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A Study of *Arbuscular Mycorrhiza* (AM) Root Colonization in the Herbaceous Vegetation of Different Age Series Sponge Iron Solid Waste Dumps

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A B S T R A C T

Mycorrhiza is one of the most important groups of microorganisms which can effectively support the vegetation development in distress habitat like sponge iron solid waste dump. Arbuscular Mycorrhiza (AM) root colonization in the herbaceous vegetation of different age series sponge iron solid waste dumps were assessed. Among the grasses all the species were associated with AM in all the waste dumps and such observation highlights the effective role of grasses as pioneer species during vegetation succession. The percentage of mycorrhizal colonization in the herbaceous plant species decreased with increasing age of the waste dumps and as age of the waste dumps increased, the percentage of plants with very high level decreased in all the dumps. Highest percentage of mycorrhizal root colonization was observed in *Eragrostis tenella* which was dominant species of the waste dumps. More numbers of plants were with hyphae & arbuscules (H+A) structure than hyphae & vesicle (H+V) in the waste dumps reflects the importance of synergistic action of hyphae and arbuscule for transfer of nutrients from low nutrient content substrate rather than the development of storage structure like vesicle. Form the study it was concluded that establishment of mycorrhizal association is quite essential for proper vegetational development and subsequent reclamation of the sponge solid waste dumps.

Keywords

Arbuscular mycorrhiza, root colonization, sponge iron solid waste, reclamation.

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Introduction

Steel is a vital component of any country's economy and is considered amongst the driving force of modernization. Sponge iron or direct reduced iron (DRI) is mostly used for steel making through secondary sector. India has emerged as the world's largest producer of sponge iron, accounting for 33% of the global production and the coal based sponge iron production contributes about 80% of the total capacity of the country (IBM, 2013). From the coal based sponge iron industry huge amount of solid waste is generated in form of char, dust, accretion material and fly ash (CPCB, 2007). Majority of these solid wastes are dumped on land which creates large areas of black calcareous derelict land (Roy *et al.*, 2002). In developing country like India, reclamation of derelict land is an urgent necessity and one of the effective strategies for the

reclamation is through revegetation (Singh *et al.*, 2002). However, revegetation of the sponge iron industry solid waste dump is a difficult task looking in to the hostile pedosphereic environment (Pandey and Maiti, 2008). The vegetation development on sponge iron solid waste dump is required for stabilization of the dump, which can be achieved through restoration of soil microorganisms (Mukhopadhyay and Maiti, 2009). One of the most important groups of microorganisms which can effectively support the vegetation development in such scenario is mycorrhiza.

Mycorrhiza is the symbiotic association between soil fungi with the roots of vascular plants (Sieverding, 1991). In mycorrhizal association, the host plant receives mineral nutrients while the fungus obtains photosynthetically derived carbon compounds (Harley, 1989). Arbuscular mycorrhiza (AM) fungi (formerly VAM) are a type of mycorrhiza characterized by forming arbuscules, hyphae and vesicles within root cortex cells (Brundrett, 1991). About 80% of all terrestrial plant species (Smith and Read, 1997) and 95% of vascular plants are characteristically mycorrhizal (Quilambo, 2000). AM are among the most common soil microorganisms and constitute an important functional component of the soil-plant system occurring in almost all habitats and climates (Barea *et al.* 1997), including disturbed soil (Bundrett *et al.*, 1996; McGonigle and Miller, 1996)

It has been well establish that AM fungi improves plant growth in terms of better nutrient uptake, specially phosphorous, water relations, stress tolerance, production of growth promoting substances and protection from root pathogen etc. (Allen and Allen, 1980; Hayman, 1982a; Schreiner *et al*., 1997; Smith and Read, 1997; Turk *et al.*, 2006). They also have important role in ecosystem establishment (Quoreshi, 2008). The importance of AM for reclamation and reestablishment of vegetation on degraded sites have been emphasized by many workers (Rao & Tak, 2002; Singh, 2003; Sheoran *et al.*, 2010; Kumar *et al.*, 2010). However reports on AM root association of the plants growing on sponge iron solid waste dumps are scanty in India, where large numbers of sponge iron industries are operating. Therefore in the present study attempt is been made to analyze the AM root colonization in the herbaceous vegetation of different age series sponge iron solid waste dumps in the light of reclamation.

