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Effect of 5-Aminolevulinic Acid (5-ALA) on Leaf Chlorophyll Fast Fluorescence Characteristics and Mineral Element Content of *Buxus megistophylla* Grown along Urban Roadsides

Hao Yang ¹, Jianting Zhang ¹, Haiwen Zhang ¹, Yi Xu ², Yuyan An ¹ and Liangju Wang ^{1,*}

¹ College of Horticulture, Nanjing Agricultural University, Nanjing 210095, China; 2019804194@njau.edu.cn (H.Y.); 2019104027@njau.edu.cn (J.Z.); 2019104008@njau.edu.cn (H.Z.); anyuyan0447@njau.edu.cn (Y.A.)

² Changzhou Park Management Center, Changzhou 213001, China; hdtang@ctie.edu.cn

* Correspondence: wlj@njau.edu.cn; Tel.: +86-25-84395265

Abstract: It is well known that trees grown on roadsides suffer from stressful environments, including poor soils, bad weather, and harmful gases from automobile exhaust. Improving the adaptability of roadside trees to adverse environments is important for urban management. An experiment was carried out with six-year-old *Buxus megistophylla* Levl. hedgerows, where 20 mg/L 5-aminolevulinic acids (5-ALA) solution was sprayed on the blade surface at the end of April. Three months later, plant morphology, chlorophyll fast fluorescence characteristics, antioxidant enzyme activities and the mineral element content were investigated. The results showed that leaf size and thickness were significantly greater with 5-ALA treatment, and the leaf color was also greener than those of the control. 5-ALA treatment significantly promoted the electron transfer activity of the PSII reaction center on the donor side, the reaction center itself and the receptor side. It reduced energy dissipation through the heat with increased photochemical quantum yields. The activities of antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) in leaves and roots, were stimulated by 5-ALA treatment. The content of soluble sugars and free proline in leaves was significantly increased by 5-ALA treatment, as were the absorption and accumulation of several kinds of mineral nutrient elements, such as nitrogen, phosphate, calcium, magnesium, iron, copper and boron. Additionally, 5-ALA application significantly increased the content of cadmium, mercury, chromium and lead in the roots but decreased them in the leaves. This implies that 5-ALA may induce a mechanism in *B. megistophylla* in which toxic elements were intercepted in roots to avoid accumulation in leaves, which ensured healthy growth of the aboveground tissues. 5-ALA may regulate the absorption and utilization of mineral nutrient elements in soil with the interception of toxic heavy metal elements in roots, promote leaf photosynthetic performance, induce the accumulation of soluble sugars and free proline, and improve the antioxidant enzyme systems for plants to adapt to the stressful environment of urban roads. These results provide a basis for 5-ALA applications alongside city roads.

Keywords: 5-aminolevulinic acid; *Buxus megistophylla*; chlorophyll fast fluorescence characteristics; mineral nutrition; urban road greening



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1. Introduction

Road greening, or landscapes alongside roads, is an important part of urban garden management. The cultivation and maintenance of vibrant roadside green landscape belts have become an important feature of modern social civilization [1]. However, the soil used in road greening is often mixed with engineering construction waste, and even the foreign soil transported by large machinery is often deep subsoil, which lacks aggregate structure and has little fertility. East China is located in a subtropical monsoon climate, cold in winter and hot in summer, with four distinct seasons. The highest temperature in summer often

exceeds 40 °C, and the minimum temperature in winter can drop to −10 °C. In summer, it rains almost throughout the whole month of June and most of July, which floods the asphalt roads and the roadside trees for lengthy periods. In winter, it often snows, and a great deal of industrial salt (NaCl) is sprinkled on the road to prevent traffic accidents, which results in the salination of soils and underground water. Additionally, the plants are exposed to exhaust fumes from heavy traffic. Such severe environments subject the roadside plants to multiple stresses, including heat, cold, excess rain, stagnant water, drought, acid or saline–alkali soils, and air and/or soil pollution [2]. How to improve plants alongside heavily traffic roads to resist these multiple stresses and beautify modern cities has become an important problem to be solved in the process of developing a civilized society.

5-Aminolevulinic acid (5-ALA) is a δ -amino acid that does not participate in protein synthesis. It is the key biosynthetic precursor of all porphyrin compounds, such as chlorophyll, heme, cytochrome and so on [3,4]. At the same time, it is also effective at improving plant stress tolerance as a new natural, nontoxic, biodegradable and environmentally friendly plant growth regulator [5]. In agriculture, 5-ALA has been known to promote plant growth and improve the yield of many crops, such as rice, radish, barley, potato, garlic, broad bean and so on [6]. Under salt stress, 5-ALA promoted the growth of spinach seedlings [7]. Under low temperature and low light, 5-ALA improved the photosynthetic capacity of melon seedlings and promoted plant growth [8]. In addition, 5-ALA improved plant tolerance to biotic and abiotic stresses, such as high temperature [9], strong light [10], UV-B [11], drought [12], waterlogging [13], alkaline soil [14], heavy metal pollution [15], pesticides [16], herbicide damage [17], and fungal infection [18]. In garden plants, 5-ALA improved cold tolerance of camphor and rhododendron [19] and heat tolerance of *Ligustrum japonicum* and *Spiraea japonica* [20]. However, to date, there are no reports of 5-ALA applications to urban road greening tree or shrub species.

Buxus megistophylla is an evergreen shrub species [21], which is widely planted along roadsides in urban and rural public green spaces, roadside hedgerows, ecological ditches, and other related landscape constructions in many regions of China [22]. It has been proposed that the species is moderately sensitive to drought [23], chilling injury [24] and air pollution [25]. Yet, no attempts to improve its stress tolerance have been reported. In this study, hedgerows of *B. megistophylla* grown on the roadsides of the main transport highway were sprayed with exogenous 5-ALA solutions in the spring at the early stage of plant growth. The 5-ALA treatment significantly improved the growth of these hedgerow plants. Therefore, analyses were carried out to clarify the possible mechanisms contributing to the improved growth to provide a theoretical basis for applying this nonprotein amino acid to urban road greening plants.

