The relationship between typical heavy metal content and physiological indexes of shrubs in bioretention facilities

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ABSTRACT

Bioretention facilities have been widely constructed, but it is unknown whether rainfall runoff containing pollutants will hurt the plants in bioretention facilities. Experiments were carried out to explore the relationship between the heavy metal (Cd and Pb) contents of four shrubs and three physiological indexes. The results show the following: (1) The heavy metal absorption of shrubs may be directly proportional to the heavy metal content in stormwater runoff. (2) For the experimental devices, sand/soil-low concentration (SS-L) and sand/soil-high concentration (SS-H), except that the contents of Cd and Pb of L. vicaryi in SS-H showed a trend of increasing first and then decreasing, the contents of heavy metals in other shrubs showed a gradual increase. (3) For SS-L, the net photosynthetic rate (NPR) and transpiration rate (TR) of R. xanthina were in direct proportion to the contents of Cd and Pb in vivo. The NPRs and TRs of the other three shrubs and the chlorophyll content (CC) of L. vicaryi presented an inversely proportional relationship. For SS-H, the NPR of L. vicaryi was in direct proportion to its Cd and Pb contents. Almost all other conditions showed an inverse relationship.

Key words: bioretention facilities, heavy metals, physiological indexes, shrubs, stormwater runoff

HIGHLIGHTS

- Whether rainfall runoff will hurt shrubs in road bioretention was answered.
- The relationship between heavy metal and shrub physiology was studied.
- Contents of Cd and Pb in shrubs gradually increased except in one case.
- Cd and Pb inhibited physiological functions of most shrubs.
- Cd and Pb promoted some physiological indexes of individual shrubs.
1. INTRODUCTION

With the acceleration of urbanization, city impermeability rates are increasing, and a large amount of stormwater cannot directly penetrate the ground, thus increasing the stormwater runoff from roads. Large stormwater runoff not only causes urban flooding (Sandoval et al. 2019) but also contains a large number of heavy metals and other pollutants, such as Cu, Cd, and Pb (Lim et al. 2015). These pollutants and heavy metals in stormwater runoff mainly come from fuel combustion, road asphalt, automobile exhaust, and so on (Reddy et al. 2014). Given this situation, the Chinese government proposed the concept of the ‘sponge city’ in 2013 and began to carry out the construction of a sponge city in 2014. Sponge city contains many low-impact development (LID) facilities, among which bioretention is one of the most commonly used facilities (Jiang et al. 2018).

A bioretention facility is a ground-based, plant-growing facility that uses a growing media layer and other plants, such as trees, shrubs, and herbs, to mitigate heavy metals and other pollutants in stormwater runoff (Sun & Davis 2007; Wang et al. 2019). The removal of heavy metals in stormwater runoff is mainly attributed to processes such as filtration and absorption by the growing media layer and plants, biological assimilation and decomposition, and volatilization (Gill et al. 2017; Chen et al. 2020; Kong et al. 2021). In addition, bioretention facilities have a certain storage capacity to receive stormwater runoff from roads, parking lots, and urban buildings to reduce flood peaks (Dietz & Clausen 2006). Therefore, bioretention facilities are widely used around the world. However, urban road stormwater runoff contains heavy metals and other pollutants, and it is unknown whether stormwater runoff will harm plants when it flows into bioretention facilities.