Materials and Methods

Study site

The study was carried out in solid waste dumping site of Scans Steels limited, Sundargrah, Odisha during 2011-2012. Geographical location of the area is between 20º11' North Latitude and 84º19' East Longitude. Altitude of the area is about 213m above the mean sea level. The area experiences tropical climate with three distinct seasons i.e. summer, rainy and winter. The mean annual rainfall in the area is 1422mm and mean air temperature of the area varies from 10ºC to 45ºC. The relative humidity fluctuates from minimum of 40% to maximum of 83%. In the sponge iron solid waste dumping site, accumulation of solid waste over years resulted in formation of different age series of dumps. Dump age is expressed as time since the establishment of dump in the site. For the present study freshly laid dump (D_0), 1 year (D_1), 3 year (D_3) and 5 year (D_5) old dumps were selected. During dumping of the solid waste, when the dump attains sufficient height, soil of the adjacent area is covered over the dump for stabilization. Thus, D_1, D_3 and D_5 were with soil cover, where as D0 was

without soil cover. A natural site adjacent to the waste dumping site was been taken as control site (C) for reference.

Sample collection

The fine roots of herbaceous plant species in different age series sponge iron solid waste dump and control site were collected randomly and mixed together to get a composite sample for each species. The root samples were kept in polythene bags and brought to the laboratory for mycorrhizal study.

Inspection of AM root colonization

The root samples were washed thoroughly with tap water in order to remove the adhering soil particles and cut in to pieces of approximately 1cm length. The root segments were cleared by heating in 10% KOH (w/v) at 90^{o}C in water bath for 30 min (Phillips and Hayman 1970). After heating, the KOH solution was poured off and the root samples were washed several times in distilled water. Roots were covered with a freshly prepared alkaline H_2O_2 solution to bleach for 60 min. Alkaline H_2O_2 was prepared by adding 3ml of NH_4OH_2 to 30ml of 10% H_2O_2 and 567ml of distilled water. The bleaching solution was discarded and the roots were rinsed with water. The root samples were then acidified by placing them in 2% HCl (v/v) for 5 min. The roots were again rinsed several times with distilled water. The cleared roots were stained with Lactoglycerol Trypan Blue (0.05%) stain and incubated for 45 mins at 90°C. After staining the root samples were washed with water and placed over glass slide for observation under compound microscope. The level of colonization in each root segment was measured by the method of Giovannetti & Mosse (1980) which involved gentle squashing of stained root segment on a microscope slide after covering with a cover slip. For each plant species 50 root pieces were observed. The percentage of mycorrhizal colonization was estimated by following formula:

$$\text{Mycorrhizal colonization (\%)} = \frac{\text{No. of root colonized with AM}}{\text{Total no. of roots inspected}} \times 100$$

$$\text{Percentage Occurance (\%) of a particular AM structure} = \frac{\text{No. of root with a particular AM structure}}{\text{Total no. of roots colonized with AM}} \times 100$$

Results and Discussion

The percentages of mycorrhizal root colonization in herbaceous vegetation (grass, sedge and forbs) of different sites were presented in Appendix-1. Freshly laid dump (D_0) was devoid of any vegetation, hence there was no scope for studying the mycorrhizal root colonization. Total of 34 herbaceous species in D_1, 63 in D_3 and 85 in D_5 and control site each, were assessed for the study of AM root colonization. It was found that in D_1 88% and in D_3, D_5 & Control site each 92% of the herbaceous plant species were associated with mycorrhiza (Figure 1). Furthere, herbaceous vegetation was divided in three catagories such as: Grass, Sedge and Forbs. In all the sites all the grass species (100%) were associated with mycorrhiza. Mycorrhizal association in among the sedges in D_1 and D_3 were 20% and 17% respectively, where

as in D_5 and control site were 14% each. With respect to forbs, the mycorrhizal association was 100% in D_1 and D_3 sites. However, in D_5 & control site, the association was 98%. The percentage of mycorrhizal colonization varies from 0-90% in different herbaceous plant species of D_1, D_3, D_5 and Control site (Appendix-1).