2. Materials and Methods

2.1. Materials and Treatment

The materials selected in this experiment were *Buxus megistophylla* Levl. hedgerows, which were planted on both sides of Nenjiang Road, Changzhou City, Jiangsu Province in 2014. The planting density was 64 plantlet cuttings per m², and the hedgerows were pruned monthly to maintain a 70–80 cm height during the growing season. Our visual assessment was that the plants were growing poorly, exhibiting weak annual growth, which is why they were selected for this study.

5-Aminolevulinic acid (5-ALA) solution at a dosage of 20 mg/L was sprayed on the leaf surface of plants on 28 April 2020. Control plants were sprayed with the same volume of water. The width of the hedge was 1.8 m, and the length of one plot was about 100 m. There were 3 plots per treatment, arranged randomly. Ninety days after treatment, plant growth and morphology of the control and 5-ALA treated plants were investigated. Because the hedgerows were dense, it was difficult to dig a single plant with a complete root system. In addition, monthly pruning did not permit measurements of shoot growth. Therefore, leaf morphology and rapid chlorophyll fluorescence were measured in the roadside plants. Then, 5–8 plants with partial roots were randomly dug up from each

plot. Mature leaf and fine root tissues were collected and prepared for physiological and biochemical analysis, rinsed in water, quickly frozen in liquid nitrogen, and then driven back to the lab and stored at $-80\text{ }^{\circ}\text{C}$ until analysis.

2.2. Determination of Leaf Morphology and Relative Chlorophyll Content

The length, width and thickness of fresh plant leaves were measured with a ruler or vernier caliper. The relative chlorophyll content was determined with a SPAD-502 Plus produced by Konica Minolta. For these measurements, fifteen mature leaves of the control and the treated plants were randomly selected from different plants from each plot, and an average value was obtained from 45 observations.

2.3. Rapid Fluorescence Determination of Chlorophyll in Leaves and Analysis of Fluorescence Parameters

The fast fluorescence OKJIP curve and 820 nm reflection fluorescence absorption curve of the chlorophyll of leaves of *B. megistophylla* were measured in vivo with a Multifunctional plant efficiency analyzer (M-PEA, Hansatech Instrument Ltd., Norfolk, UK). Before determination, the leaves were dark-adapted for 30 min and then exposed to saturated pulsed light ($3000\text{ }\mu\text{mol m}^{-2}\text{ s}^{-1}$) for 1 s. Fifteen leaves were measured repeatedly in the treatment and the control, respectively. The O-phase fluorescence (F_o) of the fast fluorescence curve was the initial fluorescence when the PSII reaction center was completely open. The fluorescence of the K phase (F_k), J phase (F_j), and I phase were measured at 300 μs , 2 ms, and 30 ms, respectively. F_m was the maximum fluorescence when the PSII reaction center was completely closed, and the time was about 300 ms. The absorption of modulated reflection fluorescence at 820 nm was expressed by its relative value, where the relative fluorescence $MR/MR_o = 1$ at 0.7 ms was set. The fluorescence parameters were calculated according to the method described previously [19,20]. The control and 5-ALA treatment measurements were both replicated 15 times from randomly selected plants, 5 per plot.

2.4. Determination of Soluble Sugars and Free Proline in Leaves

Frozen leaf samples were used for the measurements, where the content of soluble sugars was determined by anthrone colorimetry [26], while the free proline content was determined by the acid ninhydrin method [27]. Three biological repeats were conducted, and the average value was taken.

2.5. Determination of the Activities of Antioxidant Enzymes in Leaves and Roots

The frozen tissues were extracted according to the method described by Tan et al. [28]. The activities of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) were determined in the crude extract according to the methods described by Zhang et al. [29] and Change and Maehly [30], respectively. All extractions were biologically repeated 3 times, and the average value was taken.

2.6. Determination of Mineral Nutrient Elements in Leaves and Roots

The mature leaves and fine roots of *B. megistophylla*, allowed to equilibrate at ambient temperature, were rinsed thoroughly with tap water and then deionized water, respectively, then dried and ground to a fine powder to pass a 100-mesh sieve. One hundred milligrams of the powdered samples were digested with concentrated nitric acid at $100\text{ }^{\circ}\text{C}$ for 90 min. After complete clarification, the volume of the solution was fixed to 20 mL. The content of mineral elements, including phosphorus, potassium, calcium, magnesium, iron, copper, zinc, manganese, sodium, aluminum, cadmium, mercury, chromium and lead, were determined using an ICP-OES2100 system. For the nitrogen content, 0.2 g of dry powder was combined with 3 g potassium sulfate, and 5 mL concentrated sulfuric acid. After the solution was clarified, 5 mL of 4% NaOH (w/v) and 30 mL 30% boric acid (w/v) were added and determined using a Kjeldahl nitrogen meter. There were 3 biologically replicates, 1 per plot, and the average value was taken.

2.7. Statistical Analysis

The data from control and treated plants were compared using the Student's *t*-test of SPSS 20.0 software. When $p \leq 0.05$, the effect was considered significant; when $p \leq 0.01$, the effect was considered extremely significant.

3. Results

3.1. Effect of 5-ALA Treatment on the Appearance of *B. megistophylla* on Both Sides of Urban Roads

We found that some leaves of the control plants appeared chlorotic, some older branches had dried up, and parts of shrubs were dead. However, there was no plant dead in the area sprayed with 5-ALA. Even if a few leaves of individual plants fell off, the lateral buds sprouted, and new leaves were developing. When the weak plants were dug up, there was almost no new root growth evident in control, but many new roots occurred in the treated plants. In addition, it can be seen from Table 1 that the leaf length and width of the treatment were 13.54% and 40.34% higher than those of the control, respectively, and the difference was extremely significant ($p \leq 0.01$). The leaf thickness was also 16.67% higher ($p \leq 0.05$), and the relative content of chlorophyll was 61.06% higher than that of the control ($p \leq 0.01$). These results showed that the 5-ALA treatment significantly promoted the growth of *B. megistophylla*, a popular species for urban greening, with deeper green leaves and a better greening effect.

Table 1. Effects of application with 5-ALA solution on leaf morphology of *B. megistophylla*.

