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Pigment system constitution as an indicator for performance assessment of tree species growing under vehicular pollution load

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ABSTRACT

Vehicular pollution is one of the major stressors along the high-traffic urban roads that plants need to cope up to flourish. Alteration in pigment content is a prudent response of most of the plants under environmental stresses. Effect on pigment system is one of the major indicators of metabolic status of plant, and magnitude of stress effect on the constituent components of the pigment system depends on various factors. Therefore, quantification of stress effect on the pigment system could be a useful tool for determining vulnerability/endurance of the species to the stressful ambience. To determine suitability of plant species in polluted niche, often Air Pollution Tolerance Index (APTI) is used, which takes account of total chlorophyll content as the representation of pigment system. In this study, in addition to total chlorophyll content, chlorophyll a/b ratio and carotenoids content were assessed in 24 commonly planted tree species growing around crowded urban roads, and compared with their counterparts in less polluted non-urban environment. It was found that air pollution effects on chlorophyll a:b ratio, carotenoids content in addition to total chlorophyll content, and response of pigment components were different amongst species. Most of the species showed no change in total chlorophyll content over control, but remarkable increase in chlorophyll a/b ratio coupled with reduction in carotenoids content. Five different patterns of responses were observed thus the species were categorised accordingly. The pattern of response is found to have pronounced effect on stress tolerance and susceptibility of species. Tolerance or susceptibility of pigment system to air pollution is cumulative effect of response of chlorophyll a, chlorophyll b and carotenoids. It is inferred that these pigment components are important parameters to be taken into account for assessing suitability of plant species for air-polluted environment.

Keywords: *Chlorophyll a/b ratio; carotenoids; total chlorophyll; vehicular pollution; trees; pollution tolerance*

1. INTRODUCTION

Pigment content alteration is inevitable response in all environmental stresses experienced by plants (Munne´-Bosch and Alegre, 2004). The chloroplast is one of the primary site of attack by air pollutants such as SPM, SO_x and NO_X. Air pollutants penetrate into tissues through stomata causing denaturation of chloroplast and decrease in pigment content in the cells of polluted leaves. Disruption of Mg^{2+} centre of chlorophyll and formation of pheophytin upon exposure to various pollutants has been reported by several researchers (Rao and Leblanc, 1966; Tripathi and Gautam, 2007; Gitelson et al., 2016). Reductions in total chlorophyll contents of plants under ambient air pollution have been reported by several researchers. Agrawal et al. (2003) reported reduction in chlorophyll in various crops grown in suburban area versus that in rural areas of Varanasi, India and related it with air pollution status of the area. In trees like *Mangifera indica*, *Tectona grandis*, *Shorea robusta*, *Holoptelea integrifolia, Eucalyptus citridora and Mallotus philippinensis* exposed to vehicular pollution, the reduction in chlorophyll content has been documented (Joshi and Swami, 2009). Under urban air pollution, depletion of chlorophyll content was found to be correlated to pollution load with maximum depletion at heavily polluted site and so on in *Mangifera indica, Cassia fistula and Eucalyptus hybrid* (Tripathi and Gautam, 2007). Variations in chlorophyll contents have been studied by various workers with respect to air pollution stress (Pande, 1985; Kuddus et al., 2011; Adamsab et al., 2011; Panigrahi et al., 2012; Radhapriya et al., 2012; Sadeghian and Mortazaienezha, 2012; Nugraharani et al., 2012; Enete et al., 2012; Tanee and Albert, 2013). Plants having higher air pollution tolerance and sequestration capacity generally retain pigment content even under air pollution stress. This fact was studied by several researchers and it is generalized that higher chlorophyll content infers air pollution tolerance in plants

(Shannigrahi et al., 2003; Prajapati and Tripathi, 2008; Rai and Panda, 2014).

Effect of air pollution on Carotenoids pigment is also documented by several researchers (Singh et al., 1985; Singh et al., 1990; Dhir et al., 1999; Nighat et al., 1999; Saquib, 2008; Iqbal et al., 2010). Saquib et al. (2010) have reported that sensitivity of Carotenoids to air pollution stress was 4.5 times higher than that of chlorophyll in an experiment with *Croton bonplandianum*. Role of Carotenoids as a protection mechanism against ROSs is also established (Fleschin et al., 2000). Effect of pollution exposure on chlorophyll a and chlorophyll b has also been studied. Verma and Singh (2006), reported that chlorophyll a content decreased with increasing pollution load. A wide variation in chlorophyll b content was also observed in the foliar tissue of these plants. Also as suggested by Kondo et al., 1980, higher plant species synthesize more of the chlorophyll a than chlorophyll b and a ratio of chlorophyll a to chlorophyll b provides a measure of tolerance index of a tree. High value of this ratio indicates better tolerance to air pollution and vice-versa. It is reported that efficiency of these pigments is directly related to GPP of plants (Gitelson et al., 2016) and can be used to model or monitor optimum performance of plant in variety of environmental conditions including air pollution. Peng et al., (2011) in their study used total canopy chlorophyll to model Gross Primary Productivity (GPP) of maize crop using remote sensing. Importance of pigment system in maintaining GPP has been highlighted in several studies (Heath, 1969; Terry, 1980; Dawson et al. 2003). GPP is the mathematical product of Photosynthetically Active Radiation (PAR) incident on plant canopy, PAR absorbed by plant canopy and light use efficiency of plant. Pigment system significantly affects magnitude of PAR absorbed by the plant (Heath, 1969) and also directly affects light use efficiency (LUE) (Terry, 1980). Variation in chlorophyll content has been reported to affect LUE values seasonally (Dawson et al., 2003) as well as temporally (Houborg et al., 2011).

In this study effects of air pollution on total chlorophyll content, carotenoids content and chlorophyll a/b ratio were assessed to determine effect of air pollution on the pigment system of a sizeable number of tree species.

2. MATERIALS AND METHODS

2.1. Experimental plan

Study was carried out using 24 commonly used tree species (Table 1) of Central India. The experimental case study was carried out with the trees of these species existing along the crowded urban roads of Nagpur city, Maharashtra state, India. These plants, 10-15 meters height and nearly 1-2 meters girth about more than 20 years age, have been exposed to vehicular emissions for long time are considered as treated/exposed. Pigment system characters viz. total chlorophyll content, chlorophyll a/b ratio and carotenoids content affected by exposure to SO_2, NO_2 and particulate matter were assessed, in five replications each. The control counterparts of each species, with nearly similar age and morphology, were selected growing in a nearly negligible polluted area inside campus of CSIR-National Environmental Engineering Research Institute, Nagpur, Maharashtra, India. Annual average pollution status of control and experimental site is given in Table 2.

