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Soil Contamination in Randukuning Landfill: Morphological-Physiological Responses of *Celosia argentea* L. and *Cleome rutidosperma* D.C.

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Abstract. *The soil in the Landfill area has generally been contaminated by various types of pollutants, including heavy metals, microplastics, ammonia, chloride, benzene, toluene, ethylene, ethylene benzene, and xylene (BTEX). At high concentrations, the pollutant can cause toxic effects on plants. The study aimed to evaluate the morphological and physiological conditions of C. argentea L. and C. rutidosperma D.C. after being planted on Randukuning landfill soil. Plant species were planted on landfill soil in the greenhouse for two months. Morphological observations (stem length, root length, and the number of leaves) and physiological (biomass of roots, stems, and leaves) were carried out on days 0, 3, 7, 14, 21, and 28 after planting on landfill soil. The results showed that C. argentea L. and C. rutidosperma D.C. did not show morphological and physiological effects. Both species can grow well on landfill soils. Therefore, these species can be potential phytoremediation agents.*

Keywords: *biomass, contamination, C. argentea L., C. rutidosperma D.C., landfill, phytoremediation*

Citation

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INTRODUCTION

The garbage that accumulates for a long time in the landfills will release leachate water containing substances harmful to the environment (Koda et al., 2022). The content of these substances includes heavy metals (Lum et al., 2014; Wang et al., 2022), microplastics (Wan et al., 2022) ammonia (Koda et al., 2022), arsenic, sulfate, and benzene, toluene, ethylbenzene, xylene (BTEX) (Holzle, 2019), and also chloride (Koda et al., 2022). After that, the leachate can infiltrate and pollute landfill soil and its surroundings.

Toxic substances that pollute the environment will enter the plant body and harm humans and animals through the food chain (Komal et al., 2014; Sharma et al., 2021). Contaminants such as heavy metals can cause leaf chlorosis, a decrease in leaf size and thickness due to inhibition of the transpiration process in plants (Sobkowiak, 2016), besides that plants will also have oxidative stress which causes lipid and protein peroxidation and even death (Singh et al., 2016). In addition, the metal is also dangerous for humans because it can cause heart problems, metabolic dysfunction (Ertani et al., 2017), impaired kidney function (Smolders & Mertens, 2013), lung cancer, indigestion, and even death (Gonnelli & Renella, 2013).

Other contaminants, namely microplastics, can cause a decrease in soil fertility due to soil aggregation, growth disorders, and decreased plant root strength (Rillig et al., 2019). Microplastics generally do not directly affect plants' biochemical or physiological conditions (Azeem et al., 2021) but act as facilitators for other contaminants to make it easier to get into plants. Microplastics can also reduce the number of heavy metals in plants due to the ability of microplastics to bind to heavy metals (Ullah et al., 2021; Zong et al., 2021). In addition to microplastics, some nano plastics are more detrimental because they are easily absorbed and translocated into plant cell walls with the help of transpiration (Azeem et al., 2021).

Ammonia and chloride are also detrimental because they can cause a decrease in the ability of plants living around landfills (Koda et al., 2022). In addition, ammonia also causes a decrease in the amount of chlorophyll, a slow growth rate, reduced turgor pressure, and necrosis in plants (Kiraly et al., 2013). Similarly, chloride, increasing amount, can cause high soil salinity, thus causing chlorosis of the leaves, then necrosis, and then causing the tip of the leaf to burn (Geilfus, 2018).

Generally, plants have a defense mechanism against contaminants in the environment through detoxification mechanisms (Hamim et al., 2019). In addition, metal transporters are also formed to facilitate the translocation of metals in plants. Plant transporters also vary, specific to the metal absorbed (Komal et al., 2014; Shahid et al., 2017). At the same time, detoxification in ammonia involves the enzymes glutathione synthase and glutamate synthase (Kiraly et al., 2013).

Tolerant plants can also adapt morphologically as well as physiologically. Some examples are changes in leaf size (Sobkowiak, 2016), roots, and stems (Jiang et al., 2019), as well as maintaining their biomass (Dresler et al., 2017). Each plant is only tolerant to specific contaminants present in the environment (Komal et al., 2014; Shahid et al., 2017).

