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Physiological Response of Two Typical Plant Species under Combined Pb and Cd Stress in Bioretention Facilities

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Abstract: Bioretention facilities reduce stormwater runoff and pollutants, but there is a concern that plants in bioretention facilities may absorb heavy metal pollutants from stormwater runoff, which might impair the growth of the plant species. To investigate this issue, stormwater runoff containing various amounts of Pb and Cd heavy metals was used as the irrigation water in a bioretention facility. The low concentrations of Pb and Cd were 0.08 and 0.04 mg/L, and the high values were 0.68 and 0.32 mg/L. The plant heavy metal content and physiological indicators were measured. The indicators were chlorophyll content (CC), net photosynthetic rate (NPR), and transpiration rate (TR). The results showed that the changes in plant chlorophyll content (CC) were highly correlated with changes in the plants' Pb. Low concentrations of Pb and Cd slightly inhibited the *Ginkgo biloba* L. and *Ligustrum* × *vicaryi* NPRs, the effect was more obvious at high concentrations and the *Ligustrum* × *vicaryi*'s NPR decreased from the initial 8.97 μmol $CO_2/(m^2s)$ to 5.77 μmol $CO_2/(m^2s)$ under high concentration conditions. Pb and Cd increased the *Ginkgo biloba* L. and *Ligustrum* × *vicaryi*'s TRs, and the effect at low Pb and Cd concentrations was more significant. Under low concentrations of Pb and Cd stress, the *Ginkgo biloba* L.'s TRs reached 0.63 mmol $H_2O/(m^2s)$, *Ligustrum* × *vicaryi*'s TRs reached 1.30 mmol $H_2O/(m^2s)$. The TRs of the two plants in the experimental groups remained high throughout the experiment, and there was no significant inhibition. The study found that Pb and Cd in stormwater runoff did affect the physiological function of species to some extent. Different plant species behaved differently in bioretention facilities, but the stormwater runoff did not lead to the death of species. Our study may provide a better understanding of the development of typical plant species in bioretention facilities.

Keywords: Pb; Cd; bioretention facilities; physiological response; stormwater runoff; *Ginkgo biloba* L.; *Ligustrum* × *vicaryi*; chlorophyll content; net photosynthetic rate; transpiration rate

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1. Introduction

Heavy metals enter the environment in a variety of ways, including through manmade or natural sources [1–3], and are difficult to remove. They accumulate over time, posing a severe threat to the ecological environment [4,5]. Pb is a fuel additive and Cd is produced by tire wear; therefore, Pb and Cd are the most commonly encountered heavy metals in traffic. Heavy metals and other pollutants build up on urban surfaces before being washed away in stormwater runoff [6,7].

The idea of low impact development (LID) was established to mitigate the hazards of stormwater runoff to receiving water bodies. It proposes using decentralized, small-scale source control facilities to collect and purify rainfall runoff from metropolitan areas [8]. In particular, bioretention facilities have compact footprints and are landscaped, which means that they have become popular, small-scale control facilities [9,10]. Bioretention facilities mainly use soil and vegetation to reduce runoff flow and improve water quality [11–13]. They also use physical processes, such as adsorption, filtration, sedimentation, and evaporation, to achieve their aims [14,15].

Studies have been undertaken on the effectiveness of heavy metal removal in bioretention facilities. Many studies have shown that the fill and plant components of bioretention facilities play a substantial role in heavy metal removal from stormwater runoff. In phytoremediation, the removal capacity of heavy metals by plant species has been graded. Whether plant species in bioretention facilities were super-enriched plants has not yet been determined, but studies have found that concentrations of heavy metals (Cu, Zn, Mn, and Cd) in the runoff of vegetated plots are all significantly ($p < 0.05$) lower than those of un-vegetated plots and controls [16], so their ability to remove heavy metals is beyond doubt. In bioretention facilities, (i) plant species differ in their effectiveness, and (ii) native species are more effective than exotic ones [17]. Ying Mei et al. studied the uptake and partitioning of four heavy metals, copper (Cu), cadmium (Cd), lead (Pb), and zinc (Zn), by 30 plant species in North China through pot experiments [18]. The study [19] showed that the adsorption processes could remove up to 89%, 91%, and 91% of Pb, Cr, and Cd, respectively, while plant uptake of these heavy metals was 6%, 5.1%, and 5.2%. Previous studies have shown that when stormwater runoff carrying heavy metals reached the bioretention facility, 88–97% of the heavy metals were removed by the fill layer, 2.0–11.6% were in the outflow, and 0.5–3.3% were absorbed by plants [20]. Heavy metals in plants can come from the soil and/or air deposition [21]. The Cu, Zn, Pb, and Cd in the leaves of coniferous plants may originate from soil, whereas Cu, Zn, Pb, and Cd in the leaves of broad-leaved plants may be derived from significant atmospheric deposition according to an analysis of the relationship between heavy metal concentrations in soil and plant leaves and atmospheric deposition [22]. Plants such as conifers, which can potentially be used to monitor metal levels in the environment, have been investigated as potential bioindicators of environmental pollution [23,24].

