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Uroclhoa mosambincensis: A potential native phytoremediator for soils contaminated with arsenic

Sónia Isabel Ventura Guilundo[1*], Marta Alberto Aduge[1], Edmilson Enes Manuel Simango[1], Domingos Maguengue[1], Esnaider Rodríguez Suárez[2], Célia Marília Martins[1] and Orlando António Quilambo[1]

[1]Biology Science Department, Eduardo Mondlane University (DCB-UEM), Maputo, Mozambique.
[2]Chemical Department, Eduardo Mondlane University (DQ-UEM), Maputo, Mozambique.

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Industrial development has caused the contamination of the environment, leading to biodiversity loss and human health concerns. The use of native plants and/or their associated microbiota is a sustainable solution for reducing or transforming contaminants into less harmful forms. This study was conducted to evaluate the remedial potential of *Uroclhoa mosambincensis* in soils contaminated with arsenic. In a greenhouse experiment, seedlings of *U. mosambincensis* were divided into four treatments of increasing arsenic concentration. It was found out that in *U. mosambicensis,* although most physiological parameters were affected, in 200 mg.kg^{-1} arsenic trioxide concentration (As_2O_3) an increase in 23.3% of leaf biomass was observed. Chlorophyll A was not significantly affected by the presence of arsenic. It was also verified that the increase in arsenic concentration stimulated the removal of arsenic from soil to plant tissues at a percentage of 10.8, 27.7 and 30.2 higher in each treatment. This indicates the arsenic accumulator character of *U. mosambicensis* and its potential use for remediation of soils contaminated with arsenic.

Key words: Accumulation, arsenic trioxide, metalloid, phytoremediation.

INTRODUCTION

The intensification of industrial, agricultural and urbanization activities has increased the risk of soil pollution by heavy metals (Saxena et al., 2019) and metalloids including arsenic (As) (Yan et al., 2020). The most common forms of As found in plant tissues and in the soil are trivalent (III) and pentavalent (V) As. Trivalent As (III) is 60 times more toxic than inorganic pentavalent (V) form (Zmozinski, 2014; Abbas et al., 2018; Jinadasa and Fowler, 2019).

The toxicity of trivalent As is because it is retained in the body through the connection with sulfhydric groups (De Carvalho, 2004). In humans, doses between 10 and 50 µg. l-1 and > 50 µg. l-1 can be lethal (WHO, 2016), acting as a neurotoxic agent, and long-term exposure, even at low doses, has a carcinogenic effect (Flanagan et al., 2012; Khalid et al., 2017; Shahid et al., 2018; Alam et al., 2019). Various conventional physical-chemical techniques have been used to reduce the availability of As in the environment, such as soil washing by high pressure, gas extraction from the soil, carbon adsorption,

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*Corresponding author. E-mail: siventura84@gmail.com.

chemical oxidation, the electro-osmotic method, the thermal method *in situ* and *ex situ*, etc. (Wuana and Okieimen, 2011; Sharma et al., 2018). However, these methods are quite costly and also have undesirable effects on the environment (Wuana and Okieimen, 2011). Alternatively, phytoremediation, which is the use of plants, fungi and associated bacteria, in the recovery of polluted soil, water and air (Greipsson, 2011; Sharma and Pandey, 2014), have proven effective in reducing contaminant concentrations (Lambers et al., 2008; Ma et al., 2016) or in the transformation of contaminants into forms less harmful to living beings, especially in the trophic chain (Andrade et al., 2009; Sharma and Pandey, 2014).

In phytoremediation it is necessary to use plants that have certain characteristics, such as good absorption capacity, a deep root system, an accelerated growth rate, easy harvesting and high tolerance to the contaminant (Oliveira et al., 2007). Thus, in the present study, the potential of *Uroclhoa mosambicensis*, which is a grassy species abundant in the Beleluane Industrial Park, was evaluated to restore As-contaminated soils.

MATERIALS AND METHODS

The study was formally authorized by the local authorities. The soil and plant material samples were collected at the Beleluane Industrial Park, located near the Matola River, at latitude 25° 91361 and longitude 32° 41940 (Figure 1).

A transect was done and every 5 m a grid with dimensions of 1 m × 1 m was made. Soil was collected at a depth of 30 cm and placed in polyethylene bags for further physical-chemical parameters analysis and As quantification using the X-ray fluorescence (XRF) method (Kodom et al., 2012; Marchand et al., 2016).

