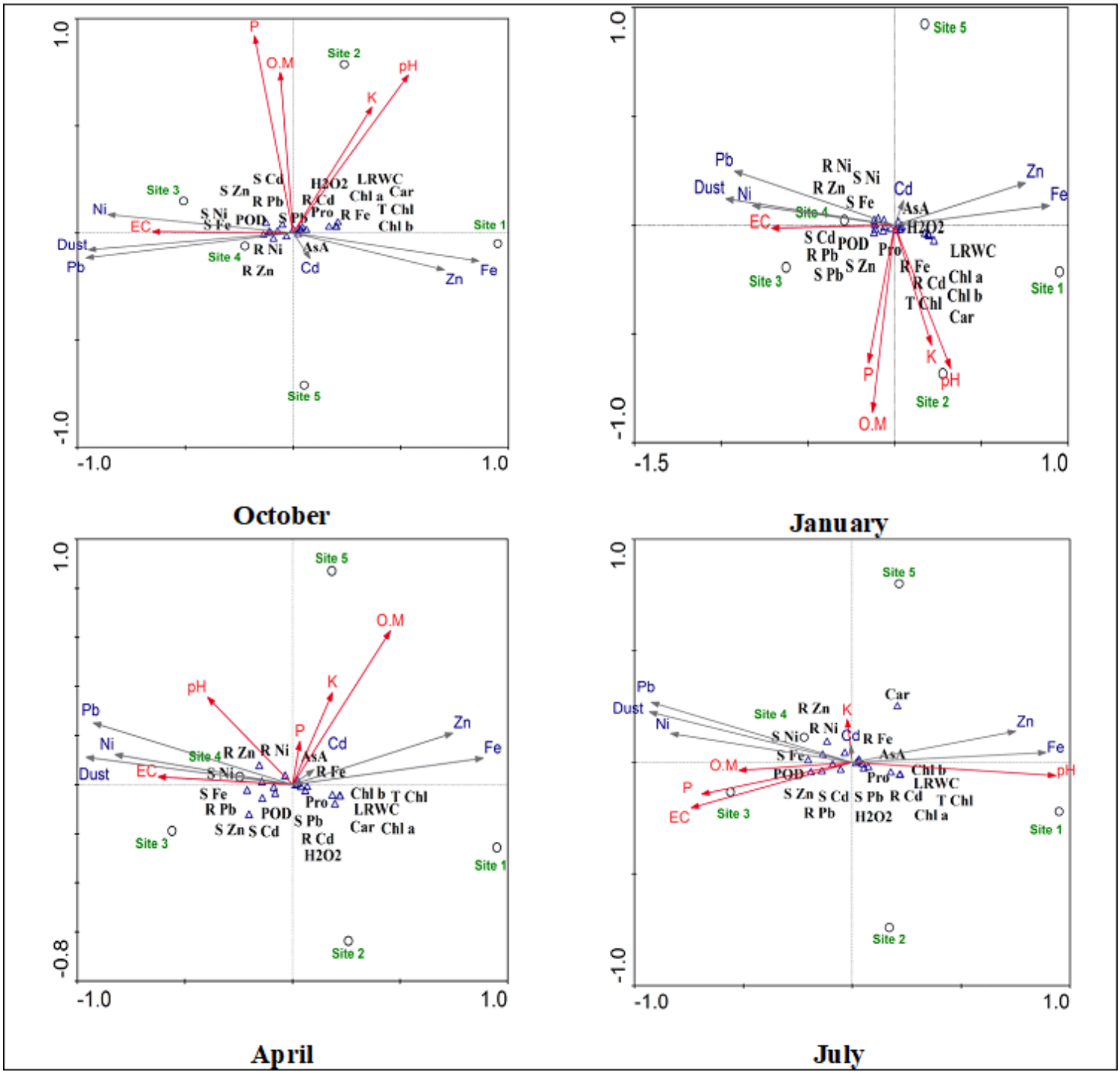

Figure 8

CCA tripolts showing physiological and biochemical attributes along with heavy metals in of *Cenchrus ciliaris* on spatial scale