Treatment	Leaf Length (cm)	Leaf Width (cm)	Leaf Thickness (cm)	SPAD
Control	3.25 ± 0.08 B ^z	2.38 ± 0.09 B	0.30 ± 0.002 b	41.70 ± 0.23 B
5-ALA	4.84 ± 0.03 A	3.34 ± 0.06 A	0.35 ± 0.002 a	67.16 ± 0.25 A

^z The data in the table are means ± SE of 45 replicate measurements. The different capital or lowercase letters represent significant differences at $p \leq 0.01$ or 0.05, respectively, between the control and the treated plants by Student's *t*-test.

3.2. Effects of 5-ALA Treatment on Chlorophyll Fast Fluorescence and 820 nm Reflection Fluorescence Absorption Curve of *B. megistophylla* Leaves

Figure 1 shows the fast fluorescence OKJIP curve and 820 nm reflection fluorescence absorption curve of *B. megistophylla* leaf chlorophyll. It can be seen from Figure 1A that the fluorescence intensity of OKJIP curves in the 5-ALA treated leaves were significantly lower than those of the control in O ($t = 50 \mu\text{s}$), K ($t = 300 \mu\text{s}$) and J ($t = 3 \text{ ms}$) phases, but significantly higher than the latter in I ($t = 30 \text{ ms}$) and P ($t \approx 300 \text{ ms}$) phases. These indicate that the heat consumption (O phase) of the photosystem II (PSII) reaction center of the control was higher than that of the 5-ALA treatment. Simultaneously, the activities of the donor side (K phase) and receptor side (J phase) were both inhibited in control. In contrast, after 5-ALA treatment, the electron transport activity of the donor side (oxygen evolved complex) and the receptor side increased, while the heat dissipation energy decreased significantly.

It can be seen from Figure 1B that the MR/MR_0 of the 820 nm reflection fluorescence absorption curve of *B. megistophylla* leaves decreased rapidly from 0.7 ms of JIP time, which decreased linearly until 3 ms, then slowly between 3 and 30 ms and down to the minimum. This dynamic reflects the loss of electrons in the photosynthetic system I (PSI) reaction center P_{700} to reduce the terminal electron receptor NADP and produce NADPH and ATP. The lower the minimum value of MR/MR_0 , the stronger the ability of the PSI reaction center to reduce the terminal electron receptor. After this, the value of MR/MR_0 increased and reached the maximum at about 300 ms. This rise reflects the reducing activity of the PSI reaction center by electron transfer between PSII and PSI. Comparing the curve between the treatment and the control, the change in magnitude of the MR/MR_0 value of the control leaves was significantly smaller than that of the treatment. The minimum MR/MR_0 value

of the treated leaves was significantly lower than that of the control at $t = 30$ ms, but the maximum MR/MR_0 value was significantly higher than that of the control at 300 ms. This indicates that the 5-ALA treatment improved the ability of the PSI reaction center to be oxidized by itself and then be reduced by the electrons transported from the PSII reaction center in the *B. megistophylla* leaves. Therefore, 5-ALA treatment had a significant promoting effect on the two photosynthetic systems in the leaves of *B. megistophylla*.

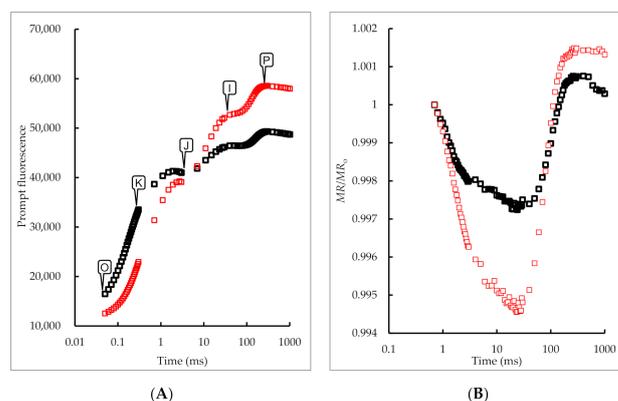


Figure 1. Effect of 5-ALA treatment on (A) leaf chlorophyll fast fluorescence and (B) 820 nm reflection absorption curve of *B. megistophylla*. Black lines indicate control, and the red lines indicate that 5-ALA treatment. O, K, J, I, and P represent different phases in the OKJIP curve.

3.3. Effect of 5-ALA on Chlorophyll Rapid Fluorescence Parameters in *B. megistophylla* Leaves

Table 2 shows that the initial fluorescence F_0 of leaves treated with 5-ALA was only 75.46% of that of the control, while the maximum fluorescence F_m and variable fluorescence F_v were 16.29% and 36.89% higher than that of the control, respectively. These differences reached an extremely significant level ($p \leq 0.01$).

Table 2. Effects of 5-ALA treatment on chlorophyll fluorescence parameters of *B. megistophylla*.

Fluorescence Parameters	Treatment		Fluorescence Parameters	Treatment	
	Control	5-ALA		Control	5-ALA
F_0	$(1.63 \pm 0.12) \times 10^4$ A ^z	$(1.23 \pm 0.03) \times 10^4$ B	ET_0/CS	$(2.79 \pm 0.19) \times 10^3$ B	$(4.51 \pm 0.19) \times 10^3$ A
F_m	$(4.91 \pm 0.22) \times 10^4$ B	$(5.71 \pm 0.17) \times 10^4$ A	DI_0/CS	$(5.83 \pm 0.83) \times 10^3$ A	$(2.65 \pm 0.01) \times 10^3$ B
F_v	$(3.28 \pm 0.22) \times 10^4$ B	$(4.49 \pm 0.15) \times 10^4$ A	ABS/RC	4.43 ± 0.40 A	2.18 ± 0.07 B
W_k	0.70 ± 0.04 A	0.43 ± 0.01 B	TR_0/RC	2.80 ± 0.14 A	1.70 ± 0.04 B
Ψ_0	0.28 ± 0.03 B	0.47 ± 0.02 A	ET_0/RC	0.73 ± 0.04 a	0.79 ± 0.02 a
M_0	2.06 ± 0.16 A	0.91 ± 0.05 B	DI_0/RC	1.64 ± 0.27 A	0.47 ± 0.02 B
ϕP_0	0.66 ± 0.03 B	0.78 ± 0.00 A	RC/CS	$(3.82 \pm 0.20) \times 10^3$ B	$(5.70 \pm 0.18) \times 10^3$ A
ϕE_0	0.19 ± 0.02 B	0.37 ± 0.01 A	V_{PSI}	$(8.72 \pm 3.22) \times 10^{-4}$ B	$(16.23 \pm 0.18) \times 10^{-4}$ A
ϕR_0	0.07 ± 0.01 B	0.11 ± 0.00 A	$V_{PSII-PSI}$	$(2.12 \pm 1.47) \times 10^{-5}$ B	$(6.72 \pm 1.38) \times 10^{-5}$ A
ϕD_0	0.34 ± 0.03 A	0.22 ± 0.00 B	PI_{ABS}	0.33 ± 0.09 B	1.62 ± 0.19 A
ABS/CS	$(1.63 \pm 0.12) \times 10^4$ A	$(1.23 \pm 0.03) \times 10^4$ B	PI_{total}	0.17 ± 0.04 B	0.66 ± 0.07 A
TR_0/CS	$(1.04 \pm 0.05) \times 10^4$ A	$(0.96 \pm 0.03) \times 10^4$ B			