Eighty seedlings of *U. mosambinsensis* (the most abundant species) were collected and grown in a greenhouse. Polyethylene pots containing 1 kg of soil from the sampling site, each one with two seedlings, were used and divided into four treatments: 0, 50, 200 and 800 mg.kg^{-1} of As_2O, following a completely randomized arrangement. The plants were acclimatized (watered with tap water) for 15 days, after which different amounts of As were added. The plants were harvested 35 days after contamination to assess their physiological and biochemical response to different concentrations of As. The content of As and other chemical elements in soil and plant tissues was also determined.

Different techniques were used to determine the physical and chemical parameters of the soil. The calcium (Ca) and magnesium (Mg) contents were determined by ammonium acetate and the sodium (Na) and potassium (K) contents were determined by the flame photometry method (Barnes et al., 1945). Organic matter and carbon (C) were determined by the wet combustion method (Walkley and Black, 1934). The concentration of nitrogen (N) was determined by the method of Kjeldahl (Bremner and Mulvaney, 1982). The texture was determined by the pipette method (Gee and Bauder, 1986). The phosphorus content was determined by the method of Olsen et al. (1954) and the particle density by the pycnometer method. The cation exchange capacity was determined by the method using ammonium acetate and calcium chloride (Raij and Küpper, 1966).

To determine the dry weight of roots and shoots, three plants were harvested per treatment and dried at 80°C in the greenhouse for 48 h. After drying, the material was weighed on the analytical balance.

The determination of chlorophyll A and B was done using a spectrophotometer. An amount of 0.1 g of fresh leaves of *U. mosambicensis* was weighed and crushed in 10 ml of 99% ethyl alcohol and the material was stored at 17°C for 24 h.

The extracts were centrifuged for 10 min at room temperature and readings were carried out using 99% alcohol as a solvent. Wavelengths of 664 and 649 nm were used for chlorophyll A and chlorophyll B, respectively, according to the following formulas (Lichtenthaler and Buschmann, 2001):

$$\text{chlorophyll A} = 13.36 \times A664.1 - 5.19 \times A648.6$$
$$\text{chlorophyll B} = 27.43 \times A648.6 - 8.12 \times A664.1$$

The XRF technique was used to determine the As content; the dry plant material was ground in a ball mill until a homogeneous powder had been formed, which was read with the aid of a computer (Kodom et al., 2012; Marchand et al., 2016; Byers et al., 2019).

Data analysis

The data were analyzed using the STATISTICA version 8.0 program. The difference between treatments was determined using *one-way* ANOVA at a significance level of 5%.

RESULTS AND DISCUSSION

Characteristics of the soil

The chemical characteristics of the soil are shown in Table 1. The soil of the industrial area has a sandy texture characterized by a higher concentration of calcium and ion exchange. Soil texture is one factor among others that can affect As mobility and availability (Marquez-Garcia et al., 2012; Abbas et al., 2018).

Sandy soils have greater availability of As, due to the lower presence of iron and aluminium oxides and hydroxides compared to clayey soils (Sheppard, 1992; Karimi and Alavi, 2016).

The As trioxide, which has greater mobility and toxicity, was used as a contaminating agent, because this compound under anaerobic conditions is most abundant and most soluble in relation to pentavalent As. The toxicity of As trioxide is 60 times greater than its pentavalent form (Abbas et al., 2018) owing to the reaction with sulfhydryl groups of enzymes and proteins, which causes the inhibition of cellular functions, contributing to the death of tissues (Jinadasa and Fowler, 2019).

Effects of different arsenic concentrations on growth

The effect of different arsenic concentrations on the leaf, stem, roots and total plant biomass is as shown in Figure 2A, B, C and D. In general, it was found that As caused reduction of growth parameters in *U. mosambinsencis* when compared with the control treatment, and this negative effect was reported in other studies (Finnegan and Chen, 2012; Abbas et al., 2018; Nabi et al., 2021).