Atriplex prostrata, A. tatarica, Portulaca oleraceae, and *Chenopodium glaucum* are tolerant to heavy metals (Koda et al., 2022). In addition, *Eichhornia crassipes* can lower the concentration of ammonium nitrate in wastewater (Ting et al., 2020). *Celosia argentea* L. (Liu et al., 2018; Oguntade et al., 2020) and *Cleome rutidoperma* D.C. (Chandra et al., 2018) can also tolerate heavy metals in the environment (Chandra et al., 2018; Liu et al., 2018; Oguntade et al., 2020). *C. argentea* L. (Liu et al., 2018; Oguntade et al., 2020) and *C. rutidosperma* D.C. (Chandra et al., 2018) can survive in heavy metal-polluted environments. Both can potentially live well in polluted environments to be used for phytoremediation.

Phytoremediation is a special effort to clean up the toxic substances in the environment sand make them harmless (Tangahu et al., 2018). Through phytoremediation, contamination of the environment, such as soil, can be reduced (Komal et al., 2014; Sharma et al., 2021). Besides being easy to use, this method is also cheap and environmentally friendly (Komal et al., 2014). *C. argentea* L. (Liu et al., 2018; Oguntade et al., 2020) dan *C. rutidosperma* D.C. (Chandra et al., 2018) are known to accumulate heavy metals on parts of their body (Chandra et al., 2018; Liu et al., 2018; Oguntade et al., 2020). However, there have been no previous studies on the morphological and physiological conditions of the two species when planted in landfill soils that contain many contaminants such as microplastics, BTEX, ammonia, and other contaminants, so it is known that the ability of these plants to survive in the unfavorable environment. This research aimed to study the morphological and physiological conditions of *C. argentea* L. and *C. rutidoperma* D.C. after being planted on polluted soil at the Randukuning landfill to know the resistance and ability of plants to live in an unfavorable environment. Thus, the potential of *C. argentea* L. and *C. rutidosperma* D.C. can be determined to become phytoremediation agents that are easier and cheaper in their implementation in polluted environments.

MATERIALS AND METHODS

Location and Time of Research

This research was conducted at Sawitsari greenhouse, Faculty of Biology, Universitas Gadjah Mada, from April to June 2021.

Research Design

A Completely Randomized Design was used to observe the effects of landfill soil on the morphological and physiological condition of *C. argentea* L. and *C. rutidosperma* D.C. Before planting in the Randukuning landfill soil, the seeds of two plants were grown for two months in the greenhouse. In the first month, the seeds of the plants were fixed on a polybag measuring 20 cm x 20 cm, which contains planting media in the form of soil mixed with compost, manure, and chaff. Then in the second month, the plant was moved to a polybag containing 1 kg of Randukuning landfill soil (n = 6). According to the airy capacity, the plant was daily watered with 100 mL of aquadest. The observations made included morphological (stem length, root length, and the number of leaves) and physiological (stem biomass, root biomass, and leaf biomass) characteristics adapted from the research of Hamim et al. (2019). The results of landfill soil testing at the BBTKLPP Yogyakarta Laboratory show that the landfill soil contains Pb, Cd, Cr, and Cu metals (Table 1). The concentrations of Cd and Cr have even exceeded the threshold of being safe for the environment. Natural Cd is 0.1-0.5 ppm (Sudarmadji et al., 2006), while Cr has a safe limit of about 2.5 ppm (Ministry of State Population and Environment of Indonesia, and Dalhousie University, 1992). The normal media sampled from a location far from the landfill also showed similar Pb, Cd, Cr, and Cu concentrations to the landfill soil. Therefore, for studying landfill soil's effect on the plants, the morphological and physiological conditions were compared before (day 0) and during planting in the landfill soil.

