

## A Brazilian Amazon Species with High Potential to Phytoextract Potential Toxic Elements

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### Abstract

The *Euterpe oleracea* Mart. has great importance in the neotropical forestry economy. Its berry is a product of great commercial value used extensively for human consumption. Most *E. oleracea* researches evaluate its food features, however, its potential use for phytoremediation and stipe use remains unknown. This research aimed to assess the seedling's phytoextraction potential and the structural chemical composition in the seedlings and mature palm trees stipes. We used the Energy-dispersive X-ray fluorescence analysis to determine the concentration of the chemical elements. *E. oleracea* seedlings showed a great phytoextraction potential for aluminum and iron. The aluminum seedlings concentration was four times higher than preconized as a hyperaccumulator species. Calcium concentration was lower than considered normal, which may represent an antagonism effect caused by the strong presence of aluminum and iron. The fast uptake and accumulation of the seedlings highlight the potential to use this species in phytoremediation programs.

**Keywords:** Phytoremediation, Mineral nutrition, Chemical characterization, X-ray fluorescence.

## 1. INTRODUCTION

The environmental contamination by potentially toxic elements and the solutions for mitigating the negative effects have been attracted worldwide attention (Bhandari et al., 2007; Ye et al., 2019). Due to the different and complex sets presented by polluted areas, different strategies regarding these adverse conditions must be found as soon as possible. Phytoremediation is an environmentally friendly technique with cost-effectiveness and potential species, or hybrids have been tested in many situations (Wani et al., 2017; Suman et al., 2018). Among the most promising strategies, phytoextraction encompasses the uptake of elements from the soil, which are translocated and accumulated in the harvestable plant parts (Pajević et al., 2016). Associated with fast-growing trees

species with high biomass production and a high commercial relevance the phytoextraction technique is a promising choice (Pulford & Watson, 2003).

*Euterpe oleracea* Mart., popularly called açai is a Brazilian species naturally occurring in the neotropical Amazon region contemplating four Brazilian states (Leitman et al., 2015). The use of this species is mostly related to the fruit, in which 25% of them are edible (epicarp and mesocarp), they are highly appreciated in food and have health benefits, mainly associated with their antioxidant capacity and phytochemical composition (Coutinho et al., 2017; Cedrim et al., 2018). Regarding the latter constitution, there are about ninety bioactive substances, including flavonoids, phenolic compounds, lignoid, and anthocyanin (Pacheco-Palencia et al., 2009; Canuto et al., 2010; Garzon et al., 2017).

The potentially toxic elements are heavy metals such as copper (Cu), iron (Fe), aluminum (Al), lead (Pb), zinc (Zn), which in minimal concentration in the soil or the water can offer risk to environmental and human health (Haghnazar et al., 2021; Verma et al., 2021). The *E. oleracea* not only resists high levels of heavy metals in the soil, but it can also remove them and transport them to the upper parts of the plant (Silva et al., 2015; Gonçalves Junior et al., 2016). In this context, *E. oleracea* presents itself as potential to be used in phytoextraction programs and its structural chemical characterization is important. Most studies regarding this species are related to its fruit. As consequence, information about the stipe and other uses have been incipient so far. Moreover, this species is usually found in seasonally flooded areas or even in marshes constantly flooded possessing a distinct metabolism (Santos et al., 2018). Sarwar et al. (2017) stated that the use of *E. oleracea* for phytoremediation can be an alternative for cleaning up these singular environments, which provides a practical procedure for removing contaminants rather than excavation and soil replacement. Among the plants used for phytoremediation, only *Oryza sativa* L. (rice) grows well in flooded fields (Almeida et al., 2019).

The determination of nutrients and the potentially toxic elements concentration in plants even at low concentrations can be done by Energy-dispersive X-ray fluorescence (EDXRF) spectroscopy (Bamford et al., 2004). This analytical technique offers a fast, non-destructive, simultaneous, and multi-element analysis with minimal sample preparation (Brouwer, 2006). Considering the incipience of studies on the commercial use of the *E. oleracea* trees stipe and remediation schemes for impacted and polluted flooded areas, we assessed the seedling's phytoextraction potential and the mineral nutrition aspects. Differences in the structural chemistry composition and physiologic impacts in younger and mature *E. oleracea* trees were also investigated.

## 2. MATERIALS AND METHODS

### 2.1. Vegetal Material

Ten seedlings of *E. oleracea* were cultivated in an uncontrolled environment in an outdoor nursery in Seropédica city (22°45'26" S; 43°41'16" W; 37 m a.s.l.). We germinated the seeds of *E. Oleracea* and then transposed them to plastic bags (10 x 20 cm) filled with a conventional substrate composed of sieved soil and mineral elements. The irrigation occurred twice a week for periods of one hour. The chemical composition of the soil is shown in Table 1. After six months, we harvested the seedlings and removed the root system. We used the upper parts (stipe and leaves) to perform the analysis.