^z The data in the table are means \pm SE of 15 replicate measurements. The different capital or lowercase letters represent significant differences at $p \leq 0.01$ or 0.05, respectively, between the control and the treated plants by Student's *t*-test.

W_k reflects the inhibition of the oxygen-evolving complex on the donor side of the PSII reaction center. The higher the W_k , the more serious the inhibition of the oxygen-evolving complex. The W_k of 5-ALA treated leaves was only 61.43% of that of the control (Table 2), indicating that 5-ALA treatment alleviated the inhibition of the oxygen-evolving complex on the donor side of the PSII reaction center. ϕ_0 represents the possibility of an exciton transferring electrons to the other electron receptors beyond Q_A^- . The ϕ_0 in the leaves treated with 5-ALA was 67.86% higher than that of the control (Table 2), indicating that 5-ALA improved the electron transport on the receptor side of the PSII reaction center. M_0 reflects the maximum closure rate when Q_A is completely reduced on the receptor

side of the PSII reaction center. The higher M_o , the faster the PSII reaction center closes. As shown in Table 2, the M_o of leaves treated with 5-ALA was only 44.17% of that of the control, indicating that 5-ALA treatment slowed the closing rate of the PSII reaction center and facilitated the transfer of photosynthetic electrons to receptors farther away from Q_A^- . The differences in these parameters show that the 5-ALA treatment improved the photosynthetic electron transport activity on the donor side and receptor side of the PSII reaction center in the leaves of *B. megistophylla*.

Variables φP_o , φE_o , φR_o and φD_o represent the maximum photochemical efficiency of the PSII reaction center, the quantum yield of absorbed light energy for photosynthetic electron transfer, the maximum photochemical efficiency of the PSI reaction center and the quantum yield for heat dissipation, respectively. Among them, the higher the first three, the higher the photosynthetic energy utilization efficiency, and the lower the φD_o , the less the energy dissipation through heat. As shown in Table 2, φP_o , φE_o and φR_o of leaves treated with 5-ALA were 18.18%, 94.74% and 57.14% higher than those of the control, respectively, while φD_o was only 64.71% of that of the control, indicating that 5-ALA treatment significantly increased the maximum photochemical efficiency of PSII and PSI in the leaves of *B. megistophylla*, with increased photochemical quantum yield and reduced heat dissipation.

The calculated results of absorption (ABS/CS_o), trap (TR_o/CS), energy transfer (ET_o/CS) and heat dissipation (DI_o/CS) per excited cross-section in the treated leaves were 75.76%, 92.31%, 161.65% and 45.45% of the control (Table 2), respectively. This means that, except for ET_o/CS , which was significantly higher in the treated leaves than in the control, the other three parameters were lower in the treated leaves than those in control ($p \leq 0.01$). Furthermore, the calculated absorption (ABS/RC_o), trap (TR_o/RC), energy transfer (ET_o/RC) and heat dissipation per active reaction center (DI_o/RC) in the treated leaves were 49.13%, 60.94%, 107.54% and 28.95% of the control, respectively. Except for ET_o/RC , which was slightly higher in the treatment than the control, although without a significant difference, the other three parameters in the treated leaves were significantly lower than those of the control. These show that 5-ALA treatment alleviated photoinhibition by relaxing energy charge per cross-section or per reaction center. Furthermore, the density of active reaction centers per cross-section (RC/CS) of treated leaves was 49% higher than that of the control. This is an important reason for the improvement of the photosynthetic capacity of leaves treated with 5-ALA.

V_{PSI} and $V_{PSII-PSI}$ represent the oxidation rate of the PSI reaction center when electrons were lost and the reduction rate of the PSI reaction center when electrons were transferred between the two photosynthetic systems, respectively. V_{PSI} and $V_{PSII-PSI}$ of leaves treated with 5-ALA were 86.12% and 216.98% higher than those of the control, respectively (Table 2), indicating that 5-ALA treatment exhibited a good promoting effect on electron transfer in PSI reaction center and a much greater promoting effect on electron transfer between the two systems. Therefore, the photosynthetic performance index, PI_{abs} , based on absorption and PI_{total} , including two photosynthetic systems in leaves treated with 5-ALA, was 390.91% and 288.24% higher than those of the control, respectively (Table 2). These data show that the 5-ALA treatment greatly improved the photosynthetic performance of *B. megistophylla* leaves.

3.4. Effects of 5-ALA Treatment on the Content of Soluble Sugar and Free Proline in *B. megistophylla* Leaves

5-ALA treatment significantly increased the content of soluble sugars and free proline in the leaves of *B. megistophylla*, where the former increased by 122.41% and the latter by 150% (Table 3). These two substances can be used as osmotic solutes, and their increased content is beneficial for enhancing the adaptability of plants to stressful environments.

Table 3. Effects of 5-ALA treatment on leaf soluble sugar and free proline content of *B. megistophylla*.