Table 1. Heavy metal content in Randukuning landfill soil

Parameters	Result (mg/kg)
Copper (Cu)	36.357
Total Chrome (Cr)	13.917
Lead (Pb)	32.772
Cadmium (Cd)	0.848

Measurement of Morphological Parameters

Morphological observations included the length of the stem, the length of the roots, and the number of leaves. Before measurements were carried out, the plant was removed from the polybag; the roots were cleaned from the soil medium. The root length was measured from the base to the end of the root. The stem length was also measured from the base to the end of the stem. The number of leaves was calculated from the first leaf that appears from the base of the stem to the last leaf at the end of the stem. Measurements were conducted on days 0, 3, 7, 14, 21, and 28.

Measurement of Physiological Parameters

Physiological observations included roots, stems, and leaves biomass. The biomass measured was the wet weight of the plant. Roots, stems, and leaves that have been removed from the polybag were washed to remove soil and other component attached. The samples werethen dried using a tissue to remove water left on the plant. The roots, stems, and leaves were then weighed using an analytical balance. Observations were carried out on days 0, 3, 7, 14, 21, and 28 after transferring the plant to landfill soil.

Statistical Analysis:

The variability of morphological (stem length, root length, and the number of leaves) and physiological (stem biomass, root biomass, and leaf biomass) data were tested with one-way analysis of variance (ANOVA) with exposure time as an independent variable, followed by the Dunnett test ($p<0.05$) if a significant difference was obtained. The result of days 3, 7, 14, 21, and 28 will be compared to day 0 to know the changes in the conditions of both species after planting on landfill soil. The value of morphological conditions of *C. argentea* L. and *C. rutidosperma* D.C. were tested using the Independent T-Test to determine which plants were most resistant to the contaminant in landfill soil and more potential as phytoremediation agents, as well as in physiological conditions.

RESULTS AND DISCUSSION

After planting on Randukuning landfill soil, the length of the stems of *C. argentea* L. and *C. rutidosperma* D.C. on days 3, and 7 did not show significant differences from day 0 (Table 2). Both drooped and withered on the stems and leaves, but on the 2nd day *C. rutidosperma* D.C. already looked upright and fresh, while *C. argentea* L. on the 4th day. It suggests that the plant undergoes an adaptation process at the beginning of the transfer from nutrient-rich media to landfill soil so that its growth becomes rather slow. Al-Faifi & El-Shabasy (2021) stated that plants exposed to toxic substances have rolling leaves because the leaf bones are elastic and the stems are shorter than plants that live in unpolluted areas. In addition, the leaves of the *Taraxacum officinale* contaminated with heavy metals (Bini et al., 2012) looked at a decreased condition in leaf thickness as well as changes in the structure of its cells.

Landfill soils have an alkaline pH (6.5-7.5) so it will be easier for heavy metals and nutrients to be absorbed by plants (Agbeshie et al., 2020). This alkaline pH is due to the soil's increased affinity for toxic ions (Komal et al., 2014). On days 14 to 28 there was a significant difference in the stem length of the two species compared to day 0, so it can be said that plants have been able to adapt to their environmental conditions (Table 2). The maximum increase in stem length in *C. argentea* L. and *C. rutidosperma* D.C occurs on the 21st day (Figure 1).

On days 14 to 28, there is a significant difference in the root length of *C. argentea* L. and *C. rutidosperma* D.C (Table 3) while insignificant differences occur on days 3 in both species. At the beginning of the transfer to a polybag containing landfill soil, the root growth also tends to be slow. It is caused by heavy metal contaminants such as Cd (Liu et al., 2018) and Cr (Yabanli et al., 2014) which are widely accumulated in the roots and will inhibit root development. The maximum addition of root length in *C. argentea* L. and *C. rutidosperma* D.C. occurs on the 28th days (Figure 1).

There was a significant difference in the number of leaves in *C. argentea* L. and *C. ru-*

tidosperma D.C. occur on days 14 to 28 (Table 4). Meanwhile, the maximum increase in the number of leaves occurs on the 28th day in *C. argentea* L. and 21st in *C. rutidosperma* D.C (Figure 2). On the 28th day, the leaves number of *C. argentea* L. increased but the extension of the stem was slow. On the other hand, the leaves of *C. argentea* D.C. on the 28th day began to dry and fall, followed by a decrease in the growth rate of the leaves. The decrease in the number of leaves is due to chlorosis, which makes the leaves fall easily (Al-Faifi & El-Shabasy, 2021).