To date, many studies (Cheng et al. 2002; Galletti et al. 2010; Zhang et al. 2020) have investigated the removal efficiency of heavy metals by plants, the heavy metal contents in plants, and the influence of heavy metals on other plant physiological indexes, but few of the relationships between the heavy metal contents of plants and plant physiological indexes have been studied. Previous studies on the contents of heavy metals in plants and the sources of heavy metals in plants have been carried out. Studies have shown that the adsorption process can remove up to 91 and 89% Cd and Pb, respectively. According to mass balance calculations, the corresponding plant absorption amounts were 5.2 and 6%, respectively (Mohammed & Babatunde 2017). Enya et al. (2019) studied heavy metal pollution in the soil-plant system of the Mersey estuary floodplain in northwest England and determined the content of heavy metals in plant tissues. The results showed that soil pollution led to an increase in heavy metal concentrations in plants. Several researchers have also studied the sources of heavy metals in plant leaves, and the results showed that heavy metals in the air are the main source of heavy metals in plant organs (Peng et al. 2020). Liang et al. (2017) also showed that the heavy metals Cu, Zn, Cd, and Pb in plant leaves not only come from the soil but also may come from the massive deposition of heavy metals in the atmosphere.
There have been some studies on the effects of heavy metals on plants. Zhang et al. (2014) showed that a high concentration of Cd significantly increased the chlorophyll content (CC) in young leaves of the energy crop king grass (Pennisetum Americanum × P. purpureum) but did not affect mature leaves. Sorrentino et al. (2018) studied three brassica species, planted with the heavy metals Cd and Pb, and showed that the photosynthesis of all three species did not drop with heavy metal contamination and that the photosynthesis of Spagnolo was fairly stable. Manios et al. (2003) showed that under the stress of Cd, Cu, Ni, Pb, and Zn, only the chlorophyll in cattail subjected to a strong concentration showed inhibition at the later stage, while the total amount of chlorophyll in cattail under stress at other concentrations showed a trend of increasing with time.

In this study, we set up bioretention facility devices and conducted stormwater runoff experiments. The specific objectives for this paper were: (1) to explore the relationship between the amount of Cd and Pb uptaking in shrubs and in stormwater runoff; (2) to investigate the changes of Cd and Pb content with time in shrubs; (3) to study the relationship between the contents of Cd and Pb in typical shrubs and their physiological indexes in bioretention facilities; and (4) to explore the influence of Cd and Pb on the physiological indicators of typical shrubs in the bioretention facility. The research results can provide theoretical support for the construction of sponge cities to some extent.

2. MATERIALS AND METHODS

2.1. Experimental devices

This experiment included three experimental apparatuses: a device with low concentration heavy metal influent, sand/soil-low (SS-L); a device with high heavy concentration metal influent concentration, sand/soil-high (SS-H); and a control device with tap water as influent, sand/soil-tap water (SS-T). SS-T was the control group, and SS-L and SS-H were the experimental group. The experimental device size (length × width × height) was 0.8 × 0.8 × 0.6 m. Figure 1 is the longitudinal profile of the experimental unit; the experimental equipment included a stent, 10 cm drainage layer, geotextile, 40 cm growing
media layer, and 10 cm water storage layer. The 40 cm growing media layer was a common combination of soil and sand with a volume ratio of 4:6. The soil and sand were proportionally mixed and then filled into the device with slight compaction. Based on an investigation of the typical shrubs on roads in Beijing, the vegetation layer included four kinds of plants, which are four typical shrubs (*R. xanthina, L. vicaryi, B. thunbergii, and B. megistophylla*). Each experimental device was separated from the next by 1 m so that all devices were under the same external conditions and experienced the same growth conditions to reduce the experimental error caused by human factors.

### 2.2. Experimental methods

For this study, mean values of low concentrations of Cd and Pb were 65.97 and 58.37 μg/L, respectively. Mean values of high concentrations of Cd and Pb were 214.83 and 151.56 μg/L, respectively. The statistical rainfall data from 1983 to 2012 in Beijing were used to design three levels of rainfall intensity and two levels of rainfall frequency. Silty loam was used as the growing media of bioretention facilities. The plants were selected by investigating the typical shrubs on the main road of the pilot sponge city construction area in Beijing. The shrubs were mixed and planted in the experimental devices.

To simulate road runoff, tap water was placed for 24 h, and the designed pollution concentration was reached by adding pure reagents. Then, a simulation of road runoff at bioretention facilities was carried out. Detailed experimental methods can be referred to the experimental methods of Gong *et al.* (2019).

### 2.3. Sample collection and determination

The net photosynthetic rate (NPR), transpiration rate (TR), and CC were selected as physiological indexes in this study. NPR, TR, and CC were measured approximately every 20 days for a total of 4 times. Two leaves were taken from the upper, middle, and lower parts of the shrub. The leaves at the same position of the same shrub were mixed for physiological index detection. This method was used for sample collection and determination of each shrub in each device; the average value of the six data points was finally taken as the physiological index value.