Treatment	Soluble Sugar (mg g ⁻¹ FW)	Free Proline (mg g ⁻¹ FW)
Control	0.58 ± 0.07 b ^z	0.12 ± 0.05 b
5-ALA	1.29 ± 0.07 a	0.30 ± 0.07 a

^z The data in the table are means ± SE of 3 replicate measurements. The different lowercase letters represent significant differences at $p \leq 0.05$ between the control and the treated plants by Student's *t*-test.

3.5. Effects of 5-ALA Treatment on the Activities of Antioxidant Enzymes in Leaves and Roots of *B. megistophylla*

The activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) in leaves and roots of *B. megistophylla* sprayed with 5-ALA were all significantly higher than those of the control (Table 4). These indicate that the 5-ALA treatment improved the antioxidant capacity of leaves and roots of *B. megistophylla*.

Table 4. Effects of 5-ALA treatment on antioxidant enzyme activities of leaves and roots of *B. megistophylla*.

Treatments	SOD (U g ⁻¹ FW)		POD (U g ⁻¹ FW min ⁻¹)		CAT (U g ⁻¹ FW min ⁻¹)	
	Leaves	Roots	Leaves	Roots	Leaves	Roots
Control	36.05 ± 0.51 b ^z	34.35 ± 0.70 b	29.73 ± 5.45 B	11.09 ± 1.95 B	15.97 ± 0.72 B	39.85 ± 4.99 B
5-ALA	38.11 ± 0.07 a	36.95 ± 0.39 a	85.69 ± 2.74 A	37.44 ± 0.68 A	62.42 ± 1.58 A	63.76 ± 14.69 A

^z The data in the table are means ± SE of 3 repeat measurements. The different capital or lowercase letters represent significant differences at $p \leq 0.01$ or 0.05, respectively, between the control and the treated plants by Student's *t*-test.

3.6. Effect of 5-ALA Treatment on the Mineral Element Content of *B. megistophylla*

The total nitrogen (N) content in roots of *B. megistophylla* was significantly higher than in leaves, and the N ratio between leaves and roots was 0.62 (Table 5), indicating that most of the N absorbed by plants remained in the roots and only a small part was transported to leaves. Compared with the control, the total N content of roots and leaves in the 5-ALA treatment increased significantly, of which leaves and roots increased by 95.58% and 26.04%, respectively. Based on the leaf and root averages, the total level of N increased by 52.68% following the 5-ALA treatment. Compared with the control, the root of N was 56.45% higher than that of the control ($p \leq 0.01$). The above results show that 5-ALA not only promoted the absorption of N but also promoted the transport and distribution of N to the leaves of *B. megistophylla*.

Table 5. Effect of 5-ALA treatment on the mineral element content in leaves and roots of *B. megistophylla*.

Element	Leaves		Roots		Ratio of Leaf to Root	
	Control	5-ALA	Control	5-ALA	Control	5-ALA
N (mg/g)	7.01 ± 0.06 B ^z	13.71 ± 0.82 A	11.29 ± 0.17 B	14.23 ± 0.21 A	0.62 ± 0.01 B	0.97 ± 0.07 A
P (mg/g)	3.20 ± 0.78 b	8.39 ± 1.44 a	0.32 ± 0.05 b	1.55 ± 0.21 a	11.50 ± 4.83 a	5.50 ± 0.82 a
K (mg/g)	10.45 ± 1.40 a	15.55 ± 3.23 a	4.67 ± 0.62 a	6.29 ± 0.62 a	2.26 ± 0.33 a	2.56 ± 0.67 a
Ca (mg/g)	27.40 ± 2.67 b	48.38 ± 5.18 a	17.73 ± 0.77 b	24.37 ± 0.81 a	1.55 ± 0.14 a	2.00 ± 0.29 a
Mg (mg/g)	0.76 ± 0.13 b	1.80 ± 0.25 a	2.40 ± 0.20 a	2.79 ± 0.34 a	0.31 ± 0.03 b	0.69 ± 0.18 a
Fe (mg/g)	0.11 ± 0.01 B	0.29 ± 0.01 A	4.39 ± 0.34 B	9.34 ± 0.47 A	0.03 ± 0.00 a	0.03 ± 0.00 a
Cu (mg/kg)	26.50 ± 0.50 b	32.30 ± 1.52 a	231.91 ± 71.74 b	463.19 ± 14.49 a	0.13 ± 0.03 a	0.07 ± 0.00 a
Zn (mg/kg)	28.21 ± 6.54 a	29.98 ± 6.08 a	66.78 ± 2.27 a	66.73 ± 1.22 a	0.43 ± 0.11 a	0.45 ± 0.09 a
Mn (mg/kg)	24.08 ± 5.23 a	23.52 ± 4.39 a	194.60 ± 37.62 a	90.67 ± 4.12 b	0.14 ± 0.01 a	0.26 ± 0.06 a
B (mg/kg)	28.07 ± 4.08 b	74.95 ± 10.61 a	121.24 ± 10.28 b	257.60 ± 18.56 a	0.23 ± 0.02 a	0.30 ± 0.07 a
Na (mg/g)	0.97 ± 0.00 a	1.04 ± 0.06 a	1.25 ± 0.04 b	1.45 ± 0.06 a	0.78 ± 0.03 a	0.72 ± 0.05 a
Al (mg/g)	0.05 ± 0.00 a	0.08 ± 0.01 a	5.11 ± 0.67 a	2.60 ± 0.27 b	0.01 ± 0.00 a	0.03 ± 0.00 a
Cd (mg/kg)	0.43 ± 0.04 a	0.28 ± 0.01 b	0.35 ± 0.10 b	0.76 ± 0.06 a	1.48 ± 0.51 a	0.37 ± 0.04 b
Hg (mg/kg)	69.45 ± 4.37 a	53.35 ± 1.46 b	213.78 ± 13.29 b	292.75 ± 20.48 a	0.32 ± 0.00 a	0.18 ± 0.01 b
Cr (mg/g)	0.36 ± 0.05 a	0.16 ± 0.01 b	1.29 ± 0.24 b	3.36 ± 0.39 a	0.30 ± 0.08 a	0.05 ± 0.01 b
Pb (mg/kg)	1.32 ± 0.10 a	0.90 ± 0.04 b	6.92 ± 1.15 b	19.11 ± 1.89 a	0.20 ± 0.04 a	0.05 ± 0.01 b

^z The data in the table are means ± SE of 3 replicate measurements. The different capital or lowercase letters represent significant differences at $p \leq 0.01$ or 0.05, respectively, between the control and the treated plants by Student's *t*-test.