Among the grasses, all were found to be associated with mycorrhiza in all the sites. Highest percentage of mycorrhizal root colonization was observed in *Eragrostis tenella* and lowest in *Bothricholoa pertusa* in all the sites. Among the sedges (Cyperaceae) only one species *Cyperus kyllingia* was associated with mycorrhiza in all the sites. Among the forbs, except *Celosia agerantea* (Amaranthaceae), all were associated with mycorrhiza and highest percentage of mycorrhizal root colonization was observed to be present in *Desmodium triflorum* in all the sites, whereas lowest percentage of association was in *Solanum surattense* in D_1 and in *Gomphrena celsiodes* in D_3, D_5 and control site. Among all the herbaceous species highest percentage of mycorrhizal root colonization was found in *Desmodium triflorum* (forbs) and lowest in *Cyperus kyllingia* (sedge). In general, the percentage of mycorrhizal colonization in the herbaceous plant species decreased with increasing age of the waste dumps.

On the basis of percentage of mycorrhizal root colonization, mycorrhizal association was divided into five levels such as: very high (≥ 70%), high (50-69%), moderate (20-49%), low (< 20%) and no association. Further, mycorrhizal association was categorized into 4 types on the basis of presence AM hyphae (H), arbuscules (A) and vesicle (V) such as: presence of hyphae only (H), hyphae & arbuscules (H + A), hyphae & vesicle (H + V) and hyphae, arbuscules & vesicle (H + A + V). The number of plant species fall under different level and category of association was represented in Table 1.

With respect to different level of association it was observed that, in D_1 50% of the plant species were with very high level, 29% with high level, 9% with moderate and 12% with no association. Plants with low level of association were absent in D_1. In D_3 16% of the plants were with very high level, 36% with high level, 6% with moderate and 5% with no association. In D_3 none of the plants were with low level of association. In D_5 8% of the plants were having very high level of association, 60% were having high level, 22% were having moderate level, 1% was having low level and 8% were having no mycorrhizal association. In control site none of the plants were with very high level of association. However, 32% of the plants were with high level, 56% with moderate level, 4% with low level and 8% with no association.

In the present study highest percentages of plants were with hyphae, arbuscules and vesicle (H+A+V) structure in all the sites. In the waste dumps more percentage (30-19%) of plants were with hyphae & arbuscules (H+A) structure and than percentage (13-10%) of plants with hyphae & vesicle (H+V), where as in the control site more percentage (35%) of plants were with hyphae & vesicle (H+V) than hyphae & arbuscules (H+A) structure (9%).

In the younger dumps i.e in D_1 and D_3 equal percentage (i.e 10 &12%) of the plants were hyphae & arbuscules (H+A) and hyphae only (H) respectively, but in older dump i.e in D_5, more percentage (13%) of plants were with hyphae & vesicle (H+V) than (10%) hyphae only (H). There was a gradual increasing trend in the percentage of plants

with hyphae & vesicle (H+V) structures with increasing age of the waste dumps. However, no specific trend was observed with respect to the percentage of plants associated with hyphae, arbuscules and vesicle (H+A+V), hyphae & arbuscules (H+A) and hyphae (H) only with age of the waste dumps. In the control site about equal percentage of the plant were associated with hyphae, arbuscule & vesicle (H + A+ V) and with hyphae & vesicle (H + V) structures.

In the present study it was observed that relatively high percentage of herbaceous plant species have AM colonization in their root. This reflects the effective role of AM association in plants colonizing the sponge iron solid waste dumps. The importance of mycorrhizal association for vegetation establishment in coal mine spoil (Kumar *et al*., 2003; Ekka & Behera, 2010), lime stone mine spoil (Singh and Jamaluddin, 2011), toxic mine tailings (Leung *et al*., 2007), sand dunes (Beena *et al*., 2001) and salt alkaline soil (Raghuwanshi and Upadhyay, 2010) are already being emphasized, which are in agreement with the observation of present study.

Among the grasses, all were found to be associated with mycorrhiza in all waste dumps which highlights the effective role of grasses as pioneer species during vegetation succession in different derelict lands (Singh, 2004; Singh and Singh, 2006; Bohre *et al*., 2012). The absence of mycorrhizal root colonization in some of the plant species could be explained by the fact that families such as Chenopodiaceae, Fumariaceae, Zygophylaceae, Cactaceae, Junceaceae, Cyperaceae, Amaranthaceae and Commelinaceae are widely thought to be non mycorrhizal, although some of the species belonging to these families on occasion to be associated with AM under certain condition (Newman and Reddell, 1987; Neeraj *et al*., 1991 and Oringa *et al*., 1997). The absence of AM association in the non-mycorrhizal plant species is might be due to the presence of antifungal compounds in the root cortical issue or in root exudates (Tester *et al*., 1987; Thangaswamy *et al*., 2005). Highest percentage of mycorrhizal root colonization in *Eragrostis tenella* among the grasses and in *Desmodium triflorum* among forbs enables them to adapt themselves better than the other species in the waste dumps and to become dominant grass and forbs respectively (Kullu and Behera, 2011).

