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Investigation of some morphological and physiologhical characteristics of *C. alternifolius*, *C. zizanioides* and *A. vera* irrigated with urban wastewater

S. Ebrahimi Nokande [1], S. M. Razavi Khosrowshahi*[2], M. Afshar Mohammadian [3]

1. 1.PhD student in Plant Physiology, Mohaghegh Ardabili University, Ardabil, Iran.
2. Full Profesor of Plant Physiology, Mohaghegh Ardabili University, Ardabil, Iran. *(Corresponding author: razavi694@gmail.com)
3. Associate Professor of Plant Physiology, Guilan University, Rasht, Iran.

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Introduction

Human activities such as the disposal of household waste, mainly import heavy metals into the soil (European Commission, 2013; EPA, 2021). Heavy metal pollution of soil can harm human life and the ecosystem through direct intake or contact with contaminated soil, drinking of contaminated water, and entering the food chain (soil-plant-human or soil-plant-animal-human) (European Commission, 2013). To remove or reduce the environmental pollutants, phytoremediation is regarded as a biological and cost-effective method employed by some plants to remove, stabilize and transfer contaminants in the soil or groundwater (Gajic and Pavlovic, 2018), So choosing suitable plant for phytoremediation is important according to the region and type of pollution and increasing expansion of urban wastewater. The province of Guilan, located in the north of Iran, has a humid subtropical climate and is a fertile province in terms of agriculture. Urban population is increasing day by day in this province and urban waste does not have a suitable place to dump so the leakage flows directly into the river or land fields. This problem is dangerous for the agricultural products of Guilan province and has created an important challenge. It seems that using plant with high phytoremediation ability and creating a green belt in the landfill site of urban wastewater of Saravan is the most feasible and low-cost method in situ. Considering the mentioned factors, in this study, we examined three plant species as candidates for phytoremediation, including *Cyperus alternifolius* and *Chrysopogon zizanioides* and *Aloe vera*, and compared their morphological and physiological characteristics.

Materials & Methods

Experimental treatments included two groups: the first treatment was urban water as the control, the second treatment was urban wastewater collected from the landfill site of Saravan. Each pot was filled with 11 kg soil pre-sieved. *C. alternifolius*, *C. zizanioides* and A. vera were gathered from the local nursery and were planted in the pots. To prevent the impact of stress on the plants and adapting the new condition, all experimental plants were irrigated with urban water for one month. After one month, control pots were irrigated with 300 mL urban water and treatment pots with 300 mL of urban wastewater twice a week for 14 months, separately. Moreover, to determine the amount of heavy metals (zinc, chromium, lead, copper, manganese, nickel and magnesium) after wastewater addition to the soil and compare it with maximum standard levels, pots without plants were considered and irrigated like other pots with plants. To assess element accumulation during irrigation, the sampling of plants and soil examination was conducted 14 months after planting. Soil samples were homogenized and dried in an oven at 30°C for 24 hours and were passed through sieve and the EC, pH and the concentration of heavy metals were examined. After harvesting the whole plants, the length of the root and shoot was measured and plant samples were dried in an oven (50 ºC for 72 hours), and then all dried plant tissues were ground into powder. Then the concentration of the metal elements in the plant samples was determined using ICP-OES according to the method of Salt et al. (1998) and AL-Oud Saud (2003).

To determine the simultaneous accumulation of metals in plants, the metal elements accumulation index (MAI) was calculated using the following formula (Liu *et al*., 2007):

$$\mathbf{MAI} = \left[\frac{1}{N}\right] \sum_{j=1}^{N} I_j$$

To calculate the uptake index, the concentration of metal in the aerial part of the plant was multiplied by the dry weight of the aerial part of the plant (Bi *et al*., 2011).

To calculate tolerance index, the dry weight of the whole plant in heavy metal-contaminated soil was divided by the dry weight of the whole plant in the control soil.

Also leaf soluble sugar (Schlegel, 1956), total phenol content (Ainsworth *et al*., 2007), total flavonoid (Chang *et al*., 2002), total anthocyanin (Masukasu *et al.*, 2003), tannin (Omidbaigi, 2008), and free radical inhibition percentage (Hatano *et al*., 1988) were investigated and compared in all three studied plants.

Results & discussion

The current results showed that the MAI of all three plants under the urban wastewater treatment was higher than the control treatment (water) after 14 months. So that, in urban wastewater treatment, the MAI in *C. zizanioides* and *A. vera* was higher than in *C. alternifolius* with values of 43.22, 40.76 and 28.30, respectively. Given that

MAI displays the general performance of plants to simultaneously accumulate metal elements for its deviation in metal uptake (Liu *et al*., 2007), it seems that *C. zizanioides* and *A. vera* were more successful in accumulating Zn, Cr, Pb, Cu, Mn, Ni, and Mg. Previous studies have reported that plant species with a high MAI value should be used as barriers between contaminated and vulnerable areas such as parks, and residential areas (Nadgorska–Socha *et al*., 2017). However, investigating the morphological and physiological indicators of the plant in the specific soil where phytoremediation takes place is also one of the necessities that are emphasized in the research of phytoremediation (Ruilian *et al*., 2012). According to the results of this study, in all three examined plants, the indices of root length, shoot length, total biomass, uptake index (UI), and tolerance index (TI) were significantly reduced compared to the control plant, and A. vera had the highest percentage of reduction. It has been reported that high levels of metals in the soil inhibit many metabolic functions of the plant and as a result cause delay in growth and ultimately limit the growth of roots and aerial organs. Plants have different sensitivities and strategies against the stress of heavy metals caused by urban sewage. The toxicity of heavy metals in different plants varies according to factors such as the type of plant, the amount of metal in wastewater and the type of soil and climate, the bioavailability of metals, the amount of metal absorption by the plant and the amount of its displacement in the plant organs (Wang *et al.,* 2006; Usman *et al*., 2005). Probably, with increasing the heavy metals, the amount of plant production, the size of cells and as a result the dry weight of the organs decreases (Sharma and Dubey, 2005; Yadollahi *et al*., 2016). In general, abiotic stresses affect the pathways involved in the biosynthesis of three main groups of secondary metabolites, including terpenes, phenols, and nitrogen-containing compounds, and possibly increase or decrease these materials (Erturk *et al*., 2007; Nokandeh *et al*., 2015; Kabiri *et al*., 2017; Mishra & Singh, 2019). In this study, the highest percentage of increase in total soluble sugar, total phenol, total flavonoid, total anthocyanin, total tannin, and DPPH radical-scavenging percentage was observed in *C. zizanioides*. Therefore, it can be said *C. zizanioides* that had better resistance and accumulated more heavy metals under urban wastewater treatment was more successful than *C. alternifolius* and *Aloe vera* for remediation of the contaminated soil.

Conclusion

According to MAI and the morphological and physiological characteristics of the three studied plants under urban wastewater treatment, *C. zizanioides* suggested as a better refining plant for cultivating and creating a green belt in Saravan's urban wastewater soil. However, due to the significant metals refining of aloe vera, it is also suggested to use this plant to create a green belt in the polluted soil of the urban sewage exit routes of Saravan municipal waste disposal site.

Keywords: *Flavonoid, Metal accumulation index, Phenol, Phytoremediation, Urban wastewater*

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Declaration of conflict of interest

The authors declare that they have no conflict of interest.