

## Chromium (Cr) Metal Absorption Using Symbiosis of *Fimbristylis globulosa* with *Agrobacterium* sp.

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Received: 06 November 2021, Revised: 17 August 2023, Available Online: 10 November 2023

**Abstract** - Chromium (Cr) metal is a pollutant caused by industrial waste disposal and agricultural activities. An alternative solution to managing chromium pollution in an environmentally friendly manner is phytoremediation using *Fimbristylis globulosa* combined with *Agrobacterium* sp. This research aims to measure 1). the ability of *Fimbristylis globulosa* combined with *Agrobacterium* sp. to absorb Cr metal; and 2). the value of the Cr metal bioconcentration factor (BCF) absorbed by *Fimbristylis globulosa* combined with *Agrobacterium* sp. This research used factorial design 2x5 with randomized design. Chromium metal analysis using atomic absorption spectrophotometer. Data were analyzed using BCF test. The results showed: 1) the highest Cr metal absorption in the treatment of *Fimbristylis globulosa*-*Agrobacterium* sp.-Cr metal content of 30 ppm (T2V3) root: 7,987 ppm and shoot: 15,649 ppm; 2) the highest value of Cr metal BCF in the treatment of *Fimbristylis globulosa*-*Agrobacterium* sp.-Cr metal content of 30 ppm (T2V3) root is 0.266 and *Fimbristylis globulosa*-*Agrobacterium* sp.-Cr metal content 10 ppm (T2V1) shoot is 0.675. Conclusions of this research are: 1) *Fimbristylis globulosa* combined with *Agrobacterium* sp. has a higher Cr metal absorption ability than without a combination of both; and 2) *Fimbristylis globulosa* combined with *Agrobacterium* sp. has a higher value of the Cr metal BCF than without a combination of both.

**Keywords:** *Agrobacterium* sp., bioconcentration factor, chromium metal, *Fimbristylis globulosa*.

### INTRODUCTION

The rapid rate of development, especially in the industrial sector, allows humans to utilize various resources to meet their daily needs accompanied by pollution. One of the pollutions is liquid waste that comes from industry to the river which affects the quality and availability of water (Aji et al., 2017).

Liquid waste that is discharged into the river if it is not in accordance with environmental quality standards and becomes a source of irrigation for agricultural land will cause pollution. The Madiun Regency area has various industries, especially textiles and has a large agricultural area (Badan Pusat Statistik Kabupaten Madiun, 2020). Textile industry wastewater pollutes rivers that are used to irrigate agricultural land, one of which is chromium (Cr) metal (Rosariastuti et al., 2013; Aji et al., 2019).

Chromium metal pollution on agricultural land has a major influence on the ecological system, namely the uptake of metals by plants in the food chain, thus affecting the health of organisms (Alghanmi et al., 2015). Chromium metal is used for industrial raw materials such as leather tanning and textiles (Pramono et al., 2012). The increase in Cr metal accumulation in agricultural land is also influenced by the activity of fertilizing and spraying chemical pesticides (Manurung et al., 2018; Laoli et al., 2021).

Chromium metal in the environment is generally found in the form of Cr (III) ion and Cr (VI) ion (Banks et al., 2006). Chromium (III) ion is less soluble, at optimal levels helps control sugar metabolism in the body (Sembel, 2015). However, excessive Cr (III) ion enters the cell causing damage to the genetic material (Eastmond et al., 2008).

Chromium (VI) ion is easily soluble, has high mobility and toxicity (Pramono et al., 2013).

The environmental quality standard for total Cr metal processed wastewater according to the Regulation of the Minister of Environment and Forestry of the Republic of Indonesia Number 6 of 2021 concerning Procedures and Requirements for the Management of Hazardous and Toxic Waste Annex XVI is 0,5 ppm. Government Regulation of the Republic of Indonesia Number 22 of 2021 concerning Implementation of Environmental Protection and Management requires environmental maintenance to overcome chromium metal pollution. Chromium metal pollution can be overcome by the application of cheap, safe, and environmentally friendly technology with phytoremediation (Glick, 2010).

Phytoremediation is the utilization of plants ability to isolate pollutants from the environment in an environmentally friendly manner (Nan et al., 2013). Plants for phytoremediation must be fast growing, absorb more than one pollutant, and have a high tolerance for pollutants (Rosariastuti et al., 2020).