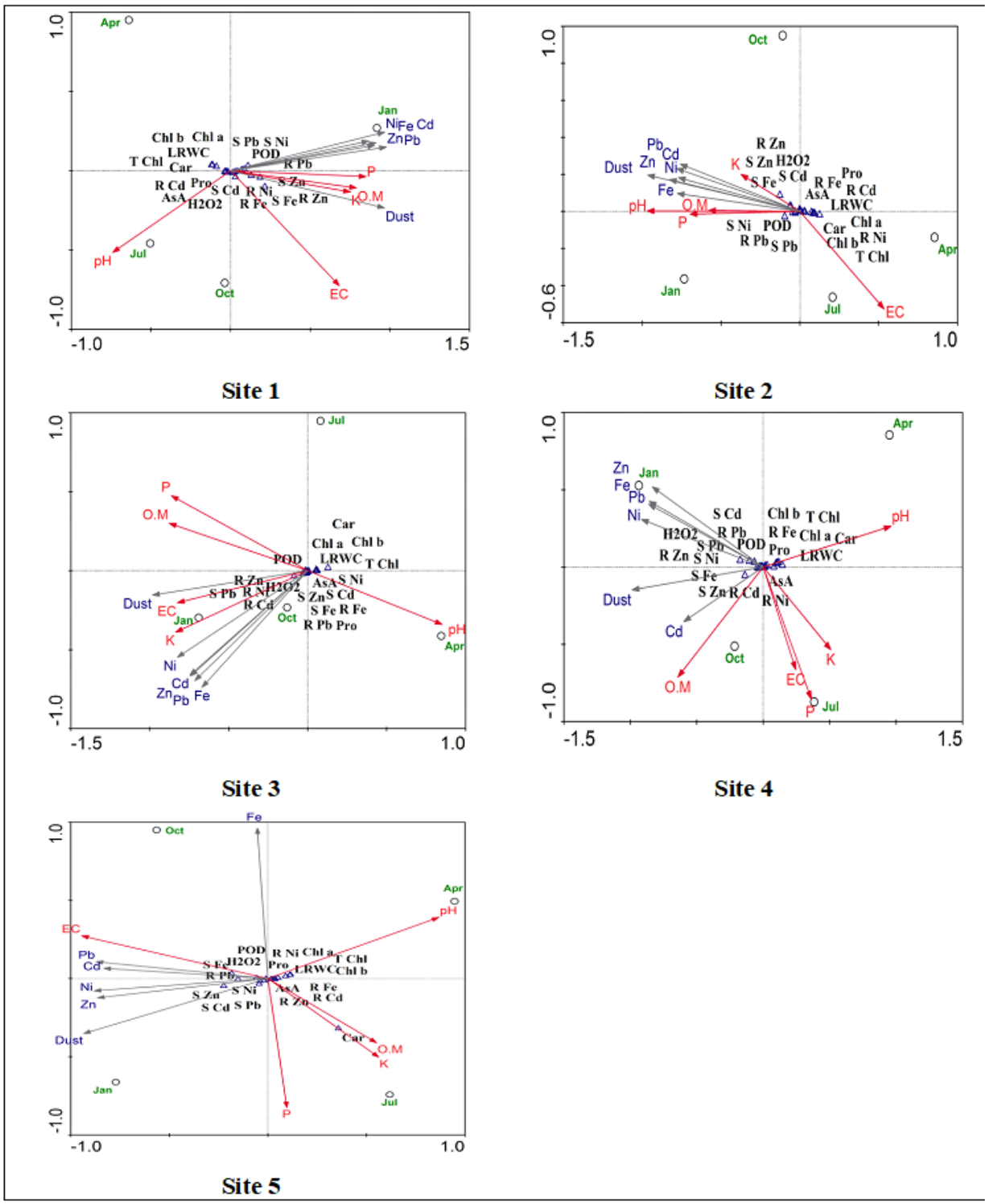

Figure 9

CCA tripolts showing physiological and biochemical attributes along with heavy metals in *Cenchrus ciliaris* on temporal scale