2.2. Biochemical parameters

Total chlorophyll content; chlorophyll a, chlorophyll b, and carotenoids content were determined using spectrophotometric method (Arnon 1949; Chouhan et al., 2012). For total chlorophyll content estimation, fresh leaves were collected from each tree species and washed with tap water to remove the dust and other particles from the surface. 500 mg of leaf sample was ground using adequate amount of 80% acetone and a pinch of acid washed sand was added. The macerate was centrifuged at 3000 rpm for 5 min and supernatant was collected. Supernatant was then made up to 25ml using 80% acetone. Optical density of the supernatant was determined by absorbance at different wavelengths viz., 480 nm, 510 nm, 645 nm, 652 nm and 663 nm for different pigemtns.

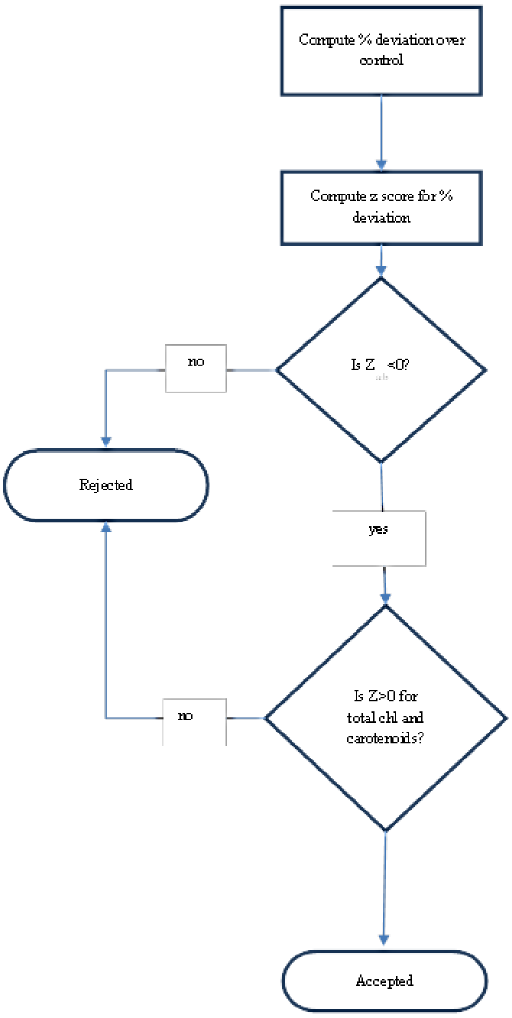

Figure 1. Procedure for selecting the tree species for air pollution tolerance using data of effect of air pollution on pigment system.

Concentration of different pigments was calculated using following formula.

$$\text{Total Chlorophyll Content} = \left(\frac{A_{652} \times 1000}{34.5}\right)\left(\frac{V}{1000 \times W}\right)$$

$$\text{Chlorophyll a Content} = [(A_{663} \times 12.7) - (A_{645} \times 2.69)]\left(\frac{V}{1000 \times W}\right)$$

$$\text{Chlorophyll b Content} = [(A_{645} \times 22.9) - (A_{663} \times 4.68)]\left(\frac{V}{1000 \times W}\right)$$

$$\text{Carotenoids Content} = [(A_{480} \times 7.6) - (A_{510} \times 1.49)]\left(\frac{V}{1000 \times W}\right)$$

Where, A_{480} - absorbance at 480 nm; A_{510} - absorbance at 510 nm; A_{645} - absorbance at 645 nm; A_{652} - absorbance at 652 nm; A_{663} - absorbance at 663 nm; V - final volume of supernatant (ml); W - weight of the leaf sample taken (g).

2.3. Statistical analysis

To compare effect of air pollution on pigment system, percent deviations of the exposed plants over the control with respect to total chlorophyll content, chlorophyll a/b ratio and carotenoids content were computed. Z-scores were computed for percent deviation for each parameter and better performing species for each parameter were identified (Saitanis et al., 2014; Saitanis et al., 2015). The procedure for selecting the species is outlined in Fig. 1. Statistical analysis was performed using the software 'SPSS Statistics for Windows', version 16.0.

Table 1. General information of tree species used in study.

S. No.	Species	Family	Common name	Habit	Growth rate	Evergreen/ Deciduous	Utility
1	*Alstonia scholaris*	Apocynaceae	Saptaparni	Tree	Quick	Evergreen	Medicinal and utility wood
2	*Anthocephalus chinensis*	Rubiaceae	Kadamba	Tree	Quick	Deciduous	Ornamental, timber, paper making, floriculture
3	*Artocarpus heterophyllus*	Urticaceae	Jackfruit	Tree	Slow	Evergreen	Food
4	*Azardiracta indica*	Meliaceae	Neem	Tree	Quick	Evergreen	Fruit, medicinal, food, organic pesticide
5	*Bauhinia purpurea*	Caesalpinaceae	Kanchan	Tree	Quick	Deciduous	Medicinal, ornamental
6	*Bougainvillea spectabilis*	Nyctaginaceae	Bogun	Shrub	Quick	Evergreen	Ornamental,
7	*Butea monosperma*	Fabaceae	Palash	Tree	Slow	Deciduous	Ornamental, timber, resin, fodder, medicine, and dye
8	*Calistremon citrinus*	Myrtaceae	Bottle brush	Tree	Slow	Evergreen	Ornamental
9	*Casuarina equisetifolia*	Casuarinaceae	Suru	Tree	Quick	Evergreen	Wood
10	*Cassia fistula*	Caesalpinaceae	Amaltas	Tree	Quick	Deciduous	Medicinal, ornamental
11	*Dalbergia sisso*	Fabaceae	Sisam	Tree	Moderate	Evergreen	Wood, medicinal
12	*Dendrocalamus strictus*	Poaceae	Bans	Shrub	Quick	Deciduous	Utility,
13	*Derris indica*	Fabaceae	Karanj	Tree	Quick	Evergreen	Bio-diesel, medicinal, ornamental
14	*Ficus religiosa*	Moraceae	Pipal	Tree	Slow	Evergreen	Medicinal
15	*Hibiscus rosasinensis*	Malvaceae	China rose	Shrub	Quick	Evergreen	Ornamental
16	*Lagerstromia speciosa*	Lythraceae	Jarul	Tree	Quick	Evergreen	Medicinal, ornamental
17	*Mangifera indica*	Anacardiaceae	Mango	Tree	Quick	Evergreen	Fruit, medicinal
18	*Mimusops elengi*	Sapotaceae	Bakul	Tree	Quick	Evergreen	Medicinal, timber
19	*Polyalthia longifolia*	Anonaceae	False ashok	Tree	Quick	Evergreen	Ornamental
20	*Saraca asoka*	Caesalpinaceae	Ashok	Tree	Quick	Evergreen	Ornamental
21	*Syzygium cumini*	Myrtaceae	Jamun	Tree	Quick	Evergreen	Fruit
22	*Tabernaemontana divaricata*	Apocynaceae	Chandani	Shrub	Quick	Evergreen	Ornamental
23	*Terminalia catappa*	Combretaceae	Indian almond	Tree	Quick	Deciduous	Ornamental, timber
24	*Thuja accidentalis*	Cupressaceae	Thuja	Tree	Quick	Evergreen	Ornamental, medicine, utility wood