Mendong (*Fimbristylis globulosa*) is a non-food plant that produces high quality natural fiber, is able to live in wetlands, and is used as a raw material for handicrafts (Suryanto et al., 2014). *Fimbristylis globulosa*

has fast growth and large biomass, it is expected to reduce Cr metal content with large and short absorption to the roots and shoots.

Efforts to maximize the potential of *F. globulosa* are by adding chelating agents to increase the plant's ability to withstand metal toxicity, increase absorption, transport, and accumulation of metals to the canopy. *Agrobacterium* sp. isolate was able to increase the absorption and accumulation of chromium to the roots and shoots of plants and was able to increase plant growth. Chromium metal is absorbed and accumulated in plant roots and shoots (Aji et al., 2017; Ferina et al., 2017).

This research aims to measure 1). the ability of *Fimbristylis globulosa* combined with *Agrobacterium* sp. to absorb Cr metal; and 2). the value of the Cr metal bioconcentration factor (BCF) absorbed by *Fimbristylis globulosa* combined with *Agrobacterium* sp.

## MATERIALS AND METHODS

### 1. Types and Design of Research

This research is included in experimental research in the laboratory. This research used factorial design 2x5 with randomized design. There were 10 treatment combinations that were repeated 3 times, so there were 30 experimental units. The research design is presented in Table 1.

Table 1. Research Design

Plant Treatment (T)	Chromium Metal Level Variation (V)	Repeat (U)		
		1	2	3
T1	V1	T1V1U1	T1V1U2	T1V1U3
	V2	T1V2U1	T1V2U2	T1V2U3
	V3	T1V3U1	T1V3U2	T1V3U3
	V4	T1V4U1	T1V4U2	T1V4U3
	V5	T1V5U1	T1V5U2	T1V5U3
T2	V1	T2V1U1	T2V1U2	T2V1U3
	V2	T2V2U1	T2V2U2	T2V2U3
	V3	T2V3U1	T2V3U2	T2V3U3
	V4	T2V4U1	T2V4U2	T2V4U3
	V5	T2V5U1	T2V5U2	T2V5U3

**Description:** T1 = *F. globulosa*; T2 = *F. globulosa* combined with *Agrobacterium* sp.; V1 = 10 ppm Cr metal solution; V2 = 20 ppm Cr metal solution; V3 = 30 ppm Cr metal solution; V4 = 40 ppm Cr metal solution; V5 = 50 ppm Cr metal solution

## 2. Preparation of *Fimbristylis globulosa* Seeds

1,5 month old of *F. globulosa* seedlings were purchased from *F. globulosa* farmer in Sragen Regency, Central Java Province.

## 3. Propagation of *Agrobacterium* sp. isolates

Inoculum propagation begins with the manufacture of LB media (luria betani). 400 mL of liquid LB media were made which were divided into 2 250 mL erlenmeyer so that each erlenmeyer contained 200 mL of liquid LB media. Adding inoculum to each erlenmeyer containing liquid LB media from the inoculum stored in the tilted media in the form of solid LB media. Next, shake the erlenmeyer containing the microbial culture for  $\pm$  72 hours on a shaker. After  $\pm$  72 hours, then multiply into 15 erlenmeyer containing liquid LB media. The addition of inoculum to carrier enrichment is as much as 250 mL of inoculum for every 1 *F. globulosa* growing medium.

## 4. Phytoremediation Stage

*Fimbristylis globulosa* was cleaned, then every 1 clump was planted in the growing media as many as 30 pieces, each containing 3000 mL of distilled water in a bucket pot. Chromium metal solutions are made in various concentrations, namely 10, 20, 30, 40, and 50 ppm. Each concentration variation was put into each *F. globulosa* growth medium, then homogenized. Adding 250 mL of *Agrobacterium* sp. inoculum to 15 treatments in bucket pots. Observations at the phytoremediation stage were carried out once a week for 4 weeks.

## 5. Chromium Metal Uptake Test by *F. globulosa*

Carefully weigh 2,5 g of fine plant samples and leave for one night. The next day, it was heated at 100 °C for 1 hour 30 minutes, cooled and added 5 mL of concentrated nitric acid and 1 mL of concentrated perchloric acid. Then it is heated to 130 °C for 1 hour, the temperature is increased again to 150 °C for 2 hours 30 minutes (until the yellow steam runs out, if there is still yellow steam add more heating time), after the yellow steam runs out the temperature is increased to 170 °C for 1 hour, then the temperature is increased to 200 °C for 1 hour (until white vapor is formed).