Heavy metals have different properties, and different plants and plant sections have different absorption capacities and heavy metal uptake potentials. Although it is widely believed that heavy metals harm plants, the study suggests that moderate quantities of heavy metals may improve some physiological indicators and the growth of plant [25]. Some previous studies have reported that stormwater runoff containing high concentrations of pollutants has no negative or significant effects on the net photosynthetic rate, chlorophyll content, and the electrolyte exudation rate in most plants, and positively affects *Iris tectorum Maxim*, whereas stormwater runoff containing low concentrations of pollutants has positive or no significant effects on the physiological indicators of herbaceous plants [26]. Perennial ryegrass fading was observed to be slightly different in response to Pb and Cd in a nutrient solution. Fading occurred at Pb concentrations of 0.2 and 0.5 mM, but was more severe at the higher Pb concentration (3.2 mM) [27]. Long-term irrigation with 5 mg/L Cd had no significant harmful effects on poplar tree development. However, the transpiration rate was higher and the photosynthetic rate was lower [28]. Pb buildup in plants has been shown to be caused by elevated Pb levels in the soil, resulting in changes in chloroplast structure and reduced photosynthetic efficiency [29]. Zhang et al. reported that high Cd contamination levels enhanced the chlorophyll content in young leaves but had no effect on mature leaves. It also increased the plant maximum net photosynthetic rate, the light compensation point, and the light saturation point [30]. Ye et al. demonstrated that plants treated with low/medium doses of heavy metals showed physiological alterations, but there were no substantial impacts and they remained viable [31].

Heavy metals from the environment enter bioretention facilities via stormwater runoff. However, some of the heavy metals are hazardous, and the question "to what extent does stormwater runoff containing heavy metals impair plant development when it enters bioretention facilities" remains unanswered. Studies on the effects of heavy metals in stormwater runoff on plant growth are scarce and inconclusive. Therefore, the primary goal of this study is to determine the impact of heavy metal concentrations in stormwater runoff on plant physiological indicators. Pb and Cd are not required for growth and are hazardous at low concentrations [32]. The Pb and Cd in stormwater runoff have been shown to be highly toxic at low levels, can undergo heavy metal accumulation at low levels, and generally have significant effects on plant growth and metabolism in bioretention facilities [33–35]. In this study, Pb and Cd were selected as research heavy metals. Two kinds of ornamental species in urban areas, *Ginkgo biloba* L. and *Ligustrum* × *vicaryi*, were selected as research objects. Chlorophyll content, net photosynthetic rate, and transpiration rate were selected as the physiological indicators. The effects of Pb and Cd on physiological indicators of *Ginkgo biloba* L. and *Ligustrum* × *vicaryi* were detected and analyzed.

2. Materials and Methods

2.1. Experimental Site

The experimental site was at the Beijing University of Architecture's Stormwater Laboratory, Daxing District, Beijing, China. In the study region, the average annual temperature was 12 °C and the average annual rainfall was 626 mm, with most of it falling from July to September, indicating a typical continental monsoon climate.

2.2. Plants and Heavy Metals

The two species used in this study were typically found in northern Chinese urban environments. These were a *Ginkgo biloba* L. arbor and one shrub, *Ligustrum* × *vicaryi*. The two species are decorative perennials with well-developed roots that are tolerant of drought and humidity.

Pb and Cd in road stormwater runoff were the heavy metals investigated in this experiment. The Pb and Cd pollution treatments used in this experiment simulated rainwater runoff with low and high concentrations of the heavy metals. Some scholars have studied the pollutant content of stormwater runoff in Beijing [7,36]; the concentrations of Pb and Cd in road stormwater runoff in Beijing were low and did not exceed CNEQS-SW-II (China National Environmental Quality Standards for Surface Water) which is Pb < 0.05 mg/L, Cd < 0.005 mg/L [7]. $Pb(NO_3)_2$ and $Cd(NO_3)_2$ were the Pb and Cd sources, the simulated stormwater runoff solution was the tap water solution fused with $Pb(NO_3)_2$ and $Cd(NO_3)_2$, and the low concentration levels in the rainwater runoff were 0.08 and 0.04 mg/L, respectively. The high concentration levels in the rainwater runoff were eight times those of the low concentrations. The experiment included six experimental treatments: two treatments that simulated low concentrations of heavy metals in rainwater runoff, two treatments that simulated high concentrations of heavy metals in rainwater runoff, and two controls that used tap water (Table 1).

Table 1. Experimental treatments information and number.

Classification	Inflow	Plants	Device Codes
Experimental groups	runoff with low concentrations of heavy metals	*Ginkgo biloba* L.	*G. biloba*-L
		Ligustrum × *vicaryi*	*L. vicaryi*-L
	runoff with high concentrations of heavy metals	*Ginkgo biloba* L.	*G. biloba*-H
		Ligustrum × *vicaryi*	*L. vicaryi*-H
Control group	tap water	*Ginkgo biloba* L. *Ligustrum* × *vicaryi*	*G. biloba*-T *L. vicaryi*-T

Note(s): n = 3.

The rainfall data for Beijing over the last 30 years were statistically analyzed to determine the experimental water intake under each treatment in this study and the number of days between water intakes in each month. The field rainfall interval time was 12 h and when the rainfall interval exceeded 12 h, it was defined as two rainfall events. Rainfall of less than 2.0 mm was considered unproductive flow and was not included in the statistical analysis. There have been 317 relevant rainfalls in Beijing over the last 30 years from August to October, with the average number of days between rainfalls in August, September, and

October being 6, 8, and 20 days, respectively (Table 2). As a result, this experiment was designed with three groups of inflow intervals. This experiment's arrangements were as follows: 21 August 2020, 27 August 2020, 2 September 2020, 10 September 2020, 18 September 2020, 26 September 2020, 5 October 2020, 24 October 2020. The simulation experiment ended on 24 October 2020, but measurements of the physiological indicators continued after the end of the experiment. The temperature variation and the maximum radiation variation on the ground during the experiment are shown in Figures 1 and 2, respectively, and the environmental variables remained the same throughout the experiment, except for the artificially set variables.