**Table 1.** Chemical composition of the conventional substrate.

N	P	K	Ca	Mg	Al					
--- g Kg <sup>-1</sup> ---										
7,90	2,19	2,13	3,73	3,52	222,85					
As	Ba	Cd	Cr	Cu	Ni	Pb	Se	Zn		
--- mg Kg <sup>-1</sup> ---										
0,20	47,60	0,20	24,2	12,5	13,3	6,3	nd	28,1		

nd = not detected.

We collected samples from harvested mature *E. oleracea* (16-years old) palm trees stipe cultivated in an agroforestry system in Paraty city (23°01'13" S; 44°40'51" W; 262 m a.s.l.). We cut the samples from three parts from the stipe: at the base (0.15 cm above the ground), at the diameter at breast height (DBH, 130 cm above the ground) and, at 5 cm below the apical meristem. Both experiments were implanted by the Federal University Rural of Rio de Janeiro, in the Rio de Janeiro State, Brazil.

### 2.2. Seedlings phytoextraction potential

We investigated the phytoextraction potential through the element's accumulation in the seedling's upper parts (stipe and leaves) after six months of growth. We also assess the levels of essential mineral elements and possible interactions between the potentially toxic elements. The upper parts of harvested seedlings were dried in a climatic chamber (60 °C) for 48h. After that, its stipe and leaves were crushed (Wiley mill) and sieved (200 µm). We used the Energy-dispersive X-ray fluorescence analysis (EDXRF) and the Fundamental Parameters method (Omote et al. 1995) to determine the mineral concentration, using three repetitions for each sample. Using an EDXRF benchtop spectrometer Shimadzu, model EDX-720, utilizing an Rh X-ray tube operated at 50 kV and 68 µA, a 5 mm diameter collimator and under vacuum (lower than 30 Pa). We acquired The X-ray spectra by a Si (Li) semiconductor detector during 500 s (live time). We used the same analysis condition for Pb determination, except the use of an Ag filter and without vacuum. We performed the analysis at the Nuclear Instrumentation Laboratory, Center of Nuclear Energy in Agriculture, University of São Paulo.

We used the Standard reference material (SRM) to assess the element's concentration accuracy. The apple leaves (NIST 1515) were used to assess the trueness for the S, K, Ca, Fe, Mn, and Cu quantification. For Si, it was utilized hay (IAEA-V-10) powder. For Al and Pb, 0.5 g cellulose powder P.A. (Cellulose Binder, Spex) was spiked five and two times separately with Al (10,000 mg kg<sup>-1</sup>) and Pb (20 mg kg<sup>-1</sup>) respectively, then drying at 60 °C in a laboratory oven for two hours and homogenized using an agate mortar. The *E. oleracea* samples

and the SRM pressed pellets were prepared using 0.5 g of the vegetal powder pressed at 7.5 ton cm<sup>-2</sup> using the press for five minutes. The acceptable ranges for elemental recovery of the standard were from 80 to 120%. Values outside this range were reported as a semi-quantitative analysis, with the approximate concentrations of the elements in the samples.

### 2.3. Chemical composition investigation

We quantified possible differences in the cellulose, holocellulose, alpha-cellulose, and total extractives concentration in the seedlings and mature palm trees. There were also investigated possible influences in the younger and mature assai palm trees caused by their different levels. We determined the structural components according to the methods described by Abreu et al. (2006). For the extractive contents, we did extraction cycles with a Soxhlet extractor for 24 hours using the organic solvents cyclohexane, ethyl acetate, and methanol sequentially. To determine the insoluble lignin contents, we did the acid-insoluble lignin reaction of the extract-free samples of the plant material with 72% sulfuric acid solution. To determine the holocellulose and alpha-cellulose content, we used the chlorination method, reacting the extract-free samples of the plant with the sodium chlorite solution (Abreu et al., 2006).

### 2.4. Data analysis

We performed the Shapiro-Wilk and the Levene test to assess the distribution of normality and homoscedasticity of data of phytoremediation and the chemical analysis. We used the analysis of variance to assess the statistical difference between the structural chemical composition of the seedlings and stipes. Then we compared using Tukey's test, with 95% of probability. For the phytoremediation, we compared the concentration of the elements of the seedlings with the concentration of the standard reference material. No data transformation was done.