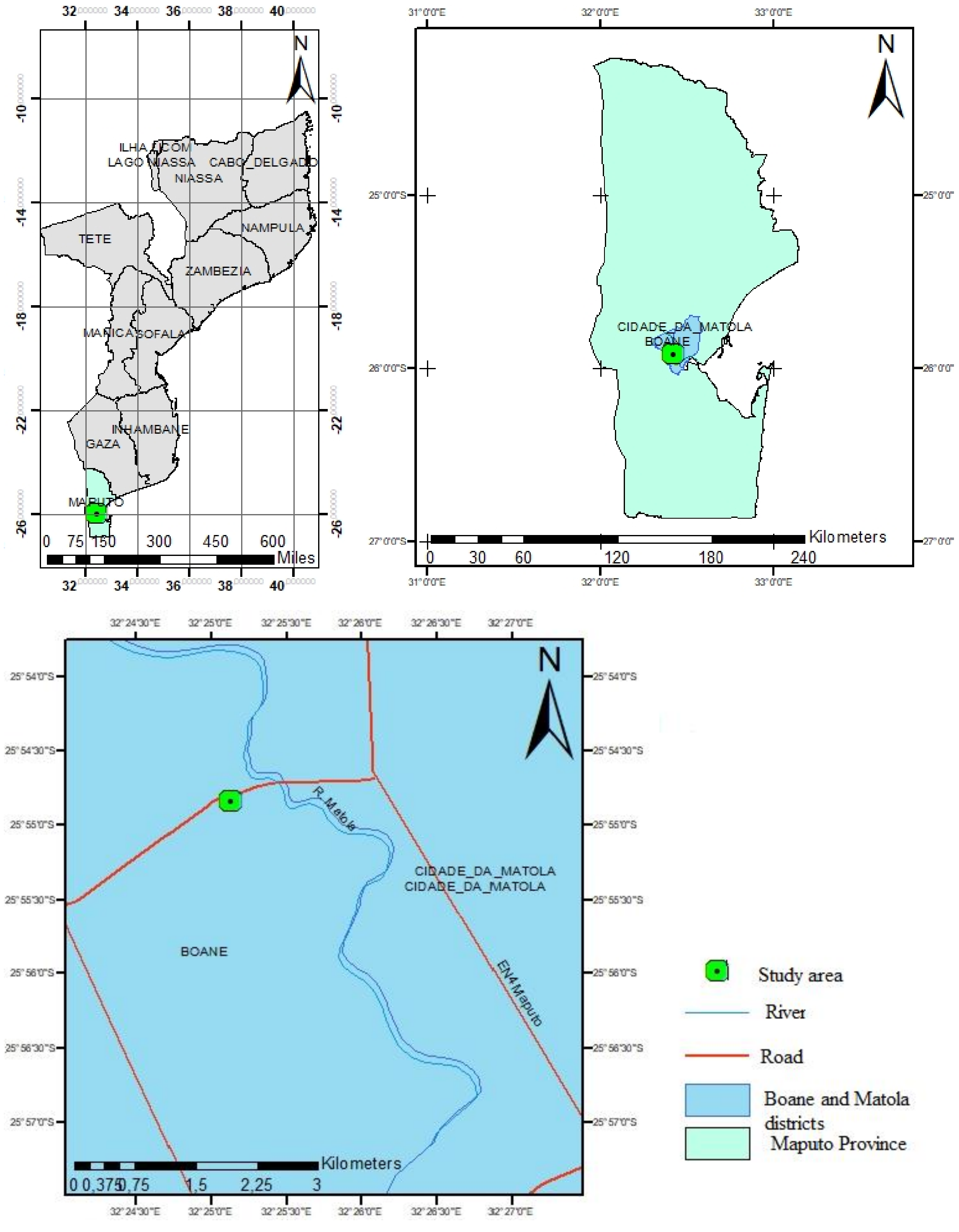

Figure 1. Location of the Beleluane Industrial Park.

Despite this general tendency, there was an indication that the concentration of 200 mg.kg^{-1} As_2O_3 was the optimum level of arsenic absorption by *U. mosambinsencis*. This idea is supported by the increase of the leaf dry weight in 23.3% after the addition of 200 mg.kg^{-1} As_2O_3 concentration ($p \leq 0.05$), suggesting that the 200 mg.kg^{-1} As_2O_3 concentration had a stimulating effect on the production of leaf dry weight, as found by Sushant and Ghosh (2010) in a study in which the increase in As concentration increased the leaf biomass

Table 1. Physical and chemical characteristics of the soil of Industrial Park.