Source: CPCB guideline for developing greenbelts, (2009)

Table 2. Annual average pollutant status of study area.

Sampling station		SO_2 (ug/m^3)	NO_2 (ug/m^3)	PM10 (ug/m^3)
Control area	NEERI Lab, Nehru Marg, Nagpur	3	14	55
Experimental area	Institute of Engineers, Nagpur	10	33	103
	MIDC office Hingana road, Nagpur	10	33	120

Source: National Ambient Air Quality Monitoring Programme (NAMP) Data, 2015

3. RESULTS

Pigment system in plants has considerable effect on GPP of plants. Adverse effect on pigment system results in reduction of GPP in turn reducing plant performance under stress. Hence, performance of pigment system under stress condition is important character imparting stress tolerance to the plant. Results of assessment of pigment system of 24 tree species showed that air pollution exposure has pronounced effect on pigment content which differs among the tree species (Fig. 2a,b,c). Estimation of total chlorophyll content, chlorophyll a:b and carotenoids content showed that all these parameters are affected by stress independently and their cumulative effect may result in deciding tolerance or susceptibility of the plant towards the air pollution stress. In present study, pigment characters were quantified in pollution exposed plants as well as their control counterparts. The difference in control and pollution exposed plants represents effect of stress. The magnitude of effect of stress was calculated in terms of percent deviation in exposed plants over control. Variability was observed among the species studied as some species exhibit positive percent deviation i.e. increase in parameter value in exposed over control whereas some show negative percent deviation i.e. decrease in parameter value thereof. Similar variability of response was observed in earlier studies by several researchers (Pande, 1985; Kuddus et al., 2011; Adamsab et al., 2011; Panigrahi et al., 2012; Radhapriya et al., 2012; Sadeghian and Mortazaienezha, 2012; Nugraharani et al., 2012; Enete et al., 2012; Tanee and Albert, 2013).

To compare the percent deviation data from all parameters among all species, data was normalized and standard Z scores were computed. In the case of, same plant species showing increase in total chlorophyll and carotenoids content under exposed condition over control, it is inferred that the plant species is able to withstand the stress or could even perform better under stress by enhancing its metabolism. Conversely, under stress condition chlorophyll b acts as accessory pigment and to combat stress a plant may trigger increasing chlorophyll b, thus the chlorophyll a/b ratio is decreased. With this premise, species showing Z score of percent deviation ≥ 0 in case of total chlorophyll content and carotenoids content, and ≤ 0 in case of chlorophyll a/b ratio is desirable (Fig. 1). Among the 24 species studied, 13 species showed affirmative response with no reduction or considerable increase in total chlorophyll content for plants dwelling in polluted area over their control counterparts. Except *Cassia fistula*, favorable response of other 12 species was coupled with similar response in chlorophyll a/b ratio or carotenoids content or both. This suggests that these two parameters support the plant to retain or increase their total chlorophyll content under stress. Chlorophyll content affecting PAR content absorption and LUE in stress in turn, this response was deemed to reduce GPP (Heath, 1969; Terry, 1980; Dawson et al. 2003; Peng et al., 2011; Gitelson et al., 2016). The results showed that response of pigment system to air pollution can be categorized in following ways.

3.1 Category 1: Gross primary productivity retained or enhanced on account of chlorophyll b and carotenoids

Some of the species studied here showed reduction in chlorophyll a/b ratio and increase in carotenoids content in exposed plants as compared to their control counterparts. These accounts for a typical stress response where accessory pigments i.e. carotenoids and chlorophyll b are synthesized to maintain the GPP productivity. Thus total chlorophyll content was maintained or increased. Increase in total chlorophyll content may be attributed to increase in chlorophyll b content. This type of response in pigment system was observed in *Mimusops elengi, Syzygium cumini, Derris indica and Mangifera indica* (Fig 3).

3.2 Category 2: GPP retained or enhanced on account of chlorophy ll b

Some of the species showed reduction in chlorophyll a/b ratio in exposed plants as compared to their control counterparts. This accounts for increase in chlorophyll b to maintain the GPP, which in turn allows total chlorophyll content to be maintained or increased. Increase in total chlorophyll content may be attributed to increase in chlorophyll b content. This event was observed in *Saraca asoka, Thuja accidentalis, Alstonia scholaris, Casuarina equisetifolia, Azardiracta indica, Dendrocalamus strictus and Butea monosperma* (Fig 4). Similar effect was also observed in Cassia fistula but the Z score of chlorophyll a:b ratio deviated slightly from zero (Zchl a:b= 0.11) making it an exceptional case.

3.3 Category 3: GPP retained or enhanced on account of carotenoids

In *Tabernaemontana divaricata* increase in carotenoids content coupled with increase in total chlorophyll in pollution exposed plants as compared to their control (Fig 5). Increase in total chlorophyll content may be attributed to increase in carotenoids content as it buffers the stress and protects other pigments from being affected from stress.