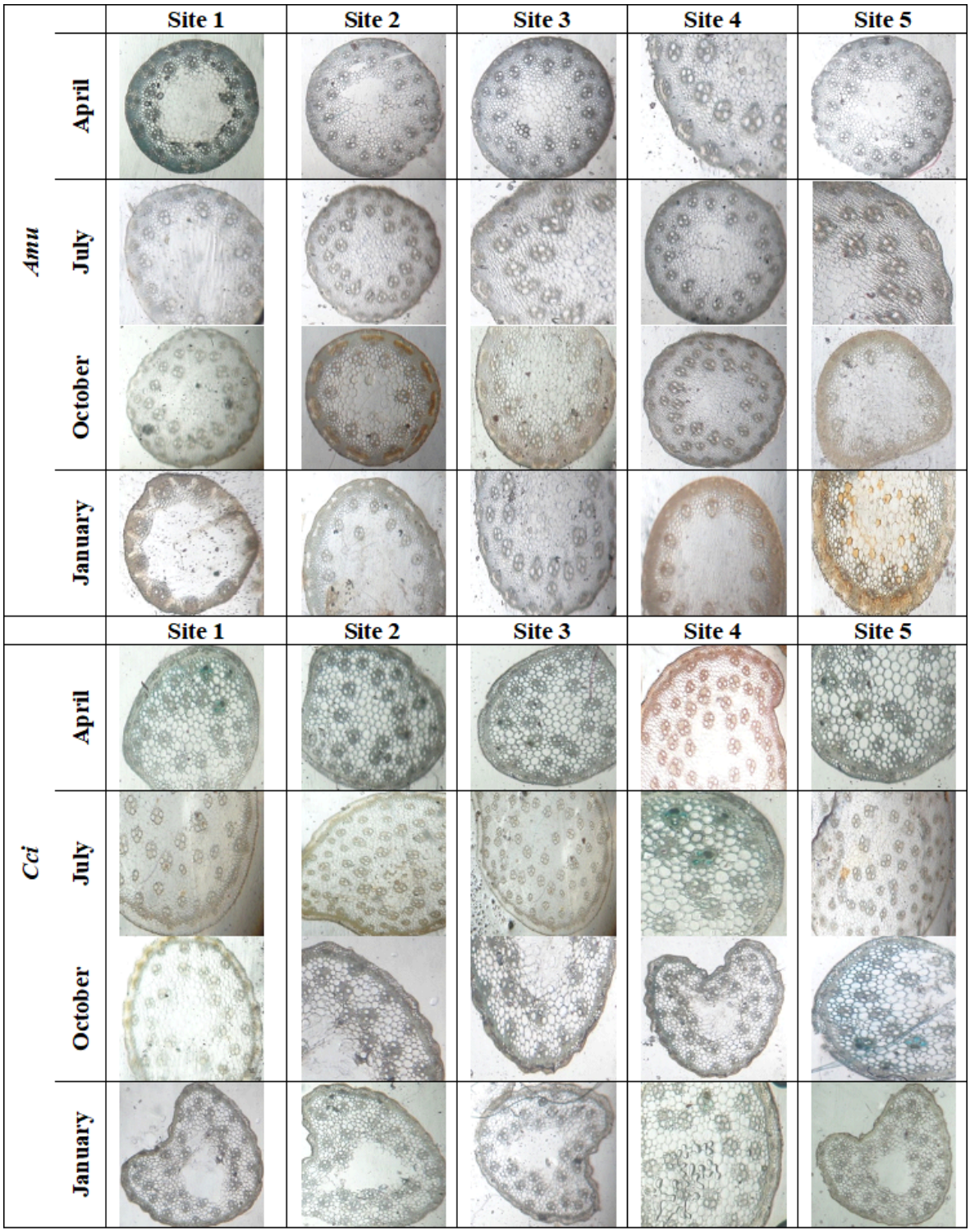

Figure 10

Seasonal variations in stem anatomical features of *Aristida mutabilis* and *Cenchrus ciliaris* at each site