Table 2. Annual rainfall statistics in August, September, and October.

Month	Rainfall Frequency	Average Monthly Frequency	Rainfall Totals (mm)	Average Rainfall per Event (mm)	Rainfall Interval (Days)
August	159	5.30	1547.71	9.73	5.85
September	111	3.70	1174.32	10.58	8.11
October	47	1.57	432.25	9.20	19.79

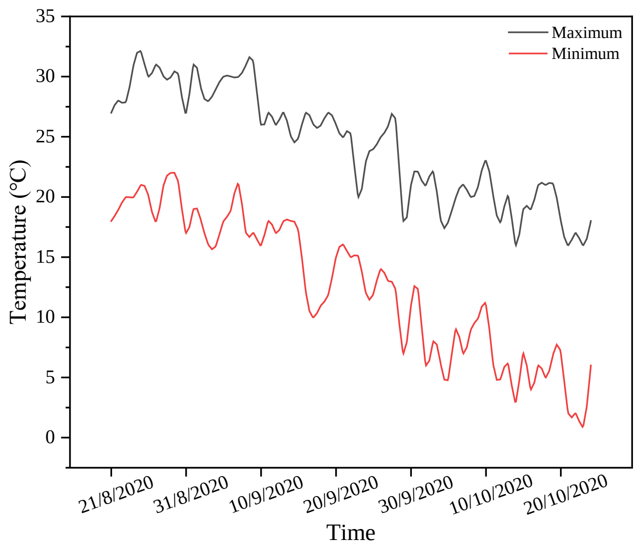

Figure 1. Changes in temperature during the experiment.

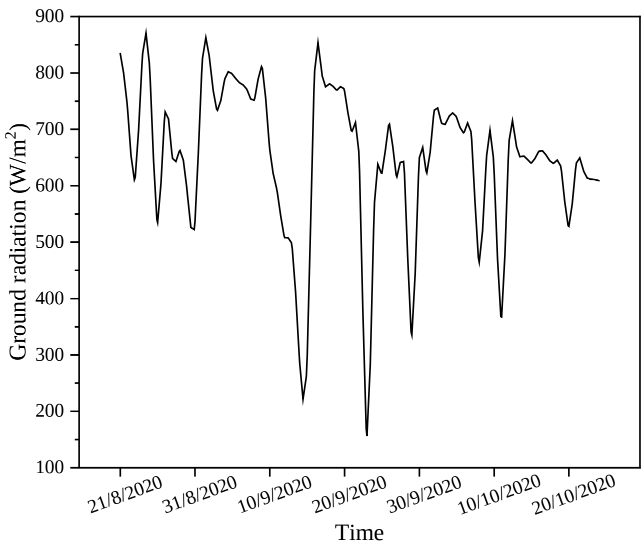

Figure 2. Changes in surface maximum radiation during the experiment.

The ratio of the bioretention facility area to the catchment area was set as 1:3, which was based on the "Sponge city construction technology guide—low impact development rainwater system construction (for trial implementation) and the urban road greening planning and design specifications (CJJ75-97)". Therefore, the inflow volumes in August, September, and October were 5.45, 5.92, and 5.15 L, respectively (Table 3).

Table 3. Experimental water inflow parameters for August, September, and October.

Month	Rainfall Interval (Days)	Average Rainfall per Event (mm)	Inflow Volume (L)
August	6	9.73	5.45
September	8	10.58	5.92
October	20	9.20	5.15

The experimental devices were made entirely of PVC, with a 28 cm water storage layer, a 40 cm filler layer, a geotextile membrane, a 5 cm gravel layer, and a 10 cm base with a 3 cm diameter perforated drainage pipe in the gravel layer. The overall height of the device was 83 cm. The uppermost layer was the water storage layer, which received rainwater runoff. The filler layer was field soil from the Daxing area and was separated from the drainage layer by a geotextile membrane, which acted as a filter layer to prevent the filler from being washed away and entering the drainage layer. The drainage layer, which was composed of gravel with a diameter of around 1 cm and a perforated drainage pipe with an inside diameter of 3 cm, was placed at the bottom of the device (Figure 3).