Physical and chemical characteristics of the soil	Value
Ca	12
Mg	0.8
Na	0.10
K	0.48
Cationic exchange capacity (meq.kg^{-1})	13.6
N	0.04
P	5.73
C	0.57
Organic matter	1.12
Electric conductivity (ms.cm^{-1})	0.209
pH (KCl)	7.37
pH (H_2O)	8.80
Sand %	79.54
Clay %	15.10
Silt %	5.36
Structure	Sandy

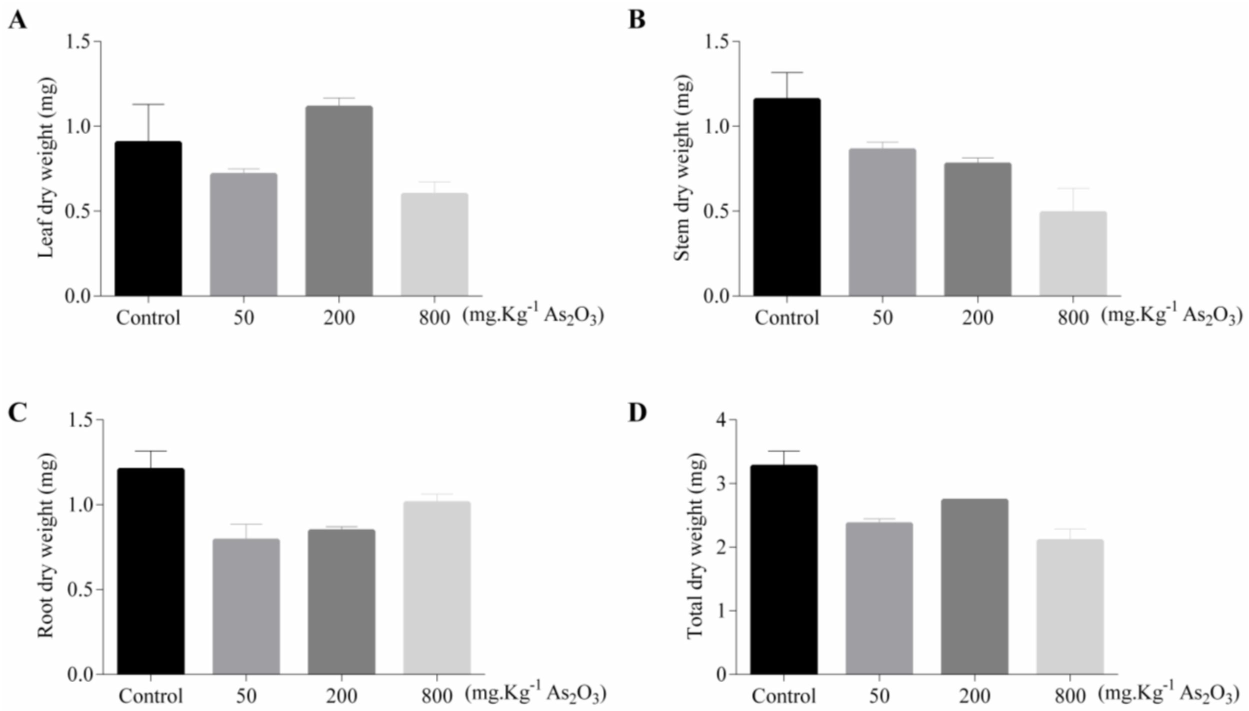

Figure 2. Effect of different As concentrations on the leaf, stem, roots and total plant dry weight is presented A, B, C and D. Data refer to the average of three replicates ± standard deviation.

in *Allium cepa*. Less reduction of root and total dry weight was also observed in the concentration when compared with the other treatments with arsenic in this study. Probably, the arsenic did not affect the root directly but interfered with the nutrients translocation to aerial parts as defended by Rehmus et al. (2014) in a study about the effect of aluminium on seedling of forests plants, in which it was verified that the root biomass increased or was not significantly affected while the shoot’s biomass was reduced. It was verified that Al caused the reduction of