It has been earlier reported by several researchers that some of the tree species tend to retain their chlorophyll content in the event of air pollution stress and therefore possess tolerance to air pollution (Shannigrahi et al., 2003; Prajapati and Tripathi, 2008; Rai and Panda, 2014). In our study it is observed that plants in category 1, 2 and 3 retain their chlorophyll, by virtue of their ability to enhance chlorophyll b and/or carotenoids content. This suggests that higher chlorophyll content is the outcome of higher chlorophyll b and/or carotenoids content to impart air pollution tolerance to the plant.

3.4 Category 4: Reduction in total chlorophyll content despite of favorable response of chlorophyll a/b or carotenoids

Some of the species in present study show reduction in chlorophyll a/b ratio and/or increase in carotenoid content, but despite of it, these plants could not manage to retain its total chlorophyll content. Conversely, this finding suggests that total chlorophyll content is not adequate to impart air pollution tolerance. These species can be considered as susceptible candidates for vehicular pollution. This response was observed in *Terminalia catappa, Lagerstromia speciosa, Hibiscus rosasinensis, Polyalthia longifolia and Dalbergia sisso*. This response shows that these plants attempt to withstand stress but either due to heavy pollution load or any other abiotic stress the plants fail to cope up and result in reduced GPP.

3.5 Category 5: Unfavorable response of all the parameters

Some species in the study show unfavorable response in all the cases. These species are highly susceptible ones as they cannot cope up with the stress at all. Such species even if survive in the polluted environment, will not perform well due to reduced GPP. This type of response was observed in *Anthocephalus chinensis, Artocarpus heterophyllus, Bauhinia purpurea, Bougainvillea spectabilis, Calistremon citrinus and Ficus religiosa*.

Reduction in chlorophyll content and GPP thereof on air pollution exposure has been reported by many researchers in different crop and forest species (Rao and Leblanc, 1966; Joshi and swami, 2009; Gitelson et al., 2016). Tripathi and Gautam (2007) have also showed that this reduction is correlated with pollution load in many species and such species can be considered as susceptible ones.

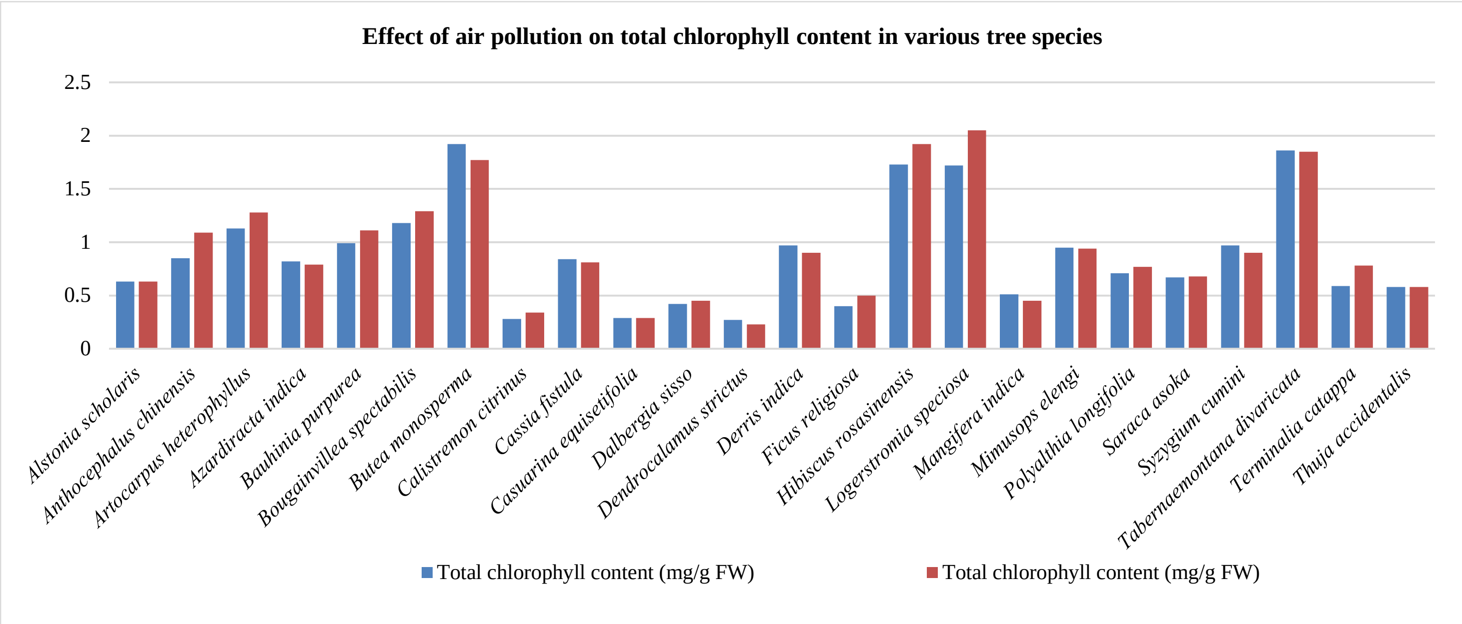

Figure 2a. Effect of air pollution on total chlorophyll content in various tree species