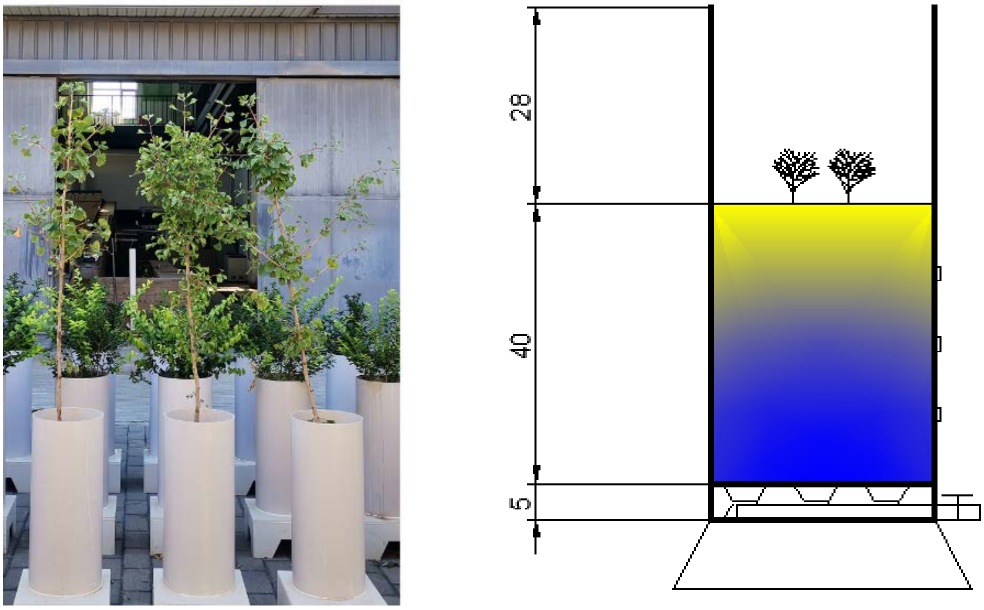

Figure 3. Actual view and cross-section of the experimental device.

2.3. Physiological Indicators

Photosynthesis is the most important physiological process occurring in plants. Most studies have demonstrated that plant photosynthesis is very sensitive to heavy metals. Stomatal closure, impaired photosynthetic mechanisms, and disruption of pigment synthesis all lead to a general decrease in photosynthetic efficiency. In this experiment, the plant physiological indicators were chlorophyll content, net photosynthetic rate, and transpiration rate, and it is hoped that the changes in the above physiological indicators will respond to the effects of heavy metals on plants.

2.4. Test of Heavy Metals and Physiological Indicators in Plants

The GFAAS method was used to determine Pb and Cd. The samples were added with 5 mL nitric acid and kept at 160 °C for 4 h in a constant temperature drying oven. Catching the acid to the liquid volume 1 mL, constant volume after cooling. The same procedure was performed with reagent blank experiment, finally using an atomic absorption spectrometer (model: SavantAA) made by GBC Scientific Equipment Pty Ltd. for determination. Only the species in the experimental groups were tested for heavy metals. In one measurement of a physiological index, three leaves were randomly found in a branch of the plant for measurement. For the measurement of the chlorophyll content, the leaves needed to be distinguished into mature leaves and young leaves. The Pb and Cd in the species were analyzed by first cutting the top, middle, and bottom leaves and branches of the species. The young leaf was the second downward leaf from the top of the plant stem and the mature leaf was the sixth leaf [37]. A SPAD-502 Plus chlorophyll meter was used to determine the CC (accuracy: ±1.0 SPAD, precision: 0.1 SPAD, manufacturer: Konica Minolta, Tokyo, Japan). A CI-340 handheld photosynthesis system (manufacturer: CID Bio-Science) was used to assess the NPR [μmol $CO_2/(m^2s)$] and TR [mmol $H_2O/(m^2s)$]. Calibrating the instruments before the experiment, a plant measured three or six leaves. Each test was administered between 10:00 a.m. and 12:00 p.m. on the test day to ensure data accuracy.

2.5. Data Analysis

The average change rate (ACT) equation was used to elucidate the influence of the heavy metals in the stormwater runoff on plant physiological markers [38].

$$ACT = \frac{\sum\left(\frac{M_{i+1}}{M_i} - 1\right)}{\tau - 1} \quad (1)$$

where M is the plant physiological indicators such as CC, NPR, or TR; i is the ith test data; τ is the total number of data.

3. Results and Discussion

3.1. Changes in Chlorophyll Content

The variations in the *Ginkgo biloba* L. chlorophyll content are shown in Figure 4a. Figure 4a shows that low concentrations of Pb and Cd could slightly alleviate decreases in *Ginkgo biloba* L. CC. The effect of high concentrations on young leaves was the same as lower concentrations but had a significant inhibitory effect on mature leaves. The CC for *G. biloba*-T-YL (YL represents young leaves) was generally lower than those for the other treatments, the CC in *G. biloba*-L-YL was higher than that in *G. biloba*-T-ML (ML represents mature leaves), and the CC in *G. biloba*-H-ML was similar to that in *G. biloba*-T-ML. The mature leaves did not significantly differ among the treatments. The ACT change curve for *Ginkgo biloba* L. CC shows that CC is less than zero at the end of the incoming water experiment, indicating that the CC in *Ginkgo biloba* L. do not increase. However, there was a significant increase in the *G. biloba*-L ACT for CC, indicating that Pb and Cd at low concentrations could partially alleviate the decrease in CC seen in young and mature *Ginkgo biloba* L. leaves.