## 3. RESULTS AND DISCUSSION

### 3.1. Seedlings phytoextraction potential

Figure 1 shows the *E. oleracea* seedlings EDXRF spectra with the respective element's peak intensities. The operating conditions used for recording the XRF spectra of the elements range Al - Cu enhanced the background at the Pb La energy peak region at 10.55 keV. The use of the Ag filter for Pb determination improves the ratio of La net intensity to noise (square root of background). That feature allows the

determination of the analytical lines with higher precision and the lowest detection limits.

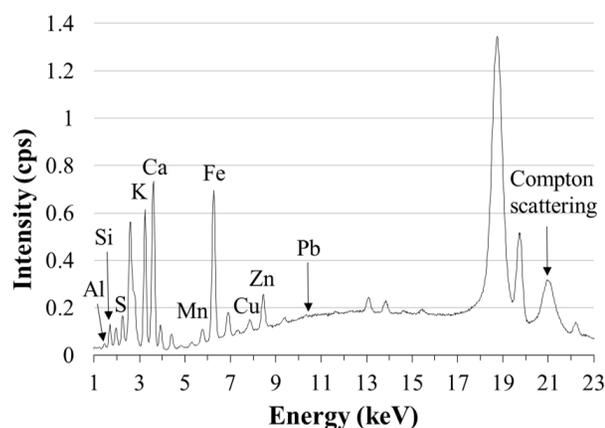


Figure 1. EDXRF spectra of the *E. oleracea* seedlings.

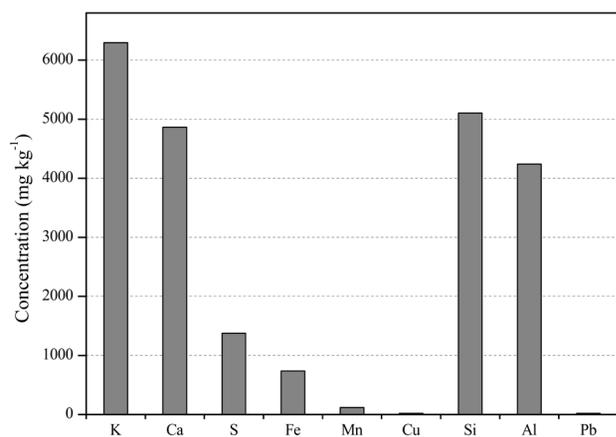
The multi-element results show that the *E. oleracea* absorbed several elements from the soil and translocate them to the upper parts of the plant. Regarding the element's concentration, there were observed acceptable ranges for elemental recovery of the standards for K, Ca, S, Fe, Mn, Cu, and Pb ranged from 82.77-109.61%. The results highlight the suitable trueness of the FP method for these elements determination by the EDXRF technique. A semi-quantitative elemental concentration was performed for Si and Al. Thus, the recovery values for those elements ranged from 54.51-55.72% outside the given range (80-120%), therefore, the concentrations were underestimated representing approximate values (Table 2).

Table 2. Recovery values for the element's concentrations determined by Fundamental Parameter method in the Standard Reference Materials.

SRM <sup>a</sup>	NIST 1515 (apple leaves)				
Analyte	K	Ca	S	Fe	Mn
C <sup>b</sup> (mg kg <sup>-1</sup> )	16355.33	14814.11	1972.90	83.96	51.96
SD <sup>c</sup>	30.43	24.34	12.79	1.17	1.26
RV <sup>d</sup> (mg kg <sup>-1</sup> )	16080	15250	1800	82.70	54.10
R <sup>e</sup> (%)	101.71	97.14	109.61	101.53	96.04
SRM	NIST 1515	IAEA-V-10 (hay)	Spike (cellulose)		
Analyte	Cu	Si	Al	Pb	
C <sup>b</sup> (mg kg <sup>-1</sup> )	4.71	981.25	5572.01	16.61	
SD <sup>c</sup>	0.17	27.40	186.791	0.26	
RV <sup>d</sup> (mg kg <sup>-1</sup> )	5.69	1800	10000	20	
R <sup>e</sup> (%)	82.77	54.51	55.72	83.05	

<sup>a</sup>SRM = Standard Reference Material; <sup>b</sup>C = concentration; <sup>c</sup>SD = standard deviation; <sup>d</sup>RV = reference value; <sup>e</sup>R = recovery.

Figure 2 shows the concentrations of the elements in the *E. oleracea* seedlings cultivated at normal conditions harvested after six months. The bars represent the average for each element concentration determined by EDXRF analysis.