5-ALA treatment also significantly increased the content of phosphorus (P) in the leaves and roots of *B. megistophylla*. The content of P in the leaves of the control was 10 times higher than that in the roots (Table 5), indicating that most of the P absorbed by roots was transported to the shoot. After 5-ALA treatment, the P content in leaves increased by 162.19%, and the root content increased by 384.38%. After the average of the whole plant, the P level of the 5-ALA treatment was 182.39% higher than that of the control. 5-ALA treatment had no effect on the ratio of P in leaves to roots. These results showed that 5-ALA treatment promoted P uptake and accumulation but had no significant effect on P distribution.

The 5-ALA treatment had no significant effect on the level of potassium (K) in *B. megistophylla*. In addition, the K content in the leaves of *B. megistophylla* was 2.26 times higher than that in the roots, indicating that the K absorbed by the plants was mainly transported to the shoot. 5-ALA treatment had no significant effect on this allocation characteristic.

Calcium (Ca) and magnesium (Mg) are both essential medium elements for plant growth. In *B. megistophylla*, the leaf–root ratio of Ca in the control plant was 1.55, indicating that Ca mainly accumulated in leaves. 5-ALA treatment did not significantly affect this characteristic but increased the Ca content in leaves and roots by 76.57% and 37.45%, respectively. This indicates that 5-ALA treatment promoted the absorption and transport of Ca but did not affect the distribution in leaves and roots. Different from Ca, the leaf–root ratio of Mg in the control plant of *B. megistophylla* was 0.31, indicating that most of the magnesium was distributed in the root system. 5-ALA treatment significantly increased Mg content in leaves (by 136.84%) but had no effect on roots. Compared with the control, the leaf–root ratio of Mg in 5-ALA treatment increased by 122.58% ($p \leq 0.05$), indicating that 5-ALA promoted the absorption of Mg by the roots of *B. megistophylla* and the distribution of Mg to the leaves.

Iron (Fe), copper (Cu), zinc (Zn), manganese (Mn) and boron (B) are essential trace elements for plant growth. The content of Fe in roots of *B. megistophylla* was much higher than that in leaves, and the ratio of leaf to root was 0.03 (Table 5). With 5-ALA treatment, the Fe of leaves was 156.45% higher than that of the control, and that in the roots also increased by 113.10% ($p \leq 0.01$), but the leaf–root ratio of Fe did not change, indicating that 5-ALA significantly promoted the absorption and transport of Fe, but did not affect the distribution ratio. The leaf–root ratio of Cu in the control plant of *B. megistophylla* was 0.13, indicating that Cu was mainly distributed in the roots. 5-ALA treatment significantly promoted the content of Cu in leaves and doubled the content in roots but had no significant effect on the distribution ratio of Cu in leaves and roots. Zn of *B. megistophylla* was mainly distributed in the roots, and the leaf content was less than half of the roots (43–45%); 5-ALA had no significant effect on Cu content and distribution. Mn was mainly distributed in the roots of *B. megistophylla*, and the leaf–root ratio was only 0.14 in the control. 5-ALA treatment had no significant effect on leaf content but significantly decreased root Mn content (about half of the control). B was also mainly distributed in the roots of *B. megistophylla*. In the control and treatment, the leaf–root ratio of B was 0.23–0.30. 5-ALA treatment significantly increased B content in leaves and roots but had no significant effect on leaf root distribution (Table 5).

Sodium (Na) and aluminum (Al) are often regarded as harmful elements. If the Na content is too high, the soil may be salinized. If the Al content is too high, the soil tends to be acidified. The Na content in roots of *B. megistophylla* was higher than that in leaves, and the content in leaves and roots increased after 5-ALA treatment, but only the root increment reached a significant level. These indicate that 5-ALA promoted Na uptake but mainly retained in the roots, without a significant increase in the leaves. For Al, 5-ALA treatment reduced the Al content in roots of *B. megistophylla* by about half and had no effect on leaves. These suggest that 5-ALA treatment reduced the absorption of Al by *B. megistophylla* as a whole.

Cadmium (Cd), mercury (Hg), chromium (Cr) and lead (Pb) are considered to be toxic heavy metal elements. In the control plant, Cd was mainly distributed in the leaves, and the leaf–root ratio was 1.48, while the other three elements were mainly distributed in the

roots, with the leaf–root ratios from 0.20 to 0.32. 5-ALA treatment reduced the leaf–root ratio of Cd to 0.37, which was only 25% of that of the control. For Hg, Cr and Pb, 5-ALA treatment significantly decreased the leaf content but increased the root content, so the leaf–root ratio decreased significantly. These results show that 5-ALA treatment can induce more toxic element storage in roots to avoid leaf accumulation. This effect is consistent with these four toxic heavy metal elements as a whole.

4. Discussion

Road greening is not only an important part of urban greening systems but also an important symbol of modern urban civilization. A good road greening system is conducive to driving safety, enjoyment of city landscapes, and environment protection [31]. However, while road traffic brings speed and convenience to people's lives, it also exerts new coercive factors on the growth of roadside plants. In addition to the conventional high or low temperatures, drought or waterlogging, salinization, and acid–alkali stress, automobile exhaust is a typical harmful factor on plant growth [32]. This is something rarely encountered by plants grown in a quiet garden. It has been proposed that the main characteristics of automobile exhaust were damage to cell membranes, decreased chlorophyll content and photosynthesis, leaf discoloration, leaf shedding and even death [33]. The plants of *B. megistophylla* used in this study were planted on both sides of the main road. However, automobile exhaust was not detected in the air. Traffic throughout the country was not particularly busy in the first half of 2020 due to the COVID-19 epidemic, so the exhaust levels were likely lower than usual. Even so, the growth of *B. megistophylla* on both sides of the road was restricted, and occasional death of shrubs occurred in the experimental site. In addition to the *B. megistophylla* reported in this paper, leaf yellowing, growth inhibition and plant death of another road greening species, *Gardenia jasminoides* Ellis, were also quite significant (data not listed). Therefore, improving the adaptability of plants used for road greening in such adverse environments, promoting plant growth, reducing mortality, and enhancing the greening effect has important practical significance.