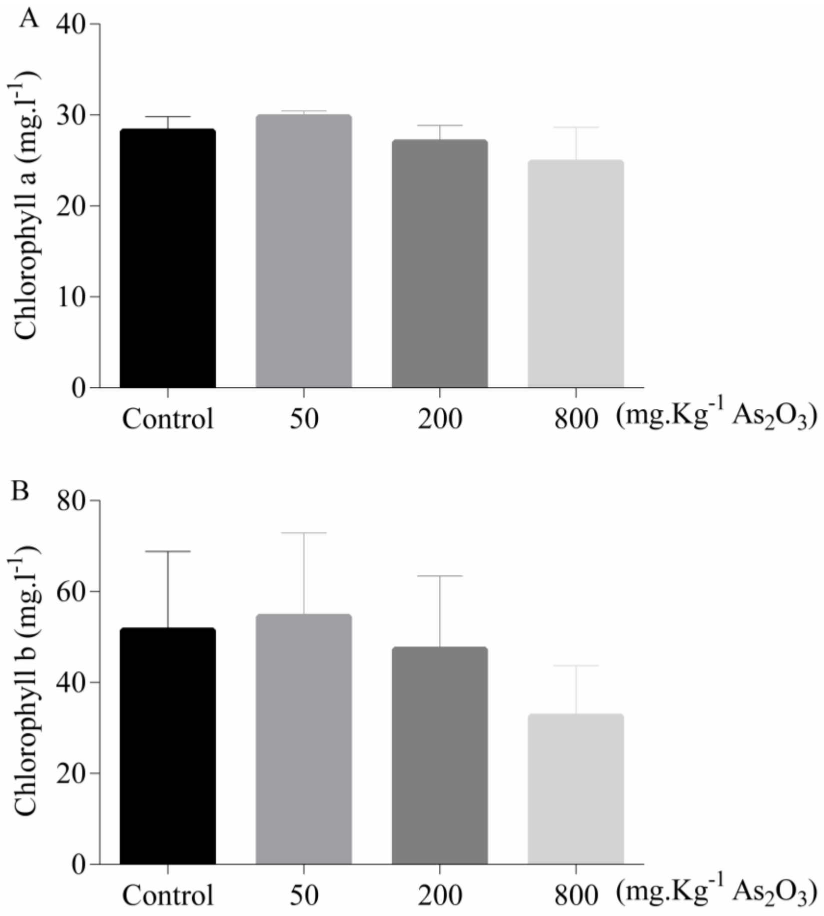

Figure 3. Effect of different As concentrations (0.0, 50, 200, 800 mg.kg^{-1} As_2O_3) on chlorophyll A (A) and chlorophyll B (B) content of *Uroclhoa mosambincensis*. The bars represent the mean of three individual plants ± SD.

the macronutrient calcium in the shoots. In the present study, it was also verified that for some nutrients as potassium, calcium, chlorine among other, increase in the plant tissues was observed with the increase of As_2O_3 concentration, but it was not possible to verify if the nutrients were accumulated in roots or aerial parts of *U. mosambicensis.*

The stem biomass decreased 25.2, 32.2 and 57.4%, respectively with an increase in As concentration ($p \leq 0.05$). A decrease by 34.7, 30.6 and 17.4% was observed in root biomass and by 27.6, 16.3 and 35.9% in total plant biomass in all As concentrations when compared with control treatment. This is in line with Melo et al. (2018), who observed a decrease by 63 and 59% in roots, and 60 and 63% in shoots of *Anadenanthera peregrina*. But an increase of root biomass was also found with the increase of arsenic and the opposite behavior in stem biomass. These results may be explained by the high toxicity of arsenic on the growth of many plants (Várallyay et al., 2015; Mawia et al., 2021). The response to the presence of As varies with the physical-chemical characteristics of the soil, the species of plant and different mechanisms of absorption, as well as toxicity and detoxifications (Abbas et al., 2018).

In a study in which several trace elements such as As were used in different plants, including *Medicado sativa* and *Phaseolus vulgaris*, it was demonstrated that the biomass was reduced in contaminated soils. Contrary to what happens with other trace elements, As is not used as a nutrient by plants and its phytotoxic effect is well known (Melo et al., 2018).

Effect of different concentrations of arsenic on chlorophyll content

Figure 3A and B shows that chlorophyll B decreased by 36.6% in 800 mg.kg^{-1} As_2O_3 concentration ($p<0.05$); however, chlorophyll A content was not significantly affected by the addition of As, indicating that *U. mosambincensis* in the presence of As is able to maintain photosynthetic activity, suggesting a tolerance mechanism.