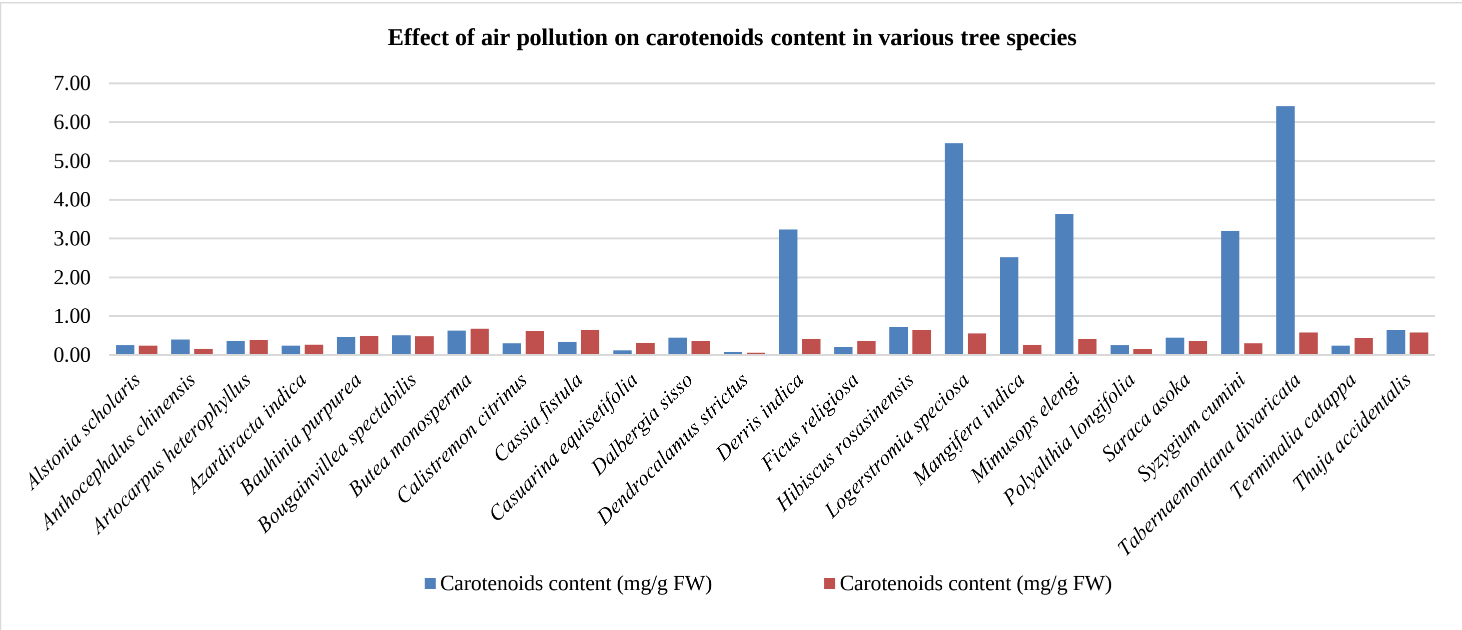

Figure 2b. Effect of air pollution on carotenoids content in various tree species

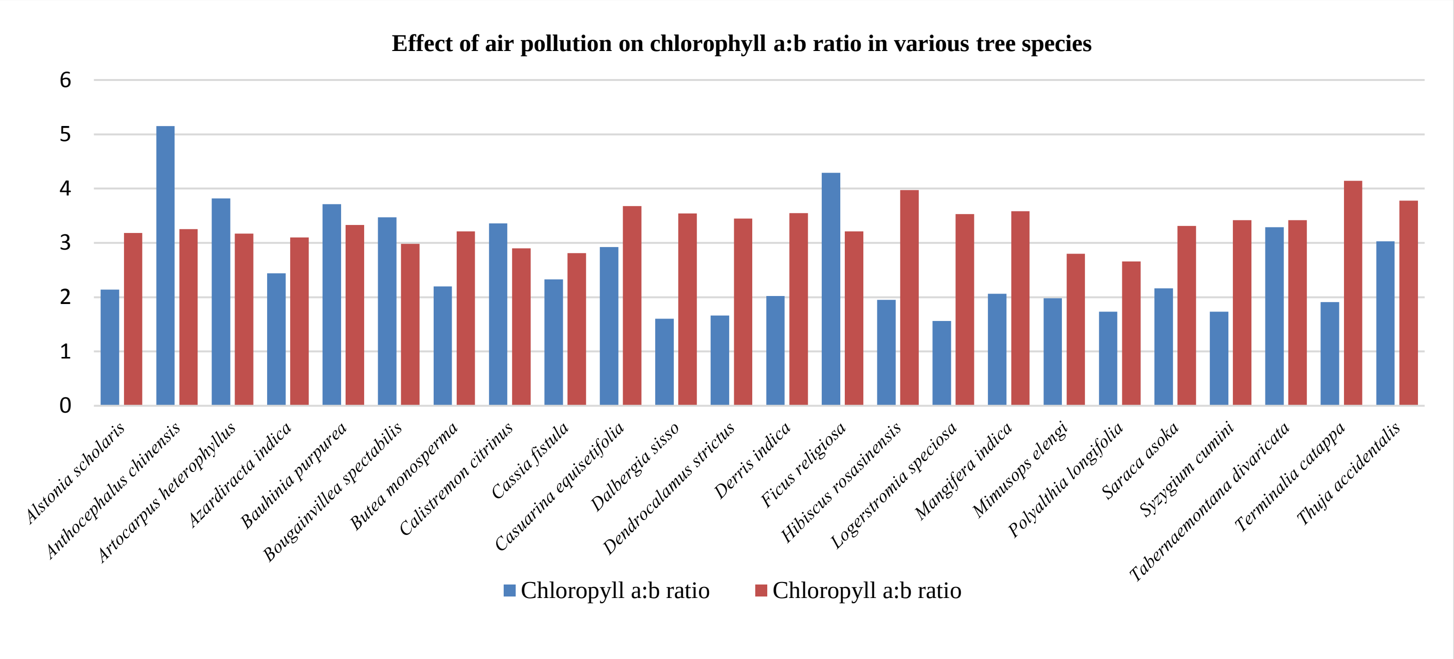

Figure 2c. Effect of air pollution on chlorophyll *a:b* ratio in various tree species