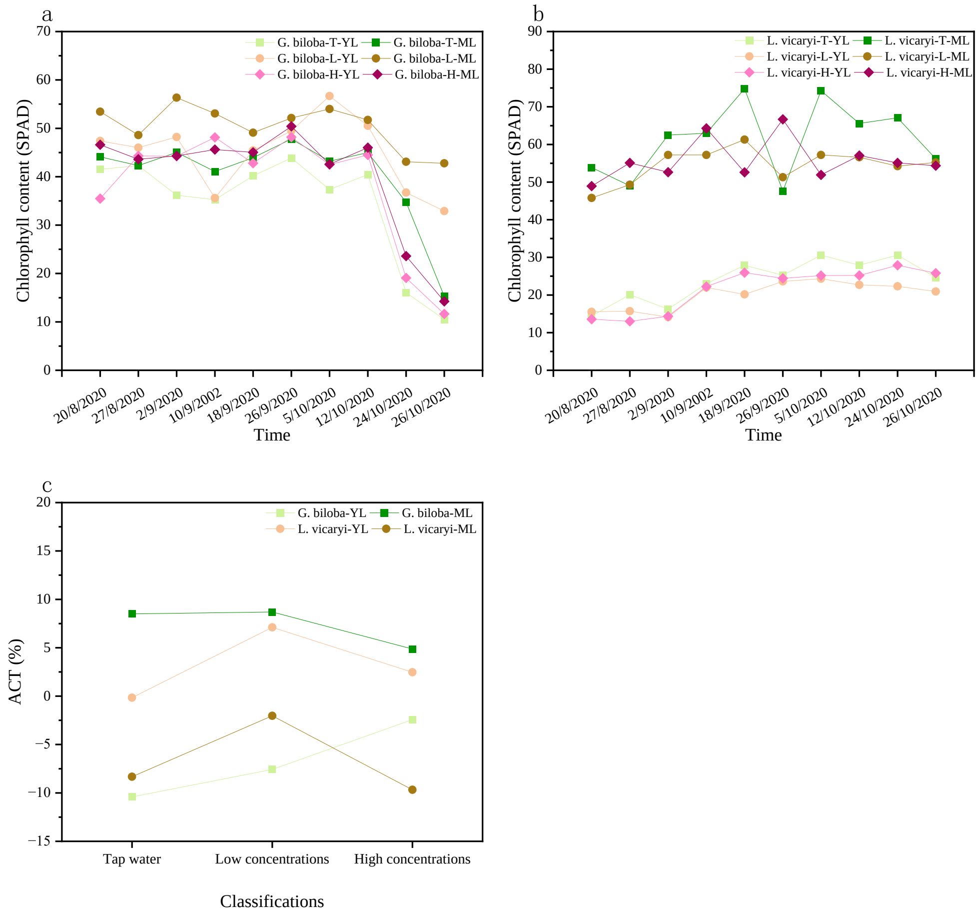

Figure 4. Changes in (**a**) *Ginkgo biloba* L. and (**b**) *Ligustrum* × *vicaryi* chlorophyll contents, n = 3; and (**c**) chlorophyll content ACT in the plants.

The changes in *Ligustrum* × *vicaryi* CC are shown in Figure 4b. Low Pb and Cd concentrations inhibited a CC rise in *Ligustrum* × *vicaryi* to some degree, but high Pb and Cd concentrations severely inhibited CC increases. The CC in *Ligustrum* × *vicaryi* showed an undulating increase, and the increase in mature leaf CC was significantly greater than in young leaves. Furthermore, CC under the *L. vicaryi*-T treatment was generally higher than that for the *L. vicaryi*-L and *L. vicaryi*-H treatments. Low concentrations of Pb and Cd had no effect on the chlorophyll contents of young leaves from the *L. vicaryi*-L treatment, but significantly inhibited CC increases in mature leaves, and the *L. vicaryi*-H treatment had a significant inhibitory effect on both young and mature leaves, according to the ACT curves. This indicated that low concentrations of Pb and Cd inhibited chlorophyll increases in *Ligustrum* × *vicaryi* to some extent, but high concentrations of Pb and Cd had significant effects. Furthermore, the results showed that chlorophyll levels in *Ligustrum* × *vicaryi* were elevated. The ACT results for heavy metal content in *Ligustrum* × *vicaryi* showed that the Pb content in *Ligustrum* × *vicaryi* decreased at low concentrations and increased at high concentrations, indicating that Pb transport was restricted. However, the higher concentration led to increased plant organ tissue damage, and thus reduced the transport restriction.

The results showed that each plant behaved differently. After analysis, the Pb content in *G. biloba*-L and *G. biloba*-H showed increases of 0.0835 mg/kg and 0.0555 mg/kg, respectively, compared to the initial experimental period, and *L. vicaryi*-L and *L. vicaryi*-H showed increases of 0.0395 mg/kg and 0.2730 mg/kg, respectively. The chlorophyll changes in both plants were highly correlated with the increase in Pb content. Plants have evolved different resistance mechanisms in response to biotic or abiotic stresses [39]. The toxic excitation effect is a plant phenomenon that can be characterized by an inverse U-shaped curve in which modest concentrations of hazardous chemicals have a stimulatory impact [40]. Honeysuckle has been shown to alleviate the effects of heavy metal stress on plant metabolic disorders by boosting leaf osmoregulatory chemicals and triggering the reactive oxygen species scavenging mechanism under a specified range of heavy metal stresses [41]. *Ginkgo biloba* L. activated this mechanism to cause harmful excitatory effects in response to low concentrations of Pb and Cd stress in this investigation. In response to the phenomenon of chlorophyll changes occurring in *Ligustrum* × *vicaryi*, based on available information, heavy metals can inhibit CC for a variety of reasons, one of which is an increase in the production of chlorophyllase, which disrupts the biosynthesis of chlorophyll and carotenoids and leads to chlorophyll breakdown [42]. The Cd was exclusively found in *Ligustrum* × *vicaryi*; therefore, it was probable that Cd stress impaired the photosynthetic organs and damaged the chloroplast ultrastructural components, such as the cystoid membranes [43,44].