5-Aminolevulinic acid (5-ALA) is a natural δ -amino acid commonly found in animals, plants, and microorganisms. It is not used to synthesize proteins but is a key biosynthetic precursor of porphyrin compounds, such as chlorophyll, heme, and cytochrome [3]. Therefore, it is closely related to plant photosynthesis and respiration [5]. A large number of studies have shown that 5-ALA can improve the resistance of plants to high temperature [9], low temperature [8], strong light [10], weak light [8], drought [12], waterlogging [13], salinization [7], heavy metal pollution [15], pesticide [16], herbicide toxicity [17], fungal diseases [18] and other biotic and abiotic stresses and has broad application potential in agricultural production [5]. However, whether 5-ALA can be used on urban road greening plants to improve environmental adaptability has never been reported. *Buxus megistophylla* is sensitive to automobile exhaust [34]. In urban roads with high concentrations of NO₂ and CO, the pH of leaf cell sap decreased, and the content of malondialdehyde (MDA), a product of membrane peroxidation, increased significantly. The results of the present work showed that 5-ALA promoted leaf growth of *B. megistophylla* (Table 1), promoted the occurrence of new roots, reduced the probability of plant death, and significantly improved urban road greening, indicating that it has an important application prospect in road greening.

5-ALA is the precursor of chlorophyll biosynthesis in plants. Exogenous application of 5-ALA increased the content of chlorophyll in leaves of *B. megistophylla* (Table 1). Chlorophyll a is the main pigment in the reaction center of the photosynthetic system (including PSI and PSII). It forms antenna pigment with chlorophyll b, carotenoids and lutein. A photosynthetic reaction center consists of about 300 chlorophyll molecules [35]. 5-ALA can increase the number of photosynthetic reaction centers by increasing the content of chlorophyll in the leaves of *B. megistophylla*. The active reaction center density (RC/CS) per cross-section increased significantly (Table 4).

The fast fluorescence OKJIP curve and 820 nm reflection fluorescence absorption curve of leaf chlorophyll were detected by M-PEA. The results showed that the O, K and J phase fluorescence of *B. megistophylla* in the leaves sprayed with 5-ALA was significantly lower than that of the control, while the I and P phase fluorescence was higher than that of the control (Figure 1A). These results showed that 5-ALA treatment increased the donor side activity, reaction center itself activity and receptor-side electron-transport activity of the PSII reaction center in leaves. Simultaneously, the minimum value of MR/MR_0 decreased significantly at $t = 30$ ms, and the maximum value of MR/MR_0 increased significantly at $t \approx 300$ ms after 5-ALA treatment (Figure 1B). This means that the activity of the PSI reaction center and the ability to reduce terminal electron receptor NADP were promoted. Fluorescence parameter analysis (Table 2) showed that W_k and M_0 decreased in the 5-ALA treated leaves, suggesting that 5-ALA alleviated the photoinhibition at the donor-side oxygen evolved complex and the receptor side, leading to a decrease in the maximum closure rate of the PSII reaction center. The increase of ϕ_0 represents an increase in the probability of excitons transferring electrons to other electron receptors downstream of Q_A . 5-ALA treatment significantly promoted the activity of the PSII reaction center in *B. megistophylla* leaves (Table 2). V_{PSI} and $V_{PSII-PSI}$ represent the rate at which the PSI itself loses electrons when it is oxidized and the rate at which the PSI reaction center is reduced by electrons transferred by PSII [19,20]. After 5-ALA treatment, both V_{PSI} and $V_{PSII-PSI}$ increased significantly, indicating that 5-ALA significantly promoted electron transfer in PSI reaction centers and between PSII and PSI reaction centers. Furthermore, 5-ALA had a significant promoting effect on the absorption-based photosynthetic performance index PI_{abs} and the total photosynthetic performance index PI_{total} , including two photosynthetic systems (Table 2). These results are similar to the effects of 5-ALA treatment at low temperatures [19] or high temperatures [20]. In addition, from the point of view of photochemical energy conversion, the quantum yield (ϕE_0) and energy transfer per excited cross-section (ET_0/CS) of *B. megistophylla* leaves treated with 5-ALA were significantly increased. Although the promoting effect of energy transfer (ET_0/RC) based on unit reaction center did not reach a significant level, energy absorption (ABS/CS or ABS/RC), trap (TR_0/CS or TR_0/RC), and heat dissipation (DI_0/CS or DI_0/RC) decreased significantly. These indicate that 5-ALA treatment can reduce the energy charge pressure of the reaction center and reduce the possibility of photoinhibition. Recently, it was reported that 5-ALA treatment could upregulate the expression of core proteins D1 and D2 of the PSII reaction center in potato leaves [36]. This may be an important reason why the core proteins of the PSII reaction center maintain higher activity. Perhaps due to the improvement of leaf photosynthetic performance, the soluble sugars in the leaves of *B. megistophylla* treated with 5-ALA increased significantly (Table 3).

Soluble sugars can be used as osmotic solutes, which are related to plant stress resistance. However, more studies have shown that proline accumulation induced by 5-ALA can improve plant stress resistance [37]. In this experiment, it was observed that 5-ALA treatment increased the proline content in the leaves of *B. megistophylla* (Table 3). This may be one of the reasons for its resistance to environmental stress on urban roads. In addition, the increases in antioxidant enzyme activities induced by 5-ALA are also important reasons for the improvement of stress resistance. In this experiment, 5-ALA significantly increased the activities of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) in leaves and roots of *B. megistophylla* (Table 4). These results are similar to previous reports [3,4], indicating that the increase of antioxidant enzyme activity is related to plant stress resistance.

