

Phytoextraction Capacity of *Chrysopogon nigritanus* Grown on Arsenic Contaminated Soil

Beda Amichalé Jean Cyrille¹, Messou Aman^{1*}, Ouattara Pétémanagnan Jean-Marie¹, Coulibaly Lacina^{1,2}

¹Laboratory of Environment and Aquatic Biology, Department of Sciences and Environment Management, Nangui Abrogoua University, Abidjan, Côte d'Ivoire

²University of Man, Man, Côte d'Ivoire

Email: *messouaman@yahoo.fr

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Abstract

This study aims to investigate the capacity of *Chrysopogon nigritanus* to accumulate As from contaminated soils. The experiment was conducted in a greenhouse. *C. nigritanus* was subjected to uncontaminated soil and As contaminated soil (50 mg/kg, 100 mg/kg and 150 mg/kg of As), for 180 days. Plant growth and biomass produced, concentration of As in soil and plant, bioaccumulation and transfer factors, as the location of As in tissues and cells of the plant have been determined. Plant growth decreased significantly with increasing of soil As concentration. *C. nigritanus* accumulated more As in roots biomass. The highest bioaccumulation factor values were found in contaminated soil at 50 mg As/kg (As 50), then contaminated soil at 100 mg As/kg (As 100) and contaminated soil at 150 mg As/kg (As 150). As was essentially fixed to the intracellular compartment of the roots, stems and leaves. In roots tissues, As was mainly retained in the rhizodermis and the pericycle. While in stems tissues, As was preferentially accumulated in the conductive bundles. In the leaves, the final destination of As was epidermis tissues.

Keywords

Phytoextraction, *Chrysopogon nigritanus*, Arsenic, Bioaccumulation Factor, Transfer Factor

1. Introduction

Over the past few decades, arsenic (As) has been a focus of environmental concerns due to its high toxicity [1]. Indeed, arsenic is classified as Group-1 carci-

nogen to humans based on strong epidemiological evidence. Its ingestion can lead to abdominal pain, hyperpigmentation of the skin, vomiting, diarrhea, cholera, an increased incidence of spontaneous abortions, late fetal deaths, prematurity and low birth weight [2]. Although arsenic occurs naturally in trace amounts in soils, anthropogenic activities including mining, coal burning and agriculture (herbicides and pesticides) lead to its high accumulation in the environment [3]. These pollutions can generate arsenic concentrations often above the toxicity threshold (12 mg/kg) in soils [4] [5].

In Côte d'Ivoire, Sako *et al.* [6] found arsenic concentrations between 11.3 and 3809 mg/kg in soils in mining areas. Under some physicochemical conditions, arsenic compounds are particularly soluble and consequently become very bioavailable and can affect agricultural production and food quality due to bioaccumulation [7]. In view that soils are limited resources and considered non-renewable on a human scale, several techniques for decontaminating contaminated soils have been developed [8]. However, most of them, in particular the physico-chemical methods (washing of soil, incineration and excavation/burying, etc.), require significant investments and considerably disrupt the biological mechanisms of the soil [9]. On the other hand, biological methods, particularly those which use higher plants (phytoremediation), could be suitable for soil remediation. Indeed, phytoremediation is a less expensive technique that does not destroy soil biodiversity and can be applied to organic or inorganic pollutants [10]. Plants can accumulate the metallic elements necessary for their development as well as those not necessary, due to physiological adaptations [11]. Moreover, hyperaccumulators are naturally capable of accumulating high levels of metals. To date, around 400 plant species have been identified as hyperaccumulating a given metal or metalloid [12]. However, few plant species are known to be hyperaccumulators of As [13]. The majority of the identified species are ferns of the Pteridaceae family [14] [15]. Due to their weak rooting and slow growth, these plants would be less efficient for large-scale use [16]. Consequently, studies are increasingly focusing on plant species that have moderate accumulations of arsenic but have rapid development with high biomass [11]. In addition, the use of endogenous plants is recommended for the remediation of contaminated local soils [17]. Among these plants, *Chrysopogon nigritanus* is of interest for conservation, stabilization and removal of some trace metals (Al, As, Cd, Cu, Cr, Pb, Zn) soils [18]. It has a high biomass production and dense root development which offers a large specific surface and delimits an important rhizosphere zone. Finally, this plant grows in all regions of Côte d'Ivoire [19]. It could therefore be suitable for remediation of arsenic-contaminated soil. This study proposed to determine the capacity of *C. nigritanus* to accumulate arsenic in contaminated soil. Specifically, this study involves in evaluating the effect of arsenic concentration on plant growth, determining the potential for extracting arsenic by *C. nigritanus* and characterizes arsenic accumulation mechanisms.