The present study revealed that with respect to the level AM root colonization, as the age of the waste dumps increased the percentage of plants with very high level of association gradually decreased. In the control site plants with very high level of association were absent and relatively high percentage of plants were with moderate level of association were present. Further, in D_1 and D_3 plants with low level of association were not present. All these observations indicated that at very early stage of vegetation succession, very high level of AM association of outmost importance for the establishment herbaceous species and as the succession precedes the level of association gradually decrease. This is supported by the findings of Ekka & Behera (2010) in different age series coal mine spoil.

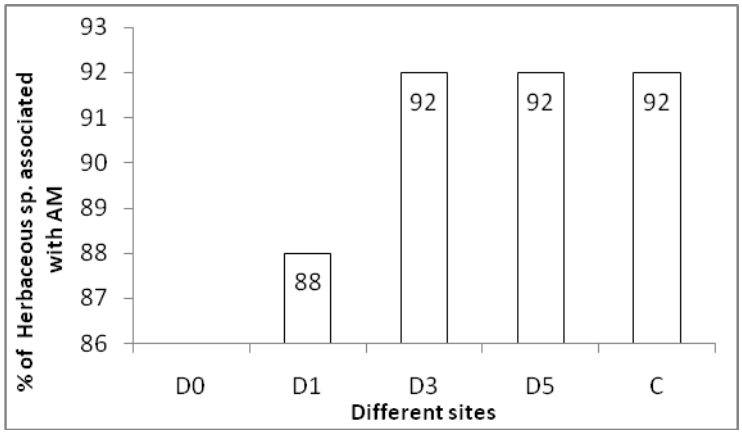
Presence of any one among the three types of AM structures hyphae, arbuscule and vesicle are normally used to designate the AM association (Pezzani *et al*., 2006). Arbuscules are ephemeral structures which are present in active root and responsible for nutrient transfer to the host plant (Cox and Tinker, 1976). AM vesicles are considered as storage organ produced in the older region of the infection (Kumar *et al*., 2003).

Table.1 Number of plant species with different level and category of AM root colonization in different age series sponge iron solid waste dumps (D0, D1, D3 and D5) and control site (C).

Categorization		Percentage of plants in different sites			
		D_1	D_3	D_5	C
Level of Association	Very high (≥ 70%)	50	25	8	0
	High (50-69%)	29	57	60	32
	Moderate (20-49%)	09	10	22	56
	Low (< 20%)	0	0	2	4
	No association	12	8	8	8
Type of Association	H	10	12	10	20
	H+A	30	16	19	9
	H+V	10	12	13	35
	H+A+V	50	60	58	36

* **Type of AM association:** hyphae only (H); hyphae & arbuscules (H + A); hyphae & vesicle (H + V); hyphae, arbuscules & vesicle (H + A + V)

Fig.1 Percentage of plant species associated with AM in different age series sponge iron solid waste dumps (D0, D1, D3 and D5) and control site (C).



Highest percentage of plants with hyphae, arbuscule and vesicle vesicle (H + A+ V) structures in all the sites indicated the importance of AM structure for nutrient transfer as well as for storage organ. More percentage of plants were with hyphae & arbuscules (H+A) structure than hyphae & vesicle (H+V) in the waste dumps reflects the importance of synergistic action of hyphae and arbuscule for transfer of nutrients from low nutrient content substrate rather than the development of storage structure like vesicle, during the early stage of vegetation succession as observed in the present study. Gradual increasing trend in the percentage of plants associated with hyphae & vesicle (H+V) structures with increasing age of the waste dumps, might be explained by the fact that with increasing age, there is gradual nutrient enrichment in the waste dumps which might reduce the necessity of nutrient absorptive structure and storage structure vesicle develops more in number. About equal percentage of the plant associated were with hyphae, arbuscule & vesicle (H + A+ V) and with hyphae & vesicle (H + V) in the control site indicate the importance of storage structure like

vesicle in stable herbaceous plant community.