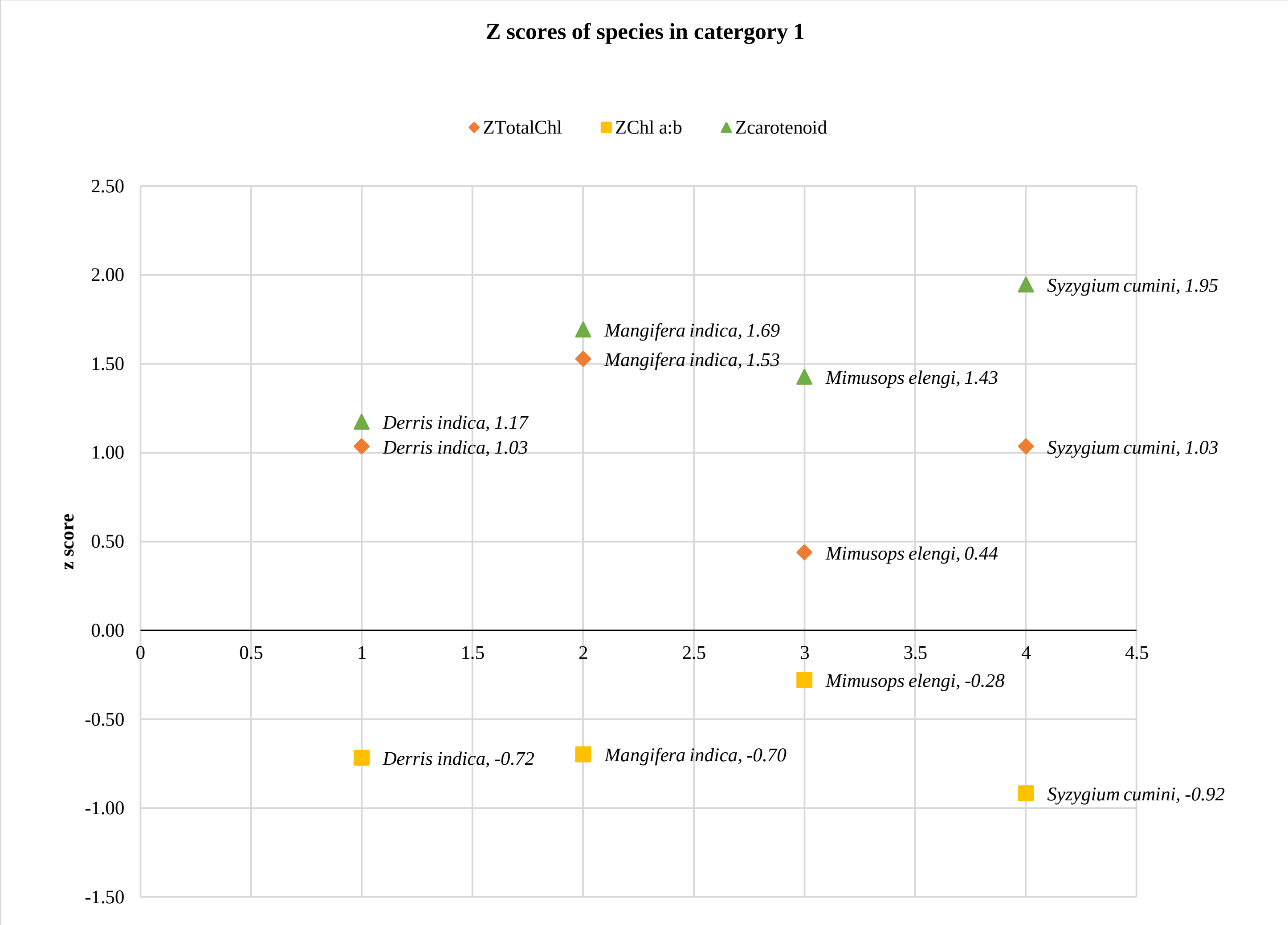

Figure 3. Representation of *Z* scores of the species in which GPP is retained on account of chlorophyll *b* and carotenoids

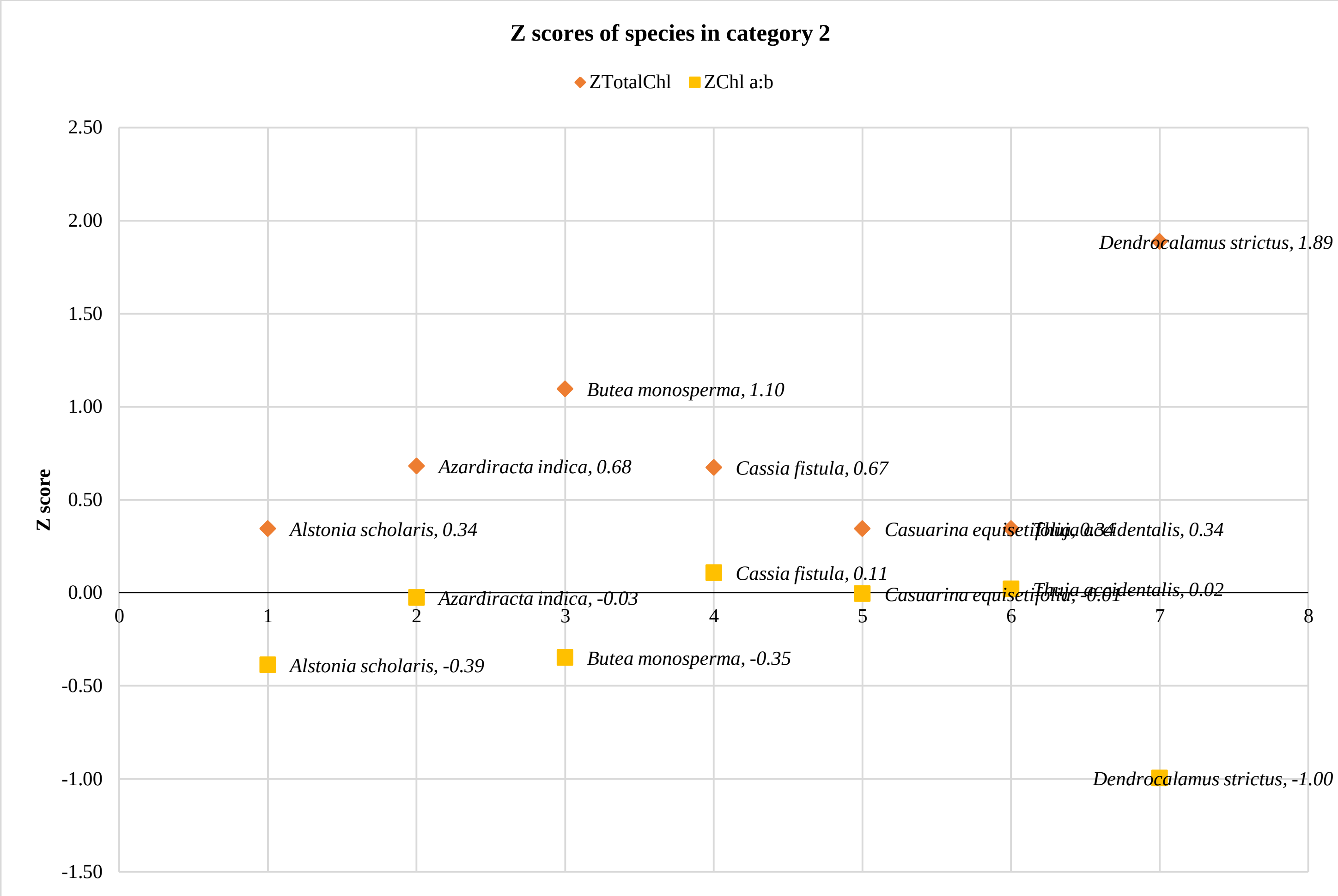

Figure 4. Representation of *Z* scores of the species in which GPP is retained on account of chlorophyll *b*