3.2. Changes in Net Photosynthetic Rate

Low concentrations of Pb and Cd slightly inhibited the *Ginkgo biloba* L. and *Ligustrum* × *vicaryi* NPRs, and excessive concentrations severely reduced their NPRs (Figure 5a,b). Figure 5a shows that the NPR for *Ginkgo biloba* L. decreased undulatingly in the *G. biloba*-T, *G. biloba*-L, and *G. biloba*-H treatments, with a maximum net photosynthetic rate of 8.57 μmol CO_2/m^2s for *G. biloba*-T, 5.97 μmol CO_2/m^2s for *G. biloba*-H and 10.90 μmol CO_2/m^2s for *G. biloba*-L. Figure 5c shows that the ACT order for the NPR under the three experimental treatments was *G. biloba*-L > *G. biloba*-T > 0 > *G. biloba*-H, but the device *G. biloba*-L did not return to its original level. Low concentrations of Pb and Cd impeded the *Ginkgo biloba* L. NPR.

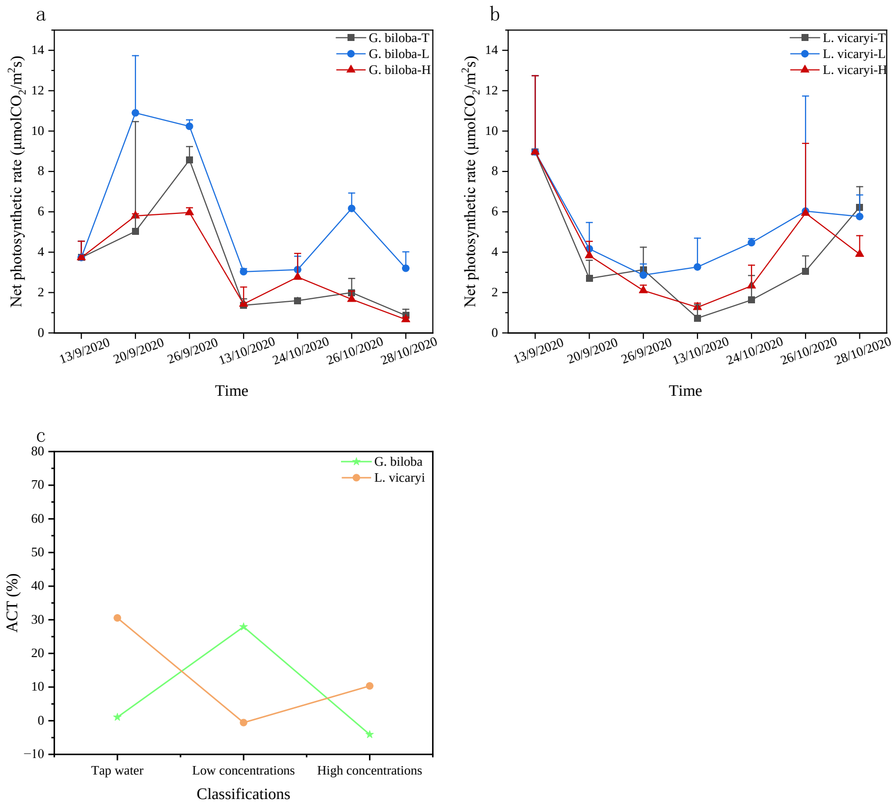

Figure 5. Changes in the (**a**) *Ginkgo biloba* L. and (**b**) *Ligustrum* × *vicaryi* net photosynthetic rates, error bar represented the measurement error of n leaf physiological index of the same plant, n = 3; and (**c**) ACT for the net photosynthetic rate.

Figure 5b shows that the NPRs for *L. vicaryi*-T, *L. vicaryi*-L, and *L. vicaryi*-H decreased at first and then increased, but none of them recovered to their initial levels. The maximum net photosynthetic rate occurred on 13 September 2020 and the value for all treatments was 8.97 μmol CO_2/m^2s. Figure 5c also shows that the ACT for the *L. vicaryi*-L NPR fell dramatically and was near 0. The ACT for the *L. vicaryi*-H NPR also decreased, and the ACT order for NPR in the three treatments was *L. vicaryi*-T > *L. vicaryi*-H > 0 > *L. vicaryi*-L. The Pb and Cd caused an overall reduction in the *Ligustrum* × *vicaryi* NPR.

In addition to variations in CC, the NPR changes could also be linked to the plant resistance mechanism [45]. Different factors influence the photosynthetic rate, but the two most important are sunlight and CO_2 [46]. Heavy metals impair photosynthesis at the whole plant level by reducing stomatal conductance and photosynthetic pigment levels. They also indirectly affect chloroplast activity and other metabolic activities [47]. Pb enters the cells and has a variety of harmful effects on plant physiological and biochemical processes, resulting in cellular abnormalities that disturb normal physiological activities. These changes lead to decreased growth and metabolism, membrane disruption, and impaired photosynthesis [48,49]. Cadmium (0 and 5 mg/L) also has been shown to dramatically lower stomatal conductance and the plant photosynthetic rate [28].