In conclusion, from the present study it was found that relatively high percentage of herbaceous plant species have AM colonization in their root. This reflects the effective role of AM association in plants colonizing the sponge iron solid waste dumps. AM association is well known to enhance plant growth by increasing nutrient uptake and stress tolerance. Solid wastes generated from iron industrial units are usually deficient in nutrients and have high metal contents, low hydrological regime and high pH. Thus, the establishment of mycorrhizal association is quite essential for proper vegetational development and subsequent reclamation of the solid waste dumps. In this connection knowledge of the AM fungi associated with the plants growing on sponge iron solid waste dump has crucial importance for their use in future reclamation and management programs.

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Appendix-1: Percentage of Arbuscular mycorrhizal (AM) root colonization in herbaceous vegetation of different age series sponge iron solid waste dumps (D_0, D_1, D_3 and D_5) and control site (C).

Sl no	Name	Family	D_0	D_1	D_3	D_5	C
	Grass						
1	*Alloteropsis cimicina* (L.) Stapf.	Poaceae	_	68	65	60	55
2	*Aristidia adscensionis* L.	Poaceae	_	_	_	56	51
3	*Aristidia hystrix* L.f	Poaceae	_	_	63	60	54
4	*Bothricholoa pertusa* L.	Poaceae	_	48	43	40	35
5	*Chloris barbata* Sw.	Poaceae	_	_	_	58	46
6	*Cynodon dactylon* (L.) Pers.	Poaceae	_	_	78	75	60
7	*Dactyloctenium aegyptium* (L.) P. Beav.	Poaceae	_	57	55	50	43
8	*Dicanthium annulatum* (Forssk) Staff.	Poaceae	_		58	52	45
9	*Dicanthium aristatum* (Poir.) Hubbard.	Poaceae	_	53	50	50	44
10	*Digitaria biformis* Willd.	Poaceae	_	_	_	53	40
11	*Digitaria cilliaris* (Retz.)Koeler.	Poaceae	_	_	57	54	48
12	*Digitaria longiflora* (Retz.) Pers.	Poaceae	_	_		46	43
13	*Echinochola colona* (L.) Link.	Poaceae	_	_	61	58	50
14	*Eleusine indica (*L.) Gaerthn.	Poaceae	_	_	_	49	42
15	*Eragrostis riparia* Willd.	Poaceae	_	78	76	73	51
16	*Eragrostis tenella* (L.) P.Beav	Poaceae	_	88	85	78	45
17	*Eragrostis unioloides* Retz.	Poaceae	_	83	78	75	53
18	*Hemarthria compressa* (L.) R. Br.	Poaceae	_	_	_	58	42
19	*Iseilema anthephoroides* Hack.	Poaceae	_	_	_	52	38
20	*Leersia hexandra* Sw.	Poaceae	_	_	_	74	52
21	*Oplismenus burmanii* (Retz.) P.Beauv.	Poaceae	_	_	_	66	46
22	*Panicum notatum* Retz.	Poaceae	_	55	53	51	40
23	*Panicum psilopodium* Trin.	Poaceae	_	_	50	47	42
24	*Panicum repens* L.	Poaceae	_	_	_	58	53
25	*Panicum triferon* Schult.	Poaceae	_	_	56	54	46
26	*Paspalidium flavidum* Retz.	Poaceae	_	73	70	65	49
27	*Paspalum scorbiculatum* L.	Poaceae	_	58	55	52	46
28	*Saccharum spontaneum* L .Mant	Poaceae	_	_	60	58	51
29	*Setaria glauca* Retz.	Poaceae	_	65	59	52	42
30	*Sporobolus diander* Retz.	Poaceae	_	_	67	60	53