NPR and TR were measured using a CI-340 photosynthetic measurement system (CI-340, CID company, USA), and all measurements were made between 9 a.m. and 12 noon during the measurement period. CC was determined by a portable chlorophyll meter (SPAD-502PLUS chlorophyll meter, KONICA MINOLTA company, Japan).

### 3. RESULTS AND DISCUSSION

#### 3.1. Analysis of Cd and Pb contents in shrubs

In the process of the experiments, the heavy metal (Cd and Pb) contents of four shrubs in SS-L, SS-T, and SS-H were detected approximately once every month. The changes in Cd and Pb contents in typical shrubs were measured and are shown in Table 1. The changing trend is presented in the form of a star awn chart in Figure 2. (Starting at 12 o’clock and moving clockwise, the four axes represent different times. The ring represents the content of the heavy metals Cd and Pb.) This section mainly covers the changes in the Cd and Pb contents in the four shrubs with increasing experimental time.

|       | *R. xanthina* |         | *L. vicaryi* |         | *B. thunbergii* |         | *B. megistophylla* |         |
|-------|---------------|---------|---------------|---------|----------------|---------|-------------------|---------|
| Date  | Heavy metal   | SS-L    | SS-T          | SS-H    | SS-L           | SS-T   | SS-H              | SS-H    |
|       |               | 0.04    | 0.02          | 0.03    | 0.04           | 0.04   | 0.06              | 0.02    |
| 2018/8/20 Cd | 1.36 | 0.79   | 1.14          | 1.14    | 1.14           | 0.86   | 1.22              | 1.22    |
| 2018/9/14 Cd | 0.18 | 0.17   | 1.46          | 0.19    | 1.08           | 0.09   | 0.03              | 0.25    |
| 2018/10/15 Cd | 1.58 | 0.95   | 4.79          | 1.07    | 2.02           | 2.12   | 2.7               | 2.7     |
| 2018/11/14 Cd | 0.25 | 0.22   | 1.04          | 0.2     | 0.23           | 1.15   | 0.71              | 0.28    |
| Pb     | 1.67 | 1.07   | 10.42         | 3.75    | 3.79           | 3.75   | 4.38              | 4.38    |

Note: The unit of Cd and Pb content in shrub is mg/kg; Date: yyyy/mm/dd.
Table 1 clearly showed that different shrubs had similar trends. Almost always, the contents of the heavy metals Cd and Pb in the shrubs increased gradually with the progression of the experiment. The Cd and Pb contents of the four shrubs in SS-H were significantly higher than those of the various shrubs in SS-L.

The Cd and Pb contents of *R. xanthina* in the SS-H experimental group gradually increased, and the rate of increase in the later period was significantly higher than that in the early and middle periods (Table 1). The Cd and Pb contents of *L. vicaryi* first increased and then decreased. The contents of Cd and Pb in *B. thunbergii* and *B. megistophylla* increased gradually, but the rate of increase decreased gradually (Table 1). Overall, the Cd and Pb contents in *L. vicaryi* first increased and then decreased, but the contents also increased as a whole compared with those on 20 August 2018. The Cd and Pb contents in *L. vicaryi*, *B. thunbergii*, and *B. megistophylla* in the devices showed an increasing trend overall (Figure 2).

The Pb content in the four shrubs in SS-L in the experimental group had the same change rule as that in *R. xanthina* in SS-H, but the Cd content in all devices increased first and then fluctuated slightly (Table 1). Overall, almost all the Cd and Pb contents in SS-L in the experimental group showed an upward trend (Figure 3).

The shrubs in the control group SS-T also contained heavy metals, which may have been caused by the deposition of heavy metals in the atmosphere, resulting in the presence of Cd and Pb in the shrubs. In the control group, SS-T, the Cd and Pb contents in the bodies of *R. xanthina*, *L. vicaryi*, and *B. thunbergii* showed an overall trend of increasing with time, but the Cd and Pb contents in *B. megistophylla* decreased first and then increased (Table 1), with values slightly higher than those on 20 August 2018, and the Cd and Pb contents in *B. megistophylla* showed little change in the whole process (Figure 4).