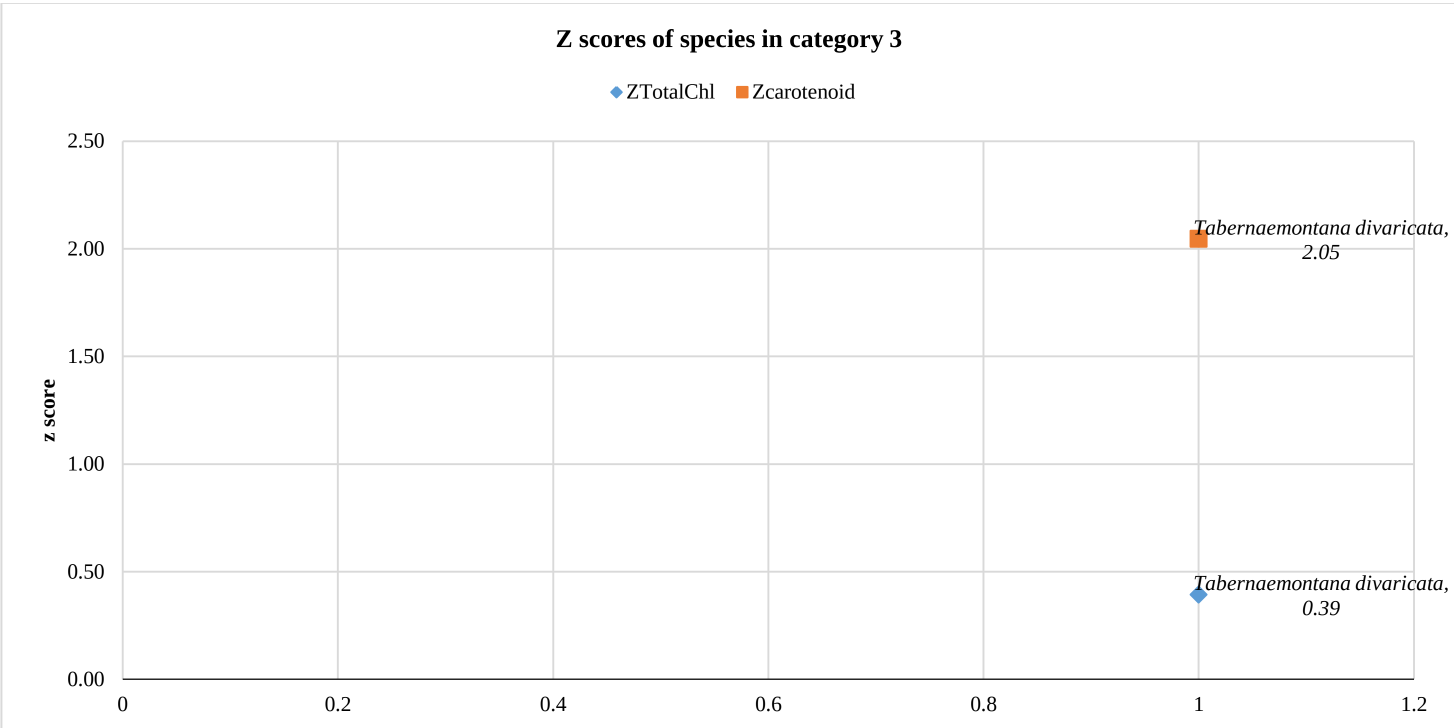

Figure 5. Representation of *Z* scores of the species in which GPP is retained on account of carotenoids