Table 2. Stem length after planting on landfill soil for 28 days

Observation Day	Species	
	***Celosia argentea* L.**	***Cleome rutidosperma* D.C.**
0	12.68 ± 0.39	3.23 ± 0.12
3	13.20 ± 0.63	3.46 ± 0.19
7	15.21 ± 1.10	3.93 ± 0.50
14	32.87* ± 1.66	12.21° ± 3.17
21	51.08* ± 11.69	25.54° ± 1.70
28	63.08* ± 13.66	36.83 ± 1.47

The symbol (*) indicates statistically a marked difference based on Dunnet test results with a 5% error.

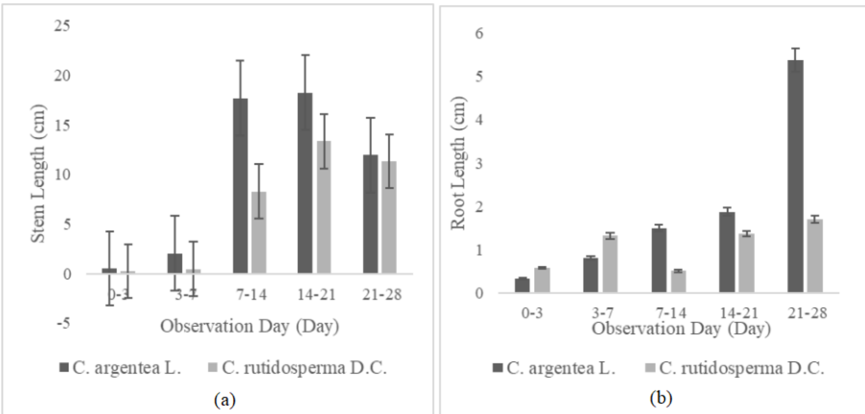

Figure 1. Morphological changes of *C. argentea* L. and *C. rutidosperma* D.C. for 28 days planted on Randukuning landfill soil (a) stem length (b) root length

The significant differences in the root biomass of *C. argentea* L. occurred also on days 14 to 28, and so did the stem length and the leaves number of *C. rutidosperma* D.C on days 21 to 28 (Table 5). In addition, the maximum increase in root biomass in both species occurred on days 21 to 28, while the maximum gain of root biomass in both species was on days 28 (Figure 3). The cause of slow increase in root biomass may also be caused by microplastics. Microplastics in the soil may also cause a decrease in root biomass. Microplastics can increase the bioavailability of heavy metals and toxic substances in the soil to be absorbed by plants (Wang et al., 2020).

The observations presented in Table 6 show a significant difference in the stem biomass of *C. argentea* L. and *C. rutidosperma* D.C that occurred on days 14 to 28. Maximum stem mass increase in both plant species found on day 28 (Figure 2). The contaminants such as Fe, As, Cd, Pb, Zn, and Hg (Agbeshie et al., 2021) are translocated not only to the root but also to the leaves and stems of plants so that perhaps they will inhibit their growth (Yabanli et al., 2014).

Table 7 shows that significant differences in *C. argentea* L. and *C. rutidosperma* D.C. biomass occurred on days 7 to 28 compared to days 0. Based on Figure 3 it is known leaf mass increase in both species occurs on day 28 just like the accretion of root and stem biomass. *Reutealis trisperma* has slightly changed morphology, physiology, and anatomy due to heavy metals in the environment, but still able to live and tolerate these environmental conditions (Hamim et al., 2019).