![Figure 2](http://iwaponline.com/hr/article-pdf/52/5/1132/950528/nh0521132.pdf)

**Figure 2** | Changes in Cd (a) and Pb (b) content in different plants in SS-H.

![Figure 3](http://iwaponline.com/hr/article-pdf/52/5/1132/950528/nh0521132.pdf)

**Figure 3** | Changes in Cd (a) and Pb (b) content in different plants in SS-L.
In this study, the Cd and Pb contents in the device with high concentration heavy metal influent were higher than those in the device with low concentration heavy metal influent, and the absorbed heavy metals of the shrubs were in direct proportion to the heavy metal contents in the runoff and stormwater.

The above analytical results also showed that for the experimental devices SS-L and SS-H, which were treated with stormwater runoff containing Cd and Pb, except that the Cd and Pb contents of *L. vicaryi* in SS-H increased first and then decreased, the contents of heavy metals under other conditions increased gradually. For the shrubs of the control group SS-T, except for the contents of Cd and Pb in *B. megistophylla*, which showed little change, the contents of Cd and Pb in other typical shrubs, including those in *B. megistophylla*, increased. Among the plants of SS-T, the contents of Cd and Pb in *L. vicaryi* increased first and then decreased, and the content of Pb in *B. megistophylla* decreased first and then increased, while the other contents increased gradually with time.

### 3.2. Analysis of changes in the physiological indexes of shrubs with the Cd and Pb contents in plants

#### 3.2.1. Net photosynthetic rate

This section mainly studies the relationship between the content of the heavy metals Cd and Pb in shrubs and NPR, as well as the effects of the heavy metals on shrub growth. The relationship between heavy metals in shrubs and NPR is shown in Figure 5. Previous studies have shown that photosynthesis is a normal reaction in plant growth, and NPR is an important physiological indicator that can reflect the growth state of plants. Since plants are sensitive to heavy metals, heavy metals may inhibit plant photosynthesis (Ralph & Burchett 1998).

The relationship between NPR and the heavy metals Cd and Pb in shrubs in the experimental group SS-L was analysed. In SS-L, the NPR of *R. xanthina* gradually increased, and the increase was in direct proportion to the contents of Cd and Pb in *R. xanthina*. However, the NPRs of *L. vicaryi*, *B. thunbergii*, and *B. megistophylla* decreased gradually, with values inversely related to the contents of Cd and Pb in *vivo*. This suggests that a low concentration of heavy metals may be beneficial to photosynthesis in *R. xanthina*, while a low concentration of heavy metals may inhibit photosynthesis in *L. vicaryi*, *B. thunbergii*, and *B. megistophylla*.

The relationship between NPR and the heavy metals Cd and Pb in shrubs in the experimental group SS-H was analysed. The NPRs of *R. xanthina* and *B. thunbergii* in SS-H in the experimental group decreased gradually, with values inversely related to the contents of Cd and Pb in these two shrubs. Although there was only a small fluctuation in the NPR of *B. megistophylla* in the whole process, the NPR of *B. megistophylla* decreased slightly on 14 November 2018 compared with that on 20 August 2018. The NPR of *L. vicaryi* increased first and then decreased, exhibiting the same change rule as Cd and Pb in *L. vicaryi*, so the NPR of *L. vicaryi* was in direct proportion to the Cd and Pb contents. This result indicates that a high concentration of heavy metals inhibited photosynthesis in *R. xanthina*, *B. thunbergii*, and *B. megistophylla* while promoting photosynthesis in *L. vicaryi*.

The relationship between NPR and the heavy metals Cd and Pb in shrubs in the experimental group SS-T was analysed. The NPRs of *R. xanthina* and *B. thunbergii* in the control group SS-T decreased with time. Although the NPR of *L. vicaryi* occurred...
decreased overall, the overall change was not significant; the Pb content in B. megistophylla decreased first and then increased, and the reduction was large. This result indicates that the NPRs of R. xanthina, L. vicaryi, and B. thunbergii gradually decreased under the condition of tap water inflow without Cd and Pb, while the NPR of B. megistophylla did not change much overall, which indicates that the photosynthetic capacity of these shrubs would also decrease under natural conditions. This effect might be caused by seasonal changes and external environmental factors. Quan & Wang (2018) also believed that NPR differed among conditions and varied with changes in environmental factors.

3.2.2. Transpiration rate

This section mainly studies the relationship between the content of heavy metals Cd and Pb in shrubs and TR, as well as the effects of the heavy metals on shrub growth. TR is an important physiological index of plants that can reflect the transpiration ability of plants. The relationship between heavy metals in shrubs and TR is shown in Figure 6.