There is currently no consensus on the effect of 5-ALA on the content of mineral elements in plants. Naeem et al. [38] suggested that 5-ALA alleviated the decrease of N, P, Ca, Mg, Zn and Fe content in rape (*Brassica napus* L.) leaves and roots under salt stress but had no effect on Mn and Cu contents. Zhang et al. [39] proposed that 5-ALA could increase Ca, Mg, Cu, Fe and Zn in apple (*Malus domestica* Borkh.) leaves but decrease the content of P, K and Na. Anwar et al. [40] observed that the content of N, P, K, Ca, Mg, Cu,

Fe, Mn and Zn in roots and leaves of cucumber (*Cucumis sativus* L.) seedlings increased significantly when 5-ALA alleviated the inhibition of low temperature on the growth of cucumber seedlings. It seems that the improvement of cold resistance of plants by 5-ALA is related to the maintenance of nutrient balance. However, Liu et al. [14] did not observe an increase of K, Fe and Mg content in leaves when they studied the improvement of alkaline tolerance of Swiss chard (*Beta vulgaris* L.) treated with 5-ALA. Therefore, the effect of 5-ALA on the content of mineral elements in plants may be related to species and types of stress. In the present work, it was observed that 5-ALA increased the level of N in leaves and roots of *B. megistophylla*. This effect was shown not only in the increase of N content in roots and leaves but also in the transport and distribution of N from roots to leaves (Table 5). It has been suggested that nitrate reductase (NR) activity and its coding gene expression was significantly upregulated in pakchoi (*Brassica campestris* ssp. *chinensis* var. *communis* Tsen et Lee) plants treated with 5-ALA, and the content of total N, amino acid and protein in leaves increased, while the content of nitrate nitrogen decreased [41]. The authors deduced that 5-ALA promoted N absorption and assimilation. Our result here is consistent with the previous report, indicating that 5-ALA can promote the absorption, transport, distribution and utilization of N in plants.

It was observed that 5-ALA increased P, Ca, Cu, Fe and B in roots and leaves of *B. megistophylla*. This is similar to the result of Naeem et al. [38]. However, 5-ALA treatment only increased leaf Mg content, had no significant effect on K and Zn content, and decreased root Mn content (Table 5). The reason for these differences is not clear. Yao et al. [42] reported that 5-ALA promoted the absorption and distribution of P in rice (*Oryza sativa* L.). We also observed that 5-ALA increased the P content of *B. megistophylla* plants (Table 5), indicating that the promoting effect of 5-ALA on P was universal. The transport of Ca by plants mainly depends on transpiration flow [43], while 5-ALA can promote stomatal opening [44] and increase transpiration rate. Therefore, it may also be common for 5-ALA treatment to promote the absorption and transport of Ca. For Mg, 5-ALA treatment led to an increase in leaf content (Table 5). This may be one reason for the increase of chlorophyll content in leaves (Table 1). For Cu, Naeem et al. [38] suggested that 5-ALA had no alleviating effect on the decrease of Cu content in rape plants under salt stress, but Anwar et al. [40] thought that 5-ALA could alleviate the decrease of Cu in cucumber induced by low temperature. Our results were similar to those of the latter. 5-ALA could significantly increase the Cu content in leaves and roots of *B. megistophylla* (Table 5). Lack of Fe will lead to leaf chlorosis. In this experiment, the leaves of the control plants were generally yellowing, while the leaves of the treated plants were green. This could not only be related to the higher N and Mg content mentioned above but to the 5-ALA treatment promoting an increase of Fe content in *B. megistophylla*. The content of Fe in both roots and leaves treated with 5-ALA was more than twice as high as that of the control. Such a huge increase is rarely reported. Similarly, 5-ALA induced a more than double increase in B content in leaves and roots of *B. megistophylla* (Table 5). These phenomena have rarely been reported before and are worthy of our attention and further study of the mechanism.

Na and Al are not essential elements for plant growth and development. Excessive accumulation will cause plant toxicity. The results showed that 5-ALA treatment increased the Na content of *B. megistophylla*, but mainly in the root system. This is similar to a previous report [45] that found 5-ALA upregulated expressions of genes, including *SOS1*, *NHX1* and *HKT1* in strawberry (*Fragaria × ananassa* Duch.) roots to intercept salt ions in root vacuoles and depress transport to the aboveground parts, avoiding leaf ion damage. The relationship between Al content and 5-ALA has not been reported before. In this experiment, the Al content in leaves was not affected by 5-ALA treatment, but it was decreased significantly in the roots, suggesting that 5-ALA treatment reduced the absorption of Al by plants. Due to the soil acidity of the experimental region, a decrease in Al content induced by 5-ALA may be related to the increase of acid tolerance of plants.

Cd, Hg, Cr and Pb are all toxic elements. Crops grown on both sides of roads are often poisoned by these heavy metal elements [46]. 5-ALA improved the tolerance of plants to

Pb [15] and Cr [47]. Recently, Xu et al. [48] proposed a mechanism of 5-ALA improving plant Cd tolerance, which is related to the upregulation of cellular antioxidant enzymes and metal carrier protein gene expression. In the present work, we observed that 5-ALA promoted the accumulation of heavy metals in the roots of *B. megistophylla* and decreased the content of leaves (Table 5). This mechanism is similar to that proposed by Wu et al. [45] that 5-ALA induces Na interception in strawberry roots under salt stress to reduce upward transport. Obviously, this mechanism is more ingenious, and it has yet to be proven. It has been reported that arbuscular mycorrhizal fungi (AMF) could improve plant tolerance against heavy metal pollution [49], where AMF can chelate part of metal elements in the rhizosphere soil [50]. Whether 5-ALA treatment improves the tolerance mediated by AMF has never been reported, but this deserves study in the future.

To sum up, 5-ALA promoted the growth of *B. megistophylla*, a road greening plant, improved plant survival, increased leaf color and enhanced the greening effect. The reason may be related to the increase of plant antioxidant enzyme activities, the improvement of plant mineral nutrition levels, and the inhibition of toxic and harmful heavy metal element accumulation in leaves. The toxic elements, such as Cd, Hg, Cr and Pb absorbed by the roots were trapped in the roots, the accumulation in leaves was reduced, the content of N, P, Ca, Mg, Cu, Fe and B in leaves were increased, and the nutritional status of plants was generally improved. The photosynthetic capacity of leaves also improved following 5-ALA treatment, the accumulation of soluble sugars and free proline was promoted, the activities of antioxidant enzymes were increased, and the stress resistance of plants was enhanced. Therefore, this nonprotein amino acid can use in urban landscapes to improve plant stress tolerance.

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