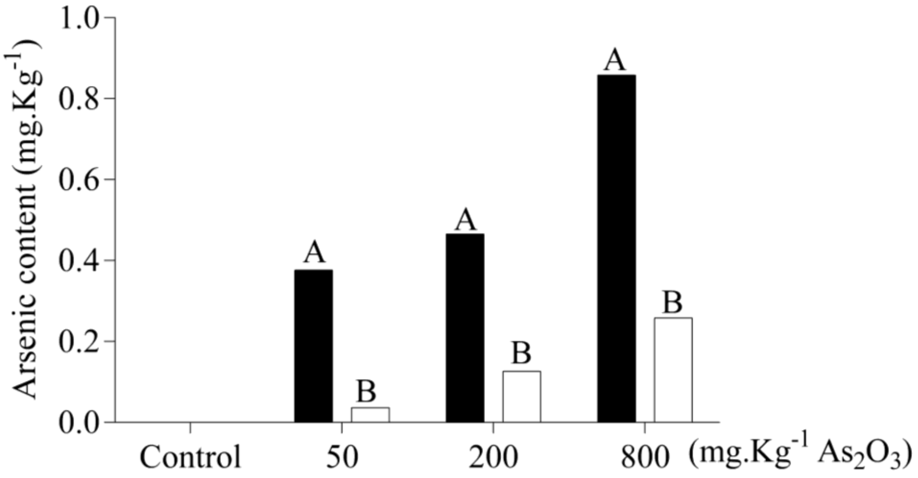

Figure 4. Arsenic concentrations in soil (A) and plant tissue (B) of *Uroclhoa mosambincensis*, submitted to different treatments (0.0, 50, 200, 800 mg.kg^{-1} As_2O_3).

The chlorophyll A and B content in 50 mg.kg^{-1} treatments was 5.8% higher compared to the control. Several studies have shown that the synthesis of photosynthetic pigments in different species is influenced in a different way by contamination with As (Abbas et al., 2018). In *A. cepa* it was shown that the increase in As concentration has a stimulating effect varied by 60 to 113% and by 14.6 and 59%, on the synthesis of chlorophylls A and B, respectively (Sushant and Ghosh, 2010). However, it was found that rice plants were sensitive to contamination with As, which caused a reduction by 57.3 and 50.2% in chlorophyll A and B, respectively (Miteva et al., 2005; Rahman et al., 2007). It is suggested that the reduction in the average levels of chlorophyll in plants under the effect of As is due to the destruction of the structure of chloroplasts by the metalloid (Miteva et al., 2005). In this study, it was not possible to establish a relation between the chlorophyll content and the biomass, except in stem dry weight where the increase of As concentration reduced the chlorophyll B content and consequently the biomass.

Arsenic concentration in the soil and in the plant

Figure 4 represents the arsenic accumulation in soil and plant tissue after 35 days of experiment. In the control, no As concentration was detected in either soil or plant tissue; however, from 50 to 800 mg.kg^{-1} As_2O_3 concentrations, the As content increased by 10.8, 27.7 and 30.2%, respectively. This result indicates that with the increase in As concentration, the stimulus for the plant to remove As from the soil increases, resulting in its accumulation in plant tissues, which is a strong indication that *U. mosambicensis* is an accumulator species.

This fact is also supported by Melo et al. (2009), using different As concentrations, they observed an increase of As in *Ricinus communis* and *Helianthus annus* tissues by 85.31 and 79.69%, in Oxisol and 32.31% and 26.03% in Entisol, respectively. This was also proven by Melo et al. (2018), observing an expressive translocation of As was observed to the aerial part of the plant *Anadenanthera peregrina*.

On the other hand, *U. mosambicensis* can be considered an accumulator, since it was found that it grows in As-contaminated soils with a concentration of 800 mg.kg^{-1} As_2O_3 (Da Silva, 2012), showing no evident signs of phytoxicity. This fact is confirmed by Melo et al. (2018), indicating that plants growing in contaminated soil with a concentration limit of 400 mg.kg^{-1} of As showed tolerance. However, other authors consider As hyperaccumulating plants as those that are able to naturally accumulate more than 1000 mg.kg^{-1} of As in dry matter (Ma et al., 2001; Gonzaga et al., 2006, 2008).

The accumulation capacity found in *U.mosambicensis* is in line with the findings of Zhao et al. (2009), who reported that As (III) is as a rule accumulated by hyperaccumulative plants. This is probably due to the fact that plants have developed different mechanisms to circumvent the toxicity caused by this metal, including compartmentalization, protein synthesis of As binding proteins and synthesis of compatible solutes (Abbas et al., 2018). On the other hand, studies carried out on *Arabidopsis thaliana* and *Brassica juncea* have proven that the accumulation of As (III) in the roots and aerial parts of plants is coordinated by sulfhydryl groups such as glutathione and phytoquelatins. Singh et al. (2006) observed that a higher level of ascobarte-glutathione pool conferred protection form oxidante in arsenic hyperaccumulator P. vittata.