Destruction is completed with the formation of a white precipitate or the remainder of a clear solution of about 1 mL. The cooled extract was then diluted with ion-free water to 25 mL, then shaken until homogeneous, and filtered using whatmann 42 filter paper, then left overnight. The clear extract was used for Cr metal measurement using the Atomic Absorption Spectrophotometer (AAS). Chromium metal content (ppm) is calculated by the formula (Balai Penelitian Tanah, 2009):

$$\text{Chromium metal content} = \text{curve ppm} \times 10 \times \text{fp} \times \text{fk}$$

### Information:

ppm curve = sample content obtained from the regression curve between the standard series grade and its reading after deducting the blank.

fp = dilution factor.

fk = water content correction factor =  $100/(100\% \text{ moisture content})$ .

## 6. Data Analysis Technique

Bioconcentration factor (BCF) analysis was used to calculate the ability of *F. globulosa* to accumulate Cr metal. According to Yoon et al. (2006), bioconcentration factor (BCF) analysis can be determined using the formula:

$$BCF = \frac{a}{b}$$

Information:

a = concentration of Cr metal accumulated in *F. globulosa*

b = initial concentration of Cr metal in water (planting medium)

## RESULTS AND DISCUSSION

### 1. Ability of *F. globulosa* Combined with *Agrobacterium* sp. to Absorb Cr Metal

Data on chromium content in *Fimbristylis globulosa* plant tissue are presented in Figure 1.

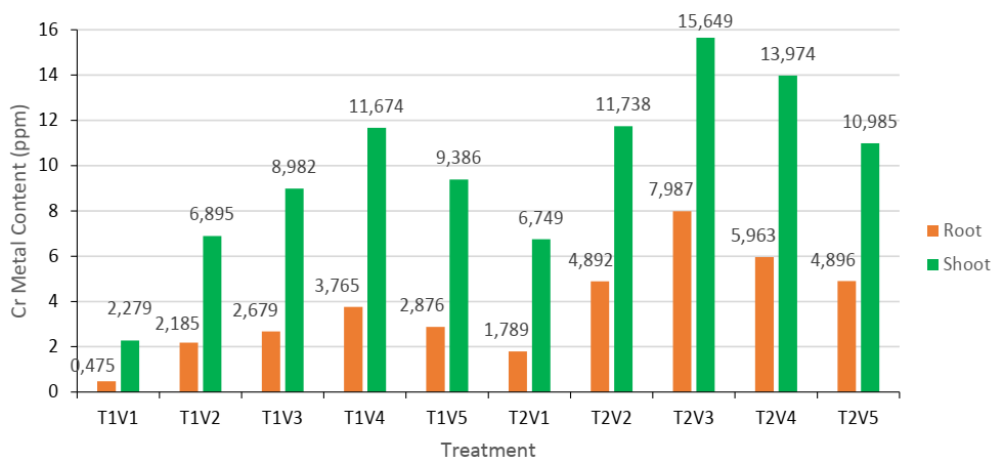


Figure 1. Cr Metal Content on *F. globulosa* Tissues

Based on Figure 1, it is known that the highest roots Cr metal content was found in the treatment of combination between *F. globulosa*-*Agrobacterium* sp.-30 ppm Cr metal content (T2V3) of 7.987 ppm and the lowest roots Cr metal content was found in the treatment of *F. globulosa*-10 ppm Cr metal content (T1V1) of 0.475 ppm.

Meanwhile, the highest shoots Cr metal content was found in the treatment of combination between *F. globulosa*-*Agrobacterium* sp.-30 ppm Cr metal content (T2V3) of 15.649 ppm and the lowest shoots Cr metal content was found in the treatment of *F. globulosa*-10 ppm Cr metal content (T1V1) of 2.279 ppm.

The high Cr metal uptake into the *F. globulosa* tissue was influenced by the activity of *Agrobacterium* sp.. *Agrobacterium* sp. is a root bacteria (rhizobacteria) that has the

ability to maintain plant life that absorbs heavy metals and helps absorb heavy metals into plant tissues (Hoflich & Metz, 1997; Safarida & Ngadiman, 2007).

Besides being able to reduce Cr metal ions, rhizobacteria can also support plant growth by synthesizing organic acids in the form of phytohormone precursors, vitamins, enzymes, siderophores, and antibiotics (Khan et al., 2009). Organic acids are chelators and play a role in the accumulation and detoxification of metals into plant tissues (Aji et al., 2017; Ferina et al., 2017).