A comparison between the two plants showed that the NPRs for *Ginkgo biloba* L. and *Ligustrum* × *vicaryi* were related to the Pb and Cd concentrations. The analysis suggested that, in addition to the inconsistency among different plants to different heavy metal treatments, the co-existence of Pb and Cd, might also have a significant effect. Pb and Cd were not detected in the two plants several times (Table 4).

It suggests that the greatest barrier to the entry of heavy metal ions into plant tissues was the physicochemical features of the plant root system [50]. The root uptake system means that root cells in plants can actively avoid Cd ions [50,51]. A second possibility is that heavy metals are retained in the roots of many plants, reducing their transfer to the aboveground tissues [52–54]. It has been found that the translocation of some specific heavy metals, such as Pb and especially Cd, was limited [54]. A third possibility is that coexisting elements reduced Cd absorption by plant roots. It has been found that the coexisting elements restrict the Cd uptake by maize roots, and their inhibitory capacity connects to the chemical characteristics of each element [55]. However, our trials did not continue throughout the year, which limited the number of accessible testing dates and could have resulted in lower Cd uptake recordings by *Ginkgo biloba* L. and *Ligustrum* × *vicaryi*.

3.3. Changes in Transpiration Rate

Pb and Cd led to an increase in the *Ginkgo biloba L. and Ligustrum* × *vicaryi* TR, and the effects of low Pb and Cd concentrations were more significant (Figure 6a,b). According to Figure 6a, the *G. biloba*-T, *G. biloba*-L, and *G. biloba*-H treatment TRs showed relatively large fluctuations, and the maximum values of the TRs for *G. biloba*-T, *G. biloba*-L, and *G. biloba*-H all occurred on the 24 October 2020. According to Figure 6c, the ACT order for the *G. biloba*-T, *G. biloba*-L, and *G. biloba*-H TRs was *G. biloba*-L > *G. biloba*-H > *G. biloba*-T > 0, indicating that Pb and Cd increased the *Ginkgo biloba* L. TR. Figure 6b shows that the *L. vicaryi*-T, *L. vicaryi*-L, and *L. vicaryi*-H TRs had similar trends, showing an increase, a decrease, and then an increase, with a maximum TR of 1 mmol H_2O/m^2s for *L. vicaryi*-T and 1.4 mmol H_2O/m^2s for *L. vicaryi*-L and *L. vicaryi*-H. Figure 6c shows that the ACT order for the *L. vicaryi*-T, *L. vicaryi*-L, and *L. vicaryi*-H TRs is *L. vicaryi*-L > *L. vicaryi*-H > *L. vicaryi*-T > 0, which indicates that Pb and Cd increase the *Ligustrum* × *vicaryi* TR and that the effect of the low Pb and Cd concentrations is more significant. Changes in the transpiration rate of plants resembled a toxic excitation effect, which is a plant phenomenon that can be characterized by an inverse U-shaped curve in which modest concentrations of hazardous chemicals had a stimulatory impact [40].

Table 4. Heavy metal level in plants based on single factor index (mg/kg).

Time	Pb				Cd			
	***G. biloba*-L**	***G. biloba*-H**	***L. vicaryi*-L**	***L. vicaryi*-H**	***G. biloba*-L**	***G. biloba*-H**	***L. vicaryi*-L**	***L. vicaryi*-H**
08/20/2020	0.1185 ± 0.0021 [a]	0.1625 ± 0.0035	0.1870 ± 0.0084	0.1450 ± 0.0028	–	–	0.01825 ± 0.0008	0.0154 ± 0.0004
09/05/2020	0.1435 ± 0.0007	0.1405 ± 0.0035	0.1575 ± 0.0021	0.1015 ± 0.0007	0.0069 ± 0.0003	0.0069 ± 0.00002	0.01395 ± 0.0004	0.008515 ± 0.00004
09/21/2020	0.1855 ± 0.0007	0.1465 ± 0.0064	0.1030 ± 0.0014	0.1305 ± 0.0007	–	–	0.008175 ± 0.0001	0.0108 ± 0.0001
10/18/2020	0.2310 ± 0.01556	0.2610 ± 0.0071	0.2825 ± 0.0077	0.2895 ± 0.0148	0.0024 ± 0.0006	0.0019 ± 0.0001	0.003775 ± 0.0001	0.004795 ± 0.0003
11/03/2020	0.3095 ± 0.0007	0.2610 ± 0.0014	0.1870 ± 0.0028	0.1495 ± 0.0035	0.0036 ± 0.0005	–	–	–
12/10/2020	0.2020 ± 0.0014	0.2180 ± 0.0014	0.2265 ± 0.0035	0.4180 ± 0.0113	–	0.0072 ± 0.0004	0.0142 ± 0.0001	0.02195 ± 0.0002

Note(s): [a] Single factor index data are mean ± SD.—Indicates that the detection value is lower than the detection limit. The detection limit of Pb is 0.02 mg/kg, the limit of quantification is 0.04 mg/kg; the detection limit of Cd is 0.001 mg/kg, the limit of quantification is 0.003 mg/kg.