Table 3. Root length after planting on landfill soil for 28 days

Observation Day	**Species**	
	***Celosia argentea* L.**	***Cleome rutidosperma* D.C.**
0	6.95 ± 0.67	4.22 ± 0.44
3	7.28 ± 0.42	4.80 ± 0.73
7	8.08* ± 0.37	6.12* ± 0.95
14	9.58* ± 0.66	6.63* ± 0.90
21	11.45* ± 1.08	8.0* ± 1.81
28	16.83* ± 1.17	9.70* ± 2.04

Table 4. Number of leaves after planting on landfill soil for 28 days

Observation Day	**Species**	
	***Celosia argentea* L.**	***Cleome rutidosperma* D.C.**
0	15 ± 0.41	9.0 ± 0.98
3	16 ± 0.98	10 ± 0.52
7	20 ± 3.10	14 ± 3.01
14	42* ± 10.57	39* ± 11.24
21	48* ± 9.39	88* ± 5.86
28	114* ± 34.65	99* ± 4.59

Table 5. Root biomass after planting on landfill soil for 28 days

Observation Day	**Species**	
	***Celosia argentea* L.**	***Cleome rutidosperma* D.C.**
0	0.48 ± 0.15	0.11 ± 0.06
3	0.68 ± 0.17	0.16 ± 0.08
7	1.20 ± 0.33	0.22 ± 0.08
14	3.23* ± 0.73	0.45 ± 0.17
21	4.48* ± 0.82	0.96* ± 0.20
28	11.25* ± 3.08	1.53* ± 0.64

In table 3, 4, 5 the symbol (*) indicates statistically a marked difference based on Dunnet test results with a 5% error.

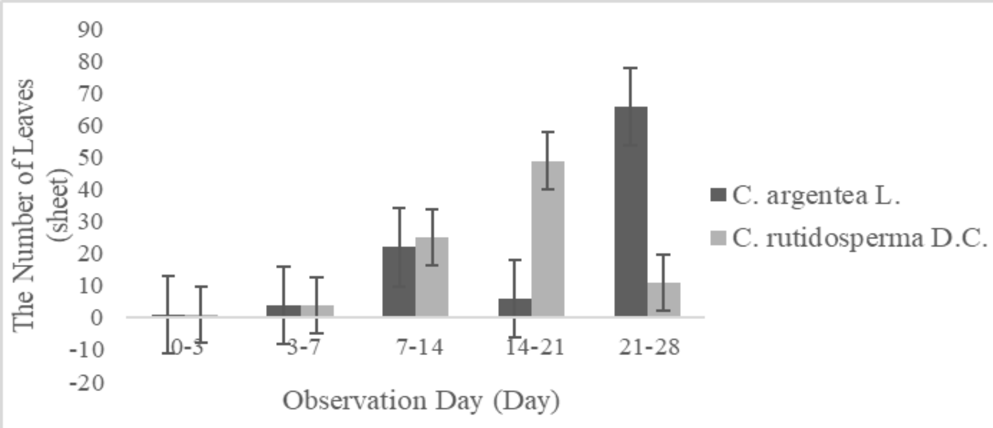

Figure 2. Changes in the number of leaves of *C. argentea* L. and *C. rutidosperma* D.C. for 28 days planted on Randukuning landfill soil

The presence of contaminants in Randukuning landfill soil has low effect on the morphological and physiological of *C. argentea* L. and *C. rutidosperma* D.C. The plants can grow well despite their slow development at the beginning of transfer to landfill soil (Figure 1, Figure 2, and Figure 3). The height of the plant increases with the day (Figure 1), but some leaves have yellow patches and are like burning on the edges. Singh et al. (2016) stated that environmental toxicants cause plant chlorosis. In this study, the plant growth remained good even though it was planted on landfill soil, and did not show significant growth inhibitions even though there were heavy metals and other contaminants in the soil. This is due to the high content of Nitrogen, Phosphates, and Potassium in the soil, as shown from the results of the analysis in BBTKLPP where the concentrations of them are respectively 1,943,304, 6,938,021, and 2,546,833 ppm.