Table 2 represents the concentration of some elements in soil and plant tissue after 35 days of experiment. The soil exhibited a high concentration of silicium (Si), iron (Fe), manganese (Mn) and aluminium (Al) in both the

Table 2. Macro, micro and other element concentrations in soil and plant tissue of *Uroclhoa mosambincensis*, under different treatments (0.0, 50, 200, 800 mg.kg^{-1} As_2O_3 concentration).

Element		00 mg.kg^{-1} As_2O_3		50 mg.kg^{-1} As_2O_3		200 mg.kg^{-1} As_2O_3		800 mg.kg^{-1} As_2O_3	
		Soil	**Plant**	**Soil**	**Plant**	**Soil**	**Plant**	**Soil**	**Plant**
Macro	K	5.475	9.936	5.329	9.101	5.451	6.769	5.865	9.172
	Ca	1.187	4.117	1.7000	4.788	1.19	3.766	1.723	5.469
	S	0	0.785	0.742	0.668	0	0.785	0	0.78
Micro	Cl	0.53	6.503	0.91	6.164	0.755	4.84	0.554	7.483
	Fe	8.271	3.425	8.905	4.103	8.434	2.704	9.081	5.781
	Mn	0.299	0.18	0.301	0.199	0.319	0.171	0.344	0.257
	Cu	0.061	0.147	0.064	0.121	0.072	0.437	0.167	0.278
	Zn	0.009	0.079	0.011	0.048	0.009	0.08	0.01	0.089
	Br	0	0.038	0	0.043	0	0.028	0	0.042
	Ni	0	0.031	0	0.034	0	0.028	0	0.044
	Si	68.205	5.942	67.489	5.446	68.768	4.572	67	7.78
	Sr	0.024	0.017	0.026	0.018	0.028	0.015	0.032	0.027
	Zr	0.165	0.016	0.142	0.024	0.222	0.024	0.167	0.042
	V	0	0.016	0	0.013	0	0	0	0.046
	Al	14.079	0	12.727	0	12.902	0	13.166	0
	Rb	0.036	0.016	0.037	0.022	0.04	0.011	0.038	0.028
Plastic and others		0	68.392	0	68.452	0	75.016	0	31.234

control and the As treatments. However, *U. mosambicensis* tissue presented high concentrations of plastic and other elements such as potassium (K), calcium (Ca), chlorine (Cl), cupper (Cu), bromide (Br) and nickel (Ni).

The difference in the nutrient content between the soil and the plant is probably due to the main factors that affect the transfer of As from the soil particles to the roots of the plants, namely the concentration of As in the soil solution, bioavailability, mass flow soil solution, pH reduction, the adsorption/desorption ratio and reduction of redox potential interaction with other ions, among others (Dabrowska et al., 2011).

It was also observed that the macro K element concentration in plants tissue decreased with As application when compared with the control. The same pattern was observed in soil, with the exception of the 800 mg.kg^{-1} As_2O_3 treatment. Similar results were obtained in a study with *R. communis* in which Mn, Fe and Cu concentrations decreased with an increase in As, probably owing to the stress effect caused by high As concentrations (Melo, 2009).

The microelement concentrations showed a different pattern; Fe increased in all As treatments when compared with the control. No Ni was detected in soil, but it appeared in all As treatments. This phenomenon was also observed in a study with the species *R. communis*, in which an increase in As concentration caused an increase in some nutrients in the plant tissues (Melo, 2009). The aluminium (Al) was only detected in soil, but was not found in plant tissue in all treatments, showing the high ionic strength and capacity of this element to compete aggressively for access through the root (Taiz and Zieger, 2014).

Conclusion

U. mosambicensis has proven to have the potential to grow in soils contaminated with As. The leaf biomass was 23.3% higher in the 200 mg.kg^{-1} As_2O_3 concentration. The stem biomass decreased with an increase in As concentration. A decrease of 34.7, 30.6 and 17.4% in root biomass and a decrease of 27.6, 16.3 and 35.9% in total plant biomass in all 50, 200, and 800 mg.kg^{-1} As_2O_3 concentrations were observed. The chlorophyll A content was not affected by different As concentrations. As accumulation was found in plant tissues at a percentage of 10.8, 27.7 and 30.2 higher than in soil in all treatments, suggesting *U. mosambicensis* as an As accumulator and its potential use for remediation of soils contaminated with As.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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