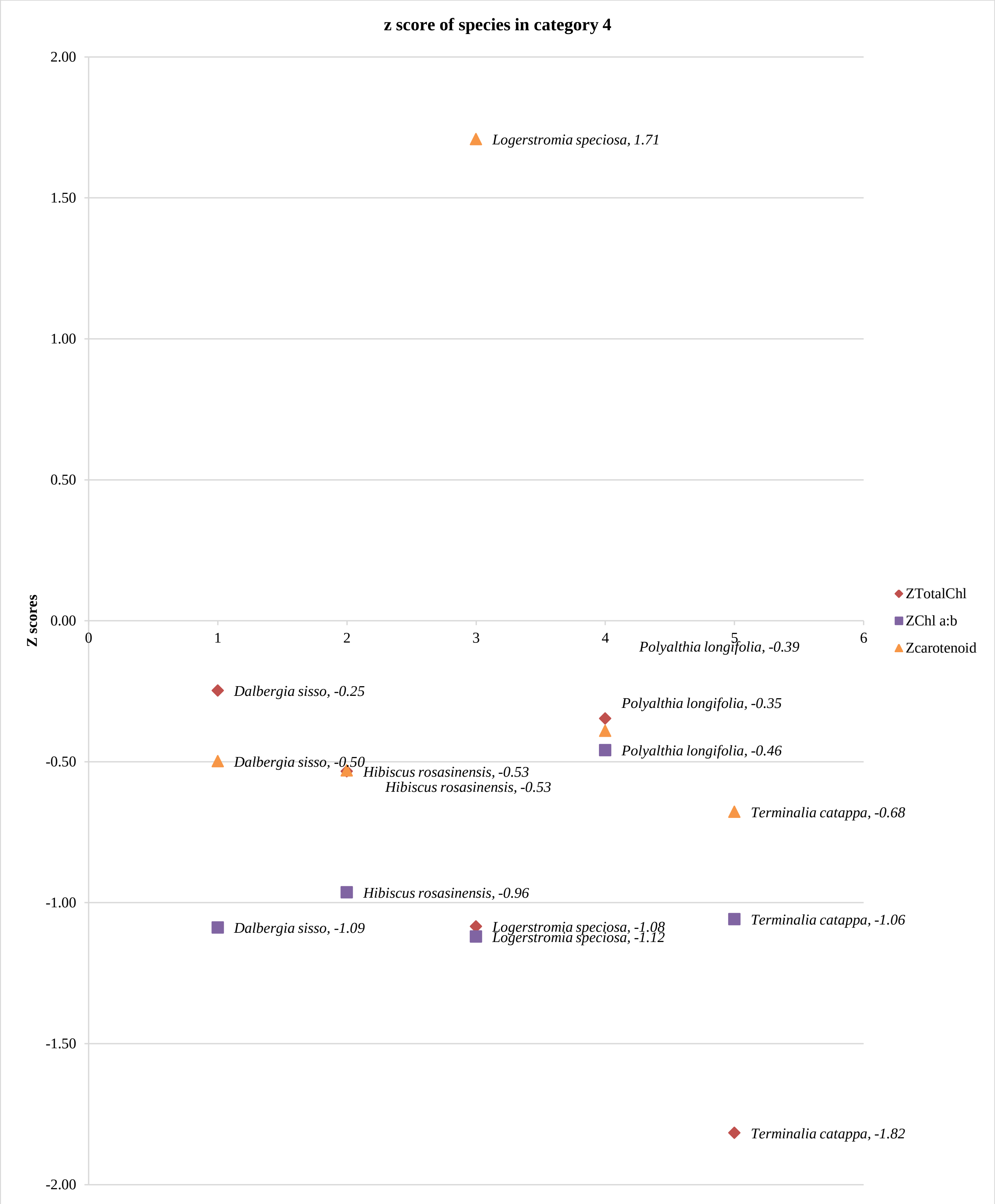

Figure 6. Representation of *Z* scores of the species in which GPP is reduced despite of favourable response of chlorophyll *b* and carotenoids

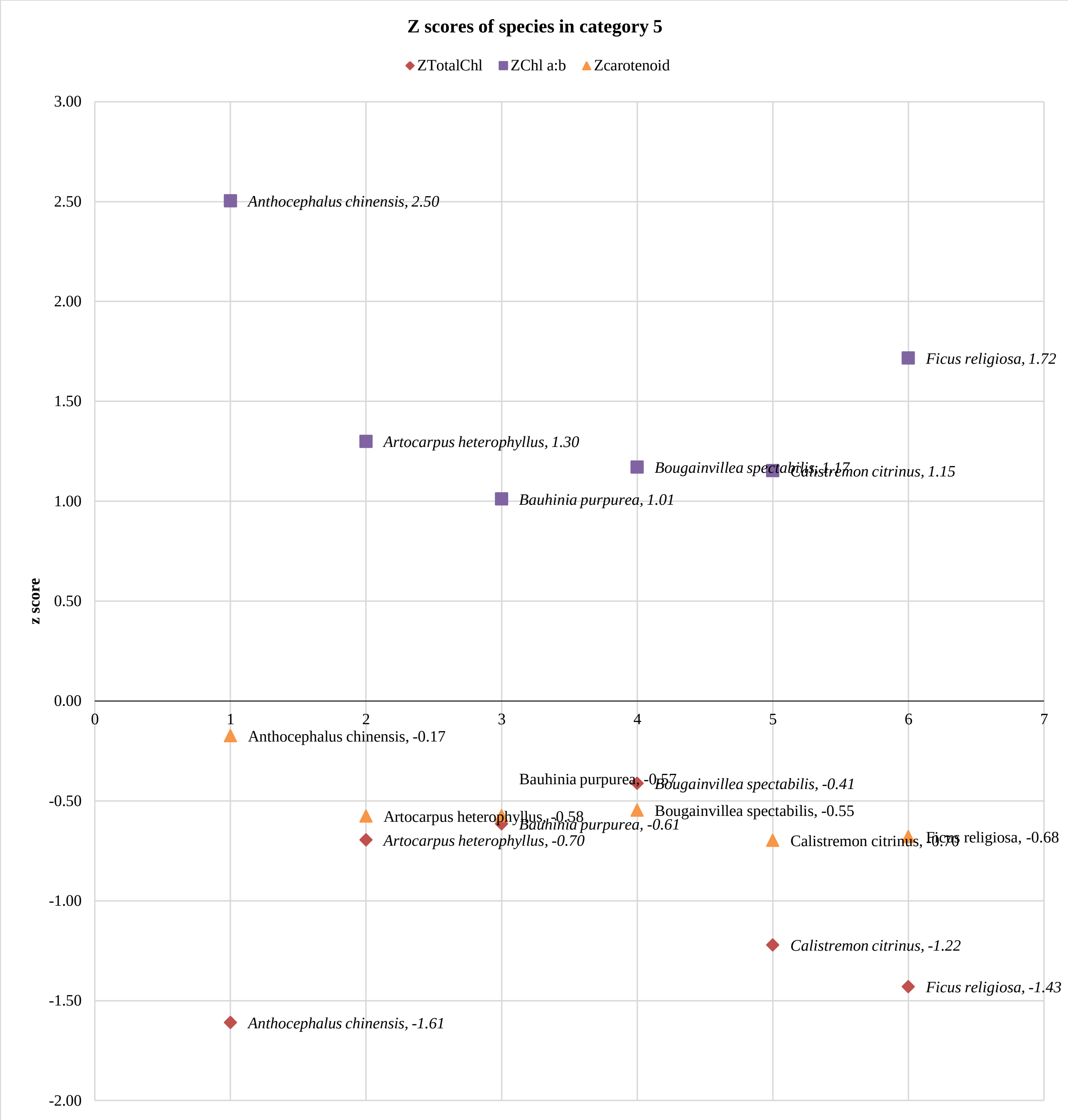

Figure 7. Representation of *Z* scores of the species in which GPP is reduced

4. CONCLUSIONS

Performance of pigment system under stress condition is important character imparting stress tolerance to the plant. Results of assessment of pigment system of 24 tree species showed that air pollution exposure has pronounced effect on pigment content which differs among the tree species. Estimation of total chlorophyll, chlorophyll a/b and carotenoids content showed that all these parameters are affected by stress independently, and their cumulative effect may result in deciding tolerance or susceptibility of the plant towards the air pollution stress. Variability was observed among the species studied as some species exhibit increase in parameter value in exposed over control whereas some show decrease in parameter value thereof. Based on the results, five categories of response of pigment system were identified. Tolerant plant retain their GPP on account of changes in chlorphyll b and/or carotenoids as these pigments combat with stress and protect the pigment system and GPP. Susceptible species either fail to retain GPP despite of changes in chlorophyll b and carotenoids or in some cases, these accessory pigments are also adversely affected by stress. It can be concluded that tolerance or susceptibility of pigment system towards air pollution is a function of cumulative behavior of chlorophyll a, chlorophyll b and carotenoids.

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