### 2. Value of the Cr Metal Bioconcentration Factor (BCF)

Data on the value of Cr metal BCF in *F. globulosa* tissue are presented in Figure 2.

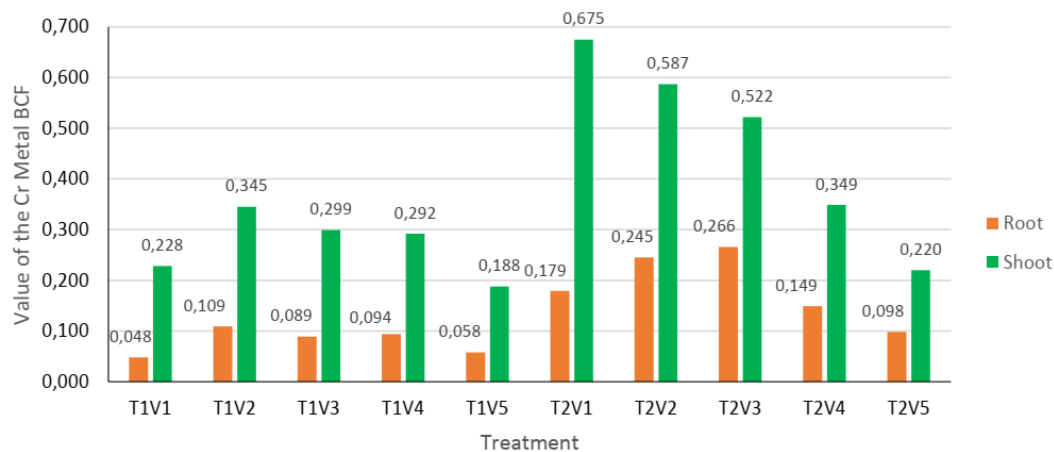


Figure 2. Value of the Cr Metal BCF

Bioconcentration factor (BCF) analysis is the tendency of a chemical to be absorbed by aquatic organisms. Bioconcentration factor (BCF) analysis is the ratio between the concentration of chemicals in aquatic organisms and the concentration of chemicals in the water (LaGrega et al., 2001). Based on Figure 2, it is known that the highest BCF value in the roots was found in the treatment of combination between *F. globulosa*- *Agrobacterium* sp.-30 ppm Cr metal content (T2V3) of 0.266 and the lowest BCF value in the roots was found in the treatment of *F. globulosa*-10 ppm Cr metal content (T1V1) of 0.048. Meanwhile, the highest BCF value in the shoots was found in the treatment of combination between *F. globulosa*- *Agrobacterium* sp.-10 ppm Cr metal content (T2V1) of 0.675 and the lowest BCF value of the shoots was found in the treatment of *F. globulosa*-50 ppm Cr metal content (T1V5) of 0.188.

Bioconcentration factor (BCF) value in both treatments were categorized as low. Based on the category of BCF value according to Van Esch (1977) pollutant properties are classified into three orders, namely very accumulative (BCF>1000), moderate accumulative (BCF among 100-1000), and low accumulative (BCF<100). Bioaccumulation of heavy metals in aquatic

organisms is a negative impact of the entry of pollutants into an ecosystem (Hidayah et al., 2014).

Accumulation of heavy metals in the body of organisms depends on the concentration of heavy metals in water (environment), temperature, pH, and dissolved oxygen (Zainuri et al., 2011). The ability of the body's organs to accumulate heavy metals are determined by the value of the bioconcentration factor index (BCF). The higher the BCF value in an organism indicates the higher the organism accumulates heavy metals (Hidayah et al., 2014).

## CONCLUSION

1) *Fimbristylis globulosa* has the highest ability to absorb Cr metal in the combination treatment of *Fimbristylis globulosa*-*Agrobacterium* sp.-Cr metal content of 30 ppm (T2V3) is 7.987 ppm in the root and 15,649 ppm in the shoot, so that it plays a role in phytoextraction and is classified as a Cr metal accumulator plant; and 2) *Fimbristylis globulosa* combined with *Agrobacterium* sp. has a higher value of the Cr metal BCF than without a combination of both, i.e. in the treatment of *Fimbristylis globulosa*-*Agrobacterium* sp.-Cr metal content of 30 ppm (T2V3) root is 0.266 and



*Fimbristylis globulosa*- *Agrobacterium* sp.-Cr metal content 10 ppm (T2V1) shoot is 0.675.

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