	Sedge						
31	*Cyperus iria* L.	Cyperaceae	_	0	0	0	0
32	*Cyperus kyllingia* Endl.	Cyperaceae	_	23	21	17	15
33	*Cyperus puntitculatus* Vahl	Cyperaceae	_	0	0	0	0
34	*Cyperus rotundous* L.	Cyperaceae	_	0	0	0	0
35	*Cyperus tenucispica* Steud.	Cyperaceae	_	_	_	0	0
36	*Fimbristylis bisumbellata* Forssk.	Cyperaceae	_	0	0	0	0
37	*Fimbristylis ovata* Burm f.	Cyperaceae	_	_	0	0	0
	Forbs						
38	*Achyranthus aspera* L.	Amaranthaceae	_	_	32	23	18
39	*Celosia agerantea* L.	Amaranthaceae	_	_	_	0	0
40	*Gomphrena celsiodes* (Ait) R.Br	Amaranthaceae	_	_	26	20	15
41	*Ageratum conyzoides* L.	Asteraceae	_	_	66	58	54
42	*Blumea lasera* (Burm.f) D.C	Asteraceae	_	_	72	65	60
43	*Caesulia axillaris* Roxb.	Asteraceae	_	_	64	58	53
44	*Eclipta prostata* L.	Asteraceae	_	_	54	50	46
45	*Emilia sonchifolia* L.DC	Asteraceae	_	_	50	46	40
46	*Tridax procumbense* L.	Asteraceae	_	74	70	65	54
47	*Borreria hispida* (L.) K. Schum.	Boraginaceae	_	70	68	64	48
48	*Commolina bengalensis* L.	Commelinaceae	_	_	_	46	35
49	*Murdania nudiflora* L.	Commelinaceae	_	_	_	44	30
50	*Toningea axilaris* (L.) Kuntze	Commelinaceae	_	_	_	40	28
51	*Evolvulus alsinoides* L.	Convolvulaceae	_	76	73	67	60
52	*Evolvulus nummularius* L.	Convolvulaceae	_	78	75	65	58
53	*Ipomea obscura* Ker-Gawl	Convolvulaceae	_	80	76	68	60
54	*Merremia tridentata* L.	Convolvulaceae	_	_	_	54	50
55	*Euphorbia hirta* L.	Euphorbiaceae	_	63	60	57	52
56	*Phyllanthus fraternus* Webster	Euphorbiaceae	_	_	59	55	41
57	*Phyllanthus simplex* Retz.	Euphorbiaceae	_	_	55	50	48
58	*Phyllanthus urinaria* L.	Euphorbiaceae	_	_	53	49	40
59	*Sebastiania chamaelea* (L.) Muell.	Euphorbiaceae	_	_	_	43	38
60	*Aeschynomene indica* L.	Fabaceae	_	68	65	58	51
61	*Alysicarpus monilifer* (L.)DC	Fabaceae	_	77	75	68	44
62	*Alysicarpus vaginalis* (L.)DC	Fabaceae	_	74	71	64	43
63	*Desmodium triflorum* (L.)DC	Fabaceae	_	90	88	85	52
64	*Indigofera enneaphylla* L. (linnaei Ali)	Fabaceae	_	_	_	65	46
65	*Medicago sativa* L.	Fabaceae	_	_	_	52	41
66	*Zornia diphylla (L.) Pers.*	Fabaceae	_	73	70	65	53
67	*Leucas aspera* (Willd.) Link.	Lamiaceae	_	68	63	56	45
68	*Leucas cephalotus* (Roth) Spreng	Lamiaceae	_	_	58	51	40

69	*Hyptis suaveolens* L.	Lamiaceae	_	_	_	48	36
70	*Ammania baccifera* L.	Lythraceae	_	_	62	55	50
71	*Sida acuta* Burm f.	Malvaceae	_	_	68	63	43
72	*Sida rhombifolia* L.	Malvaceae	_	_	65	60	41
73	*Sida cordata* Burm f.	Malvaceae	_	_	60	56	39
74	*Urena sinuota* L.	Malvaceae	_	_	58	50	43
75	*Molluga pentaphylla* L.	Molluginceae	_	_	63	56	51
76	*Ludwigia parviflora* Roxb. (Perennis)	Onagraceae	_	_	_	48	38
77	*Polygela chinesis* autt. non. L.	Polygelaceae	_	_	_	45	33
78	*Oldenlandia corymbosa* L.	Rubiaceae	_	83	76	70	44
79	*Linderina ciliata* (Colsm.)	Scrophulariaceae	_	72	69	64	50
80	*Linderina crustaceae* (L.) F.V.Muell.	Scrophulariaceae	_	75	70	68	53
81	*Scoparia dulcis* L.	Scrophulariaceae	_	58	47	45	40
82	*Solanum surattense* Burm f.	Solanaceae	_	32	30	27	22
83	*Melochia chorchorifolia* L.	Sterculiaceae	_	55	53	46	35
84	*Chorchorious aestuans* L.	Tiliaceae	_	_	54	51	43
85	*Hybanthus enneaspaermus* (L.) F.V. Muell.	Voilaceae	_	_	53	46	38

'_' indicates absence of the plant species