Toxic environments can generally cause a decrease in plant biomass and other growth disorders. Kiraly et al. (2013) showed that plants in ammonia-polluted environmental conditions experience a decrease in growth, disturbances in chlorophyll, and necrosis can occur. Hyperaccumulator plants can still live well despite polluted environmental conditions (Singh et al., 2016). For example, with *Dracaena fragrans* and *Opuntia microdasys*, the plant can absorb benzene, toluene, ethylbenzene, and xylene at a concentration of 2 ppm for 48-57 hours. This plant can potentially be a BTEX phytoremediation agent in the air (Mosaddegh et al., 2014).

The observations of Bini et al. (2012) showed that in a polluted environment, plants showed an unperfect structure of the palisade tissue of the leaves, the parenchyma tissue had many gaps between its cells, and the thickness of the leaves also decreased causing impaired growth in plants. In ammonia-polluted environments (Kiraly et al., 2013), growth is disrupted due to the stunted growth of roots and stems. The higher the concentration of contaminants in the environment, the more growth and plant biomass were inhibited. For example, Moso Bambu (*Phyllostachys pubescens*) has an increase in biomass when grown in an environment with a Cu content of 50 mg/kg, but will decrease in the biomass of roots, stems, and leaves when the Cu level in the environment was 300 mg/kg (Chen et al., 2015). *C. argentea* L. can grow healthy in soil contaminated by Cd and Mn (Liu et al., 2018). Each plant has a certain tolerance limit to the toxicity that exists in the environment.

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Table 6. Stem biomass after planting on landfill soil for 28 days

Observation Day	Species	
	***Celosia argentea* L.**	***Cleome rutidosperma* D.C.**
0	0.71 ± 0.14	0.48 ±0.27
3	1.09 ± 0.35	0.73 ±0.49
7	2.18 ± 0.32	1.14 ± 0.81
14	7.91* ± 1.35	1.68 ± 1.34
21	14.40* ±4.59	6.12* ± 0.56
28	26.08 ± 7.38	13.62* ± 3.30

The symbol (*) indicates statistically a marked difference based on Dunnet test results with a 5% error.

Table 7. Leaf biomass after planting on landfill soil for 28 days

Observation Day	Species	
	***Celosia argentea* L.**	***Cleome rutidosperma* D.C.**
0	0.73 ± 0.12	0.89 ± 0.73
3	0.96 ± 0.31	1.13 ± 0.95
7	2.87 ± 0.75	2.36 ±1.24
14	10.56* ± 2.22	4.35* ± 1.19
21	19.81 ± 11.0	9.92* ± 0.97
28	30.87* ± 12.95	18.45* ± 3.23

The symbol (*) indicates statistically a marked difference based on Dunnet test results with a 5% error.

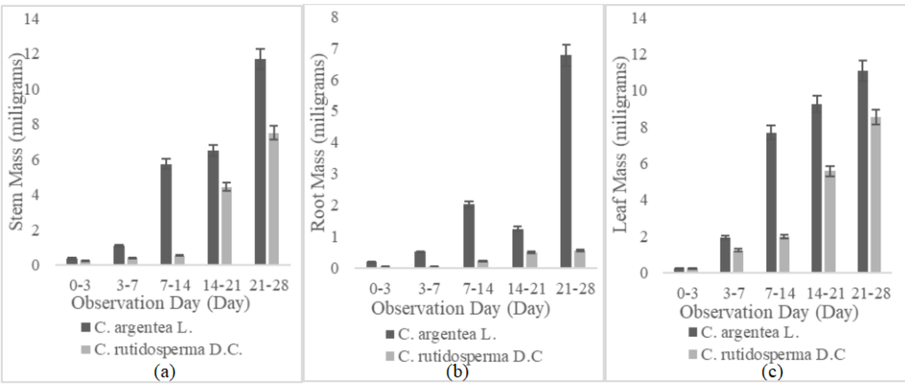

Figure 3. Physiological changes of *C. argentea* L and *C. rutidosperma* D.C. for 28 days planted on Randukuning landfill soil (a) stem mass (b) root mass (c) leaf mass

The results showed that roots, stems, and leaves biomass of *C. argentea* L. and *C. rutidosperma* D.C. continued to increase despite the small increase (Figure 3). Even though being grown in unfavorable environmental conditions, certain plants can still increase their biomass. *Echium vulgare* can grow well without a decrease in biomass even though they live in environmental conditions contaminated with Zn and Pb. This is because plants are able to secrete citric acid, malic acid, as well as phenols, and flavonoids in high concentrations as a form of self-defense (Dresler et al., 2017).