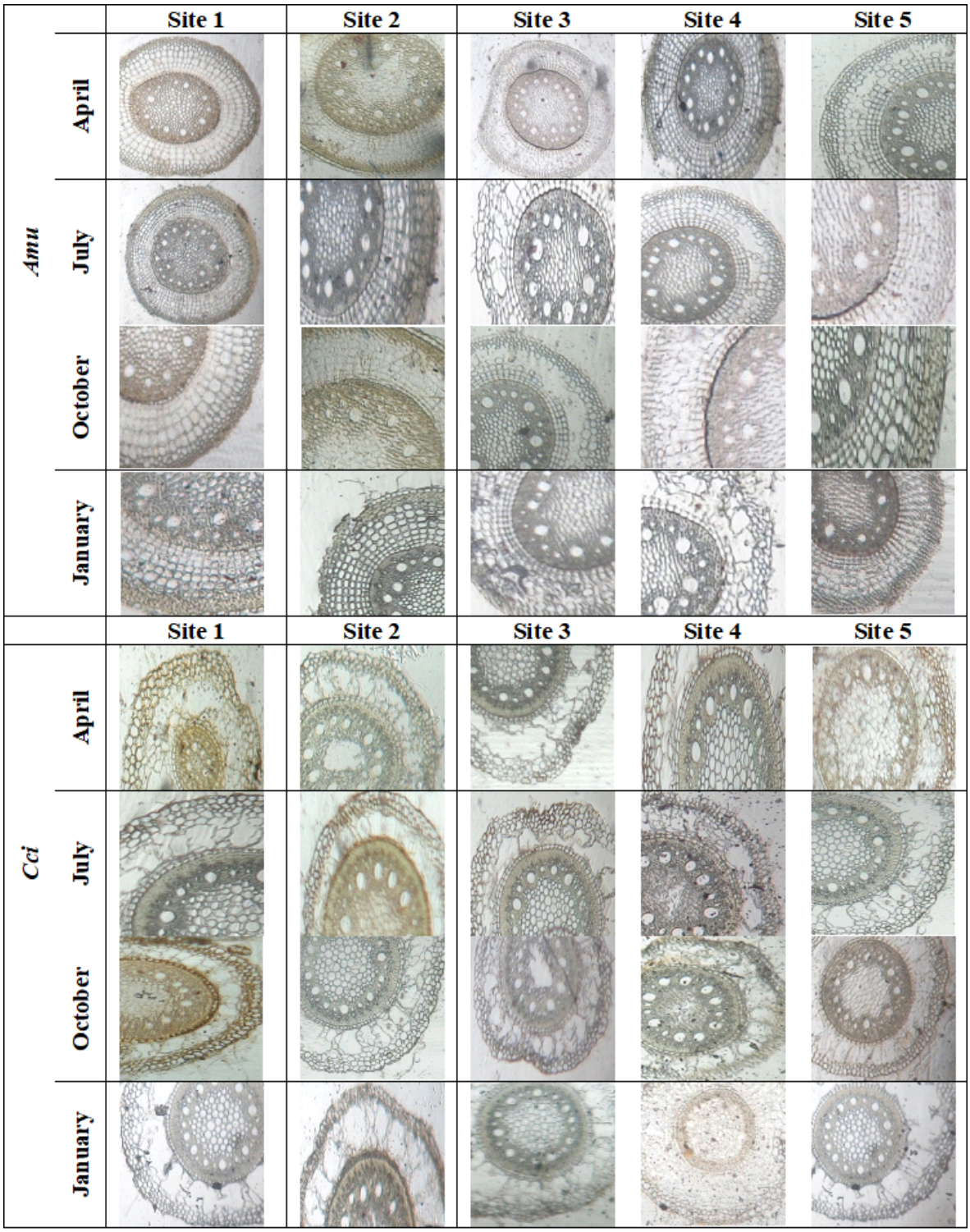

Figure 11

Seasonal variations in root anatomical features of *Aristida mutabilis* and *Cenchrus ciliaris* at each site

Supplementary Files

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- ANOVAPHYCC.xlsx