In the experimental group SS-L, the TR of R. xanthina increased gradually, and the increase was 55% overall, which was in direct proportion to the Cd and Pb contents in the shrub. The TRs of L. vicaryi, B. thunbergii, and B. megistophylla decreased gradually and were inversely proportional to the Cd and Pb contents in these shrubs. The results showed that a low concentration of heavy metals was beneficial to transpiration in R. xanthina and inhibited transpiration in other typical shrubs.

The relationship between TR and the heavy metals Cd and Pb in shrubs in the experimental group SS-H was analysed. The TRs of R. xanthina, B. thunbergii, and B. megistophylla in SS-H gradually decreased, and the change trends of Cd and Pb contents were opposite in these shrubs. With the rapid increase in the Cd and Pb contents of L. vicaryi in the early stage, the TR of L. vicaryi also decreased sharply, and the trend in the middle and late stages was the opposite. This result indicates that the TRs of the four shrubs are inversely proportional to the contents of Cd and Pb in their bodies and that high
concentrations of Cd and Pb inhibit transpiration in typical shrubs under the experimental conditions of high concentrations of heavy metals in runoff and stormwater.

In the control group SS-T, the TRs of *R. xanthina*, *B. thunbergii*, and *B. megistophylla* decreased gradually with time; the TR of *L. vicaryi* did not change much overall, but it also showed a trend of decreasing gradually, indicating that transpiration in these shrubs may also decrease gradually under natural conditions.

### 3.2.3. Chlorophyll content

This section mainly studies the relationship between the content of heavy metals Cd and Pb in shrubs and CC, as well as the effects of the heavy metals on shrub growth. Chlorophyll is the photosynthetic pigment in plants. To a certain extent, high and low CC values correspond to the level of photosynthesis, and CC is the material basis that affects plant photosynthesis. With a low CC, photosynthesis will be weak, thus making plants unable to metabolize normally. Therefore, CC has been used to monitor plant damage induced by heavy metals (Mateos-Naranjo et al. 2012). Previous studies have shown that one of the harmful effects of heavy metals on plants is to reduce CC by damaging the enzymes involved in chlorophyll biosynthesis or triggering ROS-induced enzyme degradation (Singh et al. 2006). The relationship between heavy metals in shrubs and CC is shown in Figure 7.

From Figure 7, the CCs of *R. xanthina*, *B. thunbergii*, and *B. megistophylla* increased first and then decreased with time in SS-L. The variation trend of *L. vicaryi* was that CC decreased first and then increased. The relationship between the first three shrubs and the contents of Cd and Pb in their bodies was first proportional and then inversely proportional, while the relationship for *L. vicaryi* was inversely proportional first and then proportional. This result showed that a low concentration of Cd and Pb can promote the *in vivo* synthesis of chlorophyll in *R. xanthina*, *B. thunbergii*, and *B. megistophylla*. After a certain concentration is exceeded, the inhibition of chlorophyll will be shown; *L. vicaryi* exhibits the opposite trend.
In the experimental group SS-H, the CC of *B. thunbergii* and *B. megistophylla* decreased with time. The CC of *R. xanthina* and *L. vicaryi* in SS-H and *R. xanthina* and *L. vicaryi* in SS-L showed similar trends. The CC of *R. xanthina* was first in direct proportion to its Cd and Pb contents and then in inverse proportion to its Cd and Pb contents, while the contents of Cd and Pb in *L. vicaryi*, *B. thunbergii*, and *B. megistophylla* were in inverse proportion to the CC in these shrubs. Therefore, almost all shrubs showed inverse relationships for the CC. This suggests that high concentrations of Cd and Pb in *L. vicaryi*, *B. thunbergii*, and *B. megistophylla* harmed chlorophyll synthesis in these shrubs. The synthesis of chlorophyll in *R. xanthina* was first promoted and then inhibited. This effect may be due to the strong adaptability of chlorophyll synthesis to Cd and Pb and the fact that high content of Cd and Pb can also promote the synthesis of chlorophyll in *R. xanthina*. However, if the concentration exceeds a certain level, it will also harm the synthesis of chlorophyll in *R. xanthina*. Li et al. (2009) found that a low concentration of Cd could activate antioxidant enzymes in plants, including superoxide dismutase, peroxidase, and catalase, but a high concentration of Cd could damage enzymes in plants. Mateos-Naranjo et al. (2008) also showed that a certain amount of heavy metals may inhibit the synthesis of chlorophyll in plants. This might be because heavy metals damage enzymes related to chlorophyll synthesis or trigger ROS-induced enzyme degradation (Singh et al. 2006). Redondo-Gómez et al. (2011) showed that heavy metals could significantly reduce the gas exchange and photosynthetic rate of Argentine grass leaves, and higher concentrations of heavy metals would have toxic effects on plants. Mendelsohn et al. (2001) showed that leaf expansion, aboveground biomass, and regeneration rate of *Spartina alterniflora* significantly decreased with the increase of Cd concentration in the filler.