**Figure 2.** Average elements concentrations in the six-months-old *E. oleracea* seedlings (N = 10 samples).

The *E. oleracea* seedlings effectively absorbed and translocate great amounts of Al and Fe to the upper parts (Figure 2). The Al average concentration was 4,241.42 mg kg<sup>-1</sup> of dry weight (DW), four times above than preconized to be an Al-hyperaccumulator species (Jansen et al., 2002). Al concentration in the *E. oleracea* was twice higher than found in the fine roots of *Spruce* and *Poplar* seedlings at five months of age (Brunner et al., 2008). The latter authors also observed a higher level of Al in the epidermal and cortical cells wall than in the intracellular structures.

Seedling Fe concentration was five times higher than that found in the *Euterpe edulis* leaves from conservation units in São Paulo State, Atlantic Forest biome (França et al., 2004; França et al., 2005). The Fe and Cu levels were at least five and two times, respectively, above that found in several species tested for Ni-phytoremediation purposes (Boyd & Jaffré, 2009). In addition, Cu concentration was higher than that found in three species of seedlings cultivated near a former metal smelter (Dahmani-Muller et al., 2000). The Fe and Cu concentrations were more than six and three times higher, respectively, than the normal concentrations in oil palm (*Elaeis* spp.) seedlings (Matos et al., 2016). However, the Cu concentration ranged from the normal to the excessive level, and the Pb and Mn levels were considered normal average for several species (Kabata-Pendias & Pendias, 2010). The Pb concentration in the seedlings was similar to that observed in the wood of *Pinus sylvestris* mature trees cultivated in different sites (Butkus & Baltrėnaitė, 2007). The effective uptake and transportation of Al and Fe in *E. oleracea* highlight the potential use of the

species to phytoextract both elements from soils (Baker & Brooks, 1989). Essential features to introduce the *E. oleracea* in phytoremediation programs to remove pollutants from contaminated areas (Nakbanpote et al., 2010).

A high concentration of Si was also observed in the *E. oleracea* seedlings. This beneficial element has been associated with plant stress reduction when exposed to a great amount of potentially toxic elements in the soil (Zhao et al., 2022). One of the possible mechanisms involved in increased tolerance is the compartmentalization of potentially toxic elements in the cell wall and vacuole (Emamverdian et al., 2018). Other mechanisms, such as the Si and Al complexation, were also observed in hyperaccumulator of Al *Faremea marginata*, which contributes to reducing the phytotoxic effects of the accumulation of Al in the vegetal tissue (Britez et al., 2002).

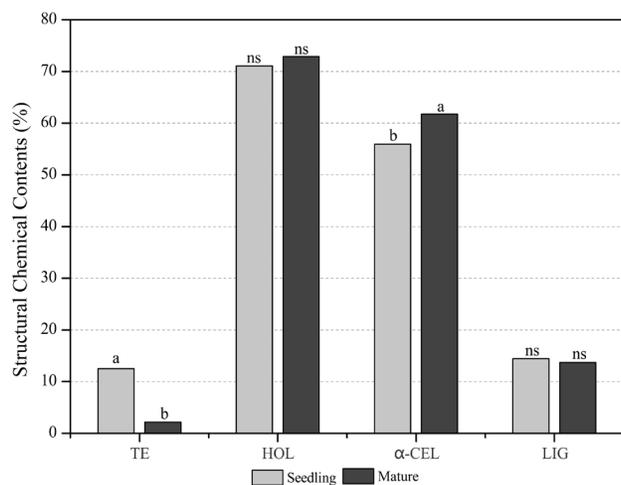
The concentrations of the essential elements K and S were found within the normal range considering similar species (Figure 2). However, the Ca concentration was lower than that found for *Elaeis* spp., maybe indicating a nutritional disturbance caused by the antagonism between Al, Fe, and Cu (Matos et al., 2016). One negative effect of non-essential elements at high concentration in the vegetal tissue is the reduction of the cation's uptake such as Ca (Sharma & Dubey, 2005). K was the element at the highest concentration in the *E. oleracea* seedlings. The essential elements concentration in the *E. oleracea* seedlings followed the descending order of K > Ca > S > Fe > Mn > Cu. Similar results were observed for Mn concentrations in the leaves of the same species. On the other hand, the authors found lower values for Ca and Cu and higher values for the K and S (Araújo et al., 2016). Similar concentrations of K were reported in the leaflet in an improved *E. oleracea* population (Brasil et al., 2008).