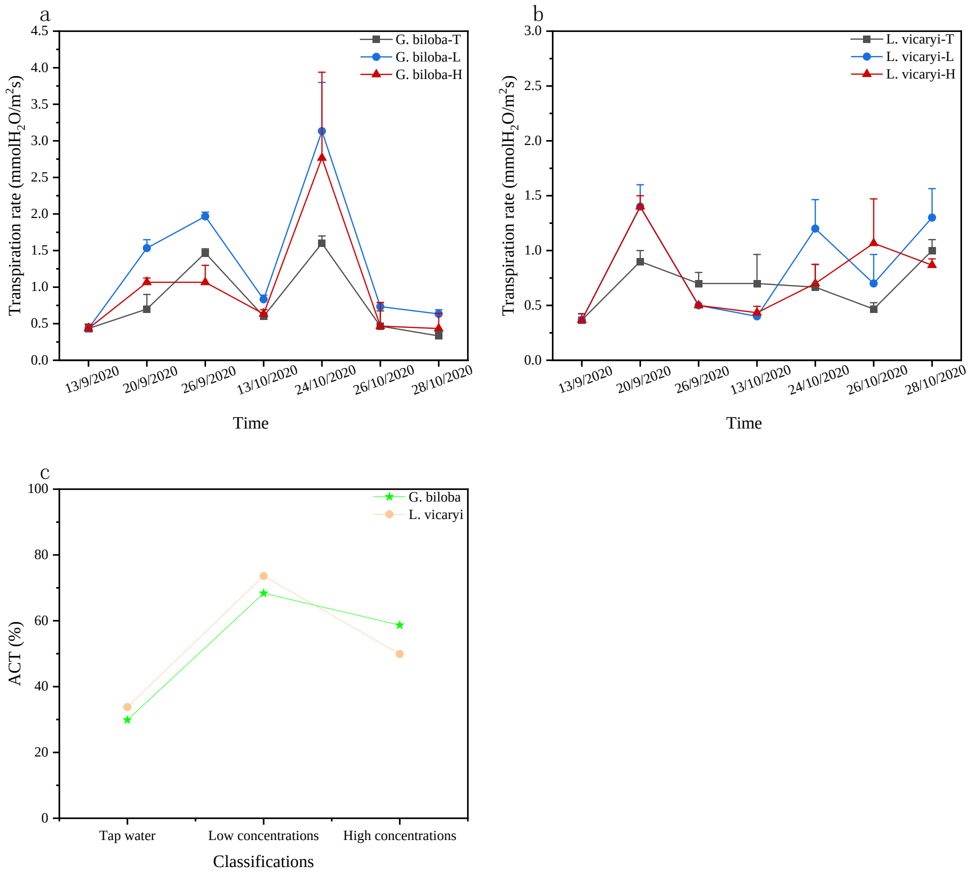

Figure 6. Changes in the (**a**) *Ginkgo biloba* L. and (**b**) *Ligustrum* × *vicaryi* transpiration rates; error bar represented the measurement error of n leaf physiological index of the same plant, n = 3; and (**c**) ACT for the plant transpiration rate.

Figure 6a and Table 4 show that the experimental treatment changes are approximately the same. Both figures show that the TR and Pb content first increased and then decreased over time in the two plants. Figure 6b and Table 4 show that the changes are relatively consistent over time. However, the correlation analysis showed that the changes in the plant physiological indicators did not significantly correlate with the changes in plant heavy metal contents. Explaining the reasons for this, the heavy metal contents in the plants were relatively small. Furthermore, the insignificant correlation also indicated that the impact of heavy metals in the stormwater runoff on plants in bioretention facilities was relatively small and that the plants could withstand the heavy metal loading. The TRs of the two plants improved when Pb and Cd were present, regardless of the Pb and Cd concentrations, and the effect was more noticeable for both plants at low concentrations. This showed that Pb and Cd had a positive and stimulating effect on plant TR.

4. Conclusions

This study examined the physiological responses of *Ginkgo biloba* L. and *Ligustrum* × *vicaryi* in bioretention facilities to compound heavy metals by simulating Pb and Cd concentrations in stormwater runoff and detecting changes in the physiological indicators for *Ginkgo biloba* L. and *Ligustrum* × *vicaryi*. Overall, the stormwater runoff containing low and high concentrations of Pb and Cd did not influence the mortality of plant species when directly contacting plant species. In fact, according to the results of the study, they did affect the physiological functions of plant species in bioretention facilities to some extent, but not all of these effects were negative. In the case of stormwater runoff containing low concentrations of Pb and Cd, bioretention facility plants showed positive changes compared to the blank control group, especially for the *Ginkgo biloba* L.

species. For stormwater runoff with high concentrations of Pb and Cd, bioretention plants performed less well compared to the blank control group, and this performance was even more pronounced on *Ligustrum* × *vicaryi*. The stormwater runoff with high concentrations of Pb and Cd harmed the growth and development of bioretention facilities plants to some extent. Specific conclusions were as follows:

(1) Low concentrations of Pb and Cd helped to prevent chlorophyll loss in *Ginkgo biloba* L. leaves, whereas high Pb and Cd concentrations may help prevent chlorophyll loss in young leaves but aggravate chlorophyll loss in mature leaves. Regardless of the concentrations, Pb and Cd reduced the CC increase in *Ligustrum* × *vicaryi* leaves. The changes in plant CC were highly correlated with the changes in plant Pb content.
(2) Low concentrations of Pb and Cd slightly inhibited the *Ginkgo biloba* L. and *Ligustrum* × *vicaryi* NPRs, whereas excessive concentrations severely reduced the NPR.
(3) Pb and cadmium increased the *Ginkgo biloba* L. and *Ligustrum* × *vicaryi* TRs and the effect of low Pb and Cd concentrations was more significant.

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