If the plant is intolerant to contaminants contained in the environment, then the population will decrease. In line with Koda et al. (2022) that plants will decrease in vegetation if they live in high-salinity environments such as landfills because they cannot be tolerant to contaminants in the environment. It occurs in the group of glycophyte plants and grasses.

As the day progresses, *C. argentea* L. and *C. rutidosperma* D.C. can live well on Randukuning landfill soil. This indicates that both plants species have the ability to be tolerant of the environment (Dresler et al., 2017). In addition, plants can adapt well and try to defend themselves from contaminants by forming antioxidants. Plants will also produce several genes that can activate certain enzymes as a defense (Singh et al., 2015).

At the beginning of exposure to contaminants or environmental stress, plants will produce ROS (*Reactive Oxygen Species*), which can cause protein and lipid peroxidation (Singh et al., 2016), as a marker that plants are under oxidative stress. After that, the plant will secrete enzymatic antioxidants such as Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Reductase (GR), Ascorbate Peroxidase (APX) (Fan et al., 2020; Li et al., 2019) as well as non-enzymatic such as Ascorbate (ASA) and Glutathione (GSH) as a form of self-defense against unfavorable conditions (Li et al., 2019). Plants will cleanse, neutralize, and dispose of ROS (Singh et al., 2016) so that plants do not get oxidative stress anymore. The presence of high ROS can lead to the peroxidation of lipids and proteins, as well as damage to plant DNA that can lead to plant death (Singh et al., 2015).

However, in this study, to be able to survive, the plants may carry out a detoxification process (Hamim et al., 2019) involving phytochelatin and metallothionein as a chelating for contaminants in the form of heavy metals (Hamim et al., 2019; Komal et al., 2014). The chelate can also come from organic acids produced by root exudates such as oxalic acid, malic acid, citric acid, tartaric acid, and succinic acid so that non-toxic components are formed and safe for plants (Li et al., 2019). Metal transporters can also be formed to facilitate metal translocation in plants (Komal et al., 2014; Shahid et al., 2017). Meanwhile, detoxification in ammonia is carried out through a reversible reaction between 2 oxoglutarate and ammonia with the help of Glutathione dehydrogenase, so that glutathione is produced to reduce ammonia toxicity in plants (Kiraly et al. 2013). Contaminants are tolerated by plants by storing them in subcellular parts of plants such as vacuoles, endoplasmic reticulum, and Golgi bodies (Li et al., 2019).

Based on the results of statistical tests using the Independent T-Test test on changes in morphological conditions of the two species showed a significance of 0.785. On the other hand, the statistical value of physiological condition was 0.209. The value of 0.785 and 0.209> 0.05 indicate that there were no significant differences in the conditions between the two species when planted on landfill soil. Both plants were in good condition even though they are grown on polluted soil, indicating that both are equally tolerant and can do the detoxification processes to decrease toxicants inside their body parts. These results also prove that *C. argentea* L. (Liu et al., 2018; Oguntade et al., 2020) can survive and be tolerant to polluted environments such as heavy metals like Cd and Mn from the environment with the condition remains good (Liu et al., 2018). *C. rutidosperma* D.C. also has the potential to accumulate Cd to plant body parts and tolerant to contaminants present in the environment (Chandra et al., 2018).

CONCLUSION

Based on the results of the study, it can

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be concluded that *Celosia argentea* L. and *Cleome rutidosperma* D.C. have good morphological and physiological conditions even though they are grown on polluted soil in Randukuning landfill, so they can potentially be a phytoremediation agent.

AUTHOR CONTRIBUTION

D.P.H. collected and analyzed the data, wrote the manuscript, A.P.N. supervised all the processes and corrected the writing of the article.

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CONFLICT OF INTEREST

There is no potential conflict of interest that occurs in the research conducted by the authors.

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