*E. oleracea* phytoextraction potential, considering the concentration of the element identified in the upper parts confirms the great capacity for the extraction of cations and anions from the soil. Those elements partially transported to the fruits can compose molecules with anti-inflammatory properties, due to the presence of flavones with bioactive antioxidants (Odendaal & Schauss, 2014; Cedrim et al., 2018). That anti-oxidant aspect has the mineral elements as precursors of the catalytic synthesis of compounds as flavones, phenolic acids, protocatechuic, p-hydroxybenzoic, vinylic, syringic, and ferulic presents in the *Euterpe* genus (Pacheco-Palencia et al., 2009). In addition, due to its complex phytochemistry composition, the stipe has been used by several communities in the Brazilian and Peru Amazon region as an important phytotherapeutic agent. Used against the snake bites damage, muscle and thoracic pains, such as tonic to combat anemia, diabetes prevention, kidney and liver disease (Bourdy et al., 2000; Deharo et al., 2004; Magalhães et al., 2020).

Until now, most of the researches regarding *E. oleracea* was focused only on the fruit composition. This is the first research published considering the *E. oleracea* potential for phytoextraction including the mineral nutrition aspects regarding possible disturbance caused by the high concentration of potentially toxic elements, and also about the structural chemical composition influences by age. Thus, this chemical research can more efficiently direct its cultivation and consumption. Or even help in the phytoremediation programs of impacted environments when the species introduction is possible. Furthermore, the potential use of the *E. oleracea* to remove potentially toxic elements from flooded and wet environments must be investigated, since it is since it is a species that tolerate flood (Sarwar et al., 2017; Santos et al., 2018). The species have been monitored by authorities mostly in the flooded and contaminated areas, in some cases, human consumption it's not indicated or even forbidden (Smith et al., 2012). This possibility could allow restoring degraded areas and the palm tree still would compress its ecological functions, certainly a health public interest issue (Wycoff et al., 2015).

### 3.2. Structural chemical composition

The age of *E. oleracea* individuals influenced the structural chemical composition and concentrations. Figure 3 shows the average values obtained for the structural chemical composition of the young and mature *E. oleracea*.



**Figure 3.** Structural chemical composition of the seedlings and stipes of mature *E. oleracea* palm trees. Averages followed by the same letter between columns do not differ by Tukey test ( $p > 0.05$ ).

Significant differences were observed between the extractive contents in the seedlings and *E. oleracea* stipe (Figure 3). The total extractive contents (TE) obtained for

the seedlings (12.5%) and the *E. oleracea* stipe (2.16%) were lower than those obtained for other monocotyledons, such as *Cocos nucifera* (33.68%) (Cardoso & Gonzalez, 2016). There was found that the extractive content tended to be inversely proportional to the *E. oleracea* age. Contrarily, the total extractive content in seedlings was higher than that found in the adult plant's stipe. This result differs from that found for wood of the species *Eucalyptus grandis*, where the total extractive content has a tendency directly proportional to age (Silva et al., 2005). The higher extractive content of the seedlings can be explained by the defense mechanism against pathogens and insects of the young plants (Zaynab et al., 2019; Singh et al., 2021).

The holocellulose content (HOL) observed was above 70%, due to the stipe present non-woody features with lower lignin content. It can be verified that no differences were found between the values for the seedlings (71.07%) and the stipe (72.89%). These values were close to those found by Barbash et al. (2016) in annual plants such as rice and wheat, which can be explained by the fact that they belong to the group of monocots. That feature enhanced the plant resistance against the strong winds, providing more flexibility to the stipe (Ramage et al., 2017). There were no differences between the values found for the lignin content in the seedlings (14.4%) and the stipe (13.7%) of *E. oleracea*. The obtained values can be considered low when compared to the values of the wood, being 16 to 24% for hardwoods and 20 to 33% for softwoods of tropical zones (Klock & Andrade, 2013; Ezeonu et al., 2017). Figure 3 also shows the alpha-cellulose content ( $\alpha$ -cel) was higher in the mature adult individuals due to the ground support mechanism. Cellulose and holocellulose have an important contribution to the glycosides carbon formation in the seeds and fruits. With may result in different concentrations of insoluble fibers in these regions (Smith et al., 2012). Glycosides carbon also act enhancing the fruits protection and the nutritional aspects (Wycoff et al., 2015).

## 4. CONCLUSIONS

The fast translocation and accumulation of Al and Fe indicate the great potential for the use of *E. oleracea* to remove these potentially toxic elements from the soil. We observed values above the recommended as an Al-hyperaccumulator species in this species seedlings. Since we carried out this research work at a laboratory scale, we recommend further investigations assess the potential use of *E. oleracea* for removing potentially toxic elements from flooded contaminated soils sites.

Except for holocellulose content, the *E. oleracea* age influenced the chemical composition. In addition, the total

extractive content in seedlings was higher than that found in mature plants. The chemical compounds related in this research can help understand the production process of many substances that can enhance human and ecosystem health conditions.

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