In the control group, SS-T, the CC in *R. xanthina* and *B. megistophylla* gradually decreased, the CC in *B. thunbergii* first increased and then decreased, and the CC in *L. vicaryi* gradually increased. This result indicates that different shrubs showed different adaptability to the external environment. The CC of some plants was promoted first and then inhibited, which may

![Figure 7](http://iwaponline.com/hr/article-pdf/52/5/1132/950528/nh0521132.pdf)
indicate that the chlorophyll of these plants has some stress resistance to Cd and Pb. Sun et al. (2018) used heavy metals of different concentrations to treat Spartina alterniflora Loisel, and the results showed that Spartina alterniflora Loisel had a certain tolerance to heavy metals and that only a high level of heavy metal treatment could reduce the CC of leaves.

4. CONCLUSIONS

To study the relationship between the contents of Cd and Pb in R. xanthina, L. vicaryi, B. thunbergii, and B. megistophylla and their NPR, TR, and CC in bioretention facilities and the effects of Cd and Pb on three physiological indexes in typical shrubs, bioretention devices were built at Beijing University of Civil Engineering and Architecture. Stormwater with low and high concentrations of heavy metals was prepared and added separately to the experimental apparatus. A device with tap water was added as a control. The average experimental period was 4 days, and the plant experimental devices were sampled to analyse the relationship between the heavy metal contents in their plants and the indexes NPR, TR, and CC. The conclusions of this study are as follows:

1. The Cd and Pb contents in the device with high concentration heavy metal influent were higher than those in the device with low concentration heavy metal influent, and the heavy metal absorption amount of shrubs may be in direct proportion to the heavy metal content in stormwater runoff.

2. For the experimental devices SS-L and SS-H, which were treated with stormwater runoff containing Cd and Pb, except that the contents of Cd and Pb of L. vicaryi in SS-H showed a trend of increasing first and then decreasing, the heavy metal contents of other shrubs showed a gradual increase.

3. Under the treatment of low concentrations of Cd and Pb, the NPR and TR of R. xanthina were in direct proportion to the contents of Cd and Pb in vivo. The NPR and TR values of the other three shrubs and the CC of L. vicaryi presented an inversely proportional relationship. The contents of Cd and Pb with respect to the CC of R. xanthina, B. thunbergii, and B. megistophylla were proportional first and then inversely proportional. Under the treatment of high concentration of Cd and Pb, the NPR of L. vicaryi was in direct proportion to its Cd and Pb contents, while that of R. xanthina was in direct proportion first and then in inverse proportion. Almost all other conditions showed inverse relationships.

4. Low or high concentrations of Cd and Pb inhibited the physiological indexes of different shrubs to different degrees but could promote some physiological indexes of individual shrubs. Low concentrations of Cd and Pb may promote the photosynthesis and transpiration of R. xanthina and inhibit the photosynthesis and transpiration of the other three shrubs, as well as the synthesis of chlorophyll of L. vicaryi. However, the synthesis of chlorophyll of three shrubs (R. xanthina, B. thunbergii, and B. megistophylla) was promoted first and then inhibited. High concentrations of Cd and Pb inhibited photosynthesis, transpiration, and chlorophyll synthesis in the typical shrubs but positively promoted photosynthesis in L. vicaryi and promoted and then inhibited chlorophyll synthesis in R. xanthina.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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