2. Material and Methods

2.1. Material

2.1.1. Soil

The topsoil used for this study was air dried and then sieved to 2 mm, then homogenized and placed in each pot. The soil was artificially contaminated with arsenic trioxide (As_2O_3) in order to obtain 50 mg/kg, 100 mg/kg and 150 mg/kg. For each concentration, the soils were saturated with an amount of arsenic salt determined according to Equation (1) [20] [21].

$$m_{\text{arsenic trioxide}} = \frac{C \times m_{\text{soil}} \times M_{\text{arsenic trioxide}}}{M_{\text{As}}} \quad (1)$$

$m_{\text{arsenic trioxide}}$ = Mass of arsenic trioxide (mg);

$M_{\text{arsenic trioxide}}$ = Molar mass of arsenic trioxide (g/mol);

M_{As} = Molar mass of arsenic (g/mol);

m_{soil} = Mass of soil in the pot (kg);

C = Theoretical concentration of arsenic (mg/kg).

2.1.2. Plant Selection

Chrysopogon nigritanus chosen for this study is a plant that grows in all regions of Côte d'Ivoire [19]. It has a high production of biomass and a dense root development which offers a large specific surface and delimits a significant rhizosphere [18]. *C. nigritanus* has a high tolerance for arsenic [18].

2.2. Methods

2.2.1. Experimental Design

The experimental was conducted in a greenhouse (Length = 13 m and width = 11 m) at the experimental site of the Biotechnology and Environmental Engineering Research Unit of Nangui Abrogoua University (Abidjan, Côte d'Ivoire). It was performed with plants grown in 48 pots (capacity = 0.024 m³). These include twelve (12) pots per dose of soil contamination by arsenic (50 mg/kg, 100 mg/kg and 150 mg/kg), *i.e.* 36 pots, and twelve (12) pots containing uncontaminated soil. *C. nigritanus* plants used were taken from nurseries (4 weeks old) established at the experimental site. Plants with the same morphological development were selected and cultured in the pots. The experiment lasted 180 days.

2.2.2. Data Collection

Growth monitoring was carried out by weekly measurement of the height of the studied plant stems using a tape measure in millimeters. As for the plant biomass produced, two (2) replicates of plants per arsenic dose were harvested monthly (30 days) and the plant biomasses (shoot and root) produced were determined by weighing on a 10⁻³ precision Sartorius EB150FEG-I scale.

Arsenic concentrations in the soils were determined monthly (30 days). Four (4) composite samples were taken by coring at the [0 - 10], [10 - 20], [20 - 30] and [30 - 40] horizons of experimental pots. The samples were kept in hermetically sealed jars until analysis.

To assess the arsenic accumulation by *Chrysopogon nigritanus*, two (2) plant replicas (shoot and root) per dose of arsenic were taken from the culture pots every month (30 days). In each pot, the harvested plants were separated into shoot and root parts. Each plant sample was washed with distilled water and high purity water to remove dust and soil. After air-drying, each plant sample was dried at 65 EC to a constant weight. The dried samples were crushed using a plant tissue grinder (RESPSCH S100).

2.2.3. Samples Analysis

Arsenic concentrations in soils were carried out according to the standard NF ISO 11466: 1995. The soil sample (0.5 g) was digested with 10 mL of aqua regia (7.5 mL of HCl and 2.5 mL of HNO₃). The content was filtered at 0.45 µm and diluted up to 50 mL with distilled water. Arsenic concentrations were determined by Plasma-Coupled Induction Atomic Emission Spectrometry (ICP-AES).

The mineralization of plant samples was made according to the standard NFX 31-151: 1993. Subsample (20 g) of crushed plant material was oven-dried at 500 EC for 2 hrs and 0.5 g of that burned sample was digested with 10 mL of aqua regia. Then, the sample was put in an oven at 180 EC for 30 min for ending digested process. The filtrate obtained after cooling was used for arsenic analysis by Plasma Coupled Induction Atomic Emission Spectrometry (ICP-AES).

Table 1 summarizes the different methods and standards used to analyze soil and plants samples.

2.2.4. Phytoextraction Efficiency

Two factors were calculated to evaluate plant phytoextraction efficiency. The Bioaccumulation Factor (BF) was calculated to estimate arsenic uptake in the plant. It presents an index of the ability of a plant to accumulate arsenic relative to the concentration in the medium [22]:

$$BF = \frac{\text{Arsenic concentration in roots} + \text{Arsenic concentration in shoots}}{\text{Arsenic concentration in soil}} \quad (2)$$

The Transfer Factor (TF) defined as the ratio between arsenic concentration in plant shoots and its concentration in roots [23]. It determined the relative movement of metal from roots to shoots:

$$TF = \frac{\text{Arsenic concentration in shoots}}{\text{Arsenic concentration in roots}} \quad (3)$$

Table 1. Methods and standard for sample analysis.

Samples type	Parameter	Methods of analysis	Standards
Soil	Arsenic	Digestion with aqua regia and reading with a Plasma-Coupled Induction Atomic Emission Spectrometry (ICP-AES)	NF ISO 11466: 1995 [24].
Plant	Arsenic	Calcination, digestion with aqua regia and reading with a Plasma-Coupled Induction Atomic Emission Spectrometry (ICP-AES)	NFX 31-151: 1993 [25].

- $TF > 1$: accumulation of As in the shoot biomass of plants;
- $TF < 1$: accumulation of As in the root biomass of plants.

2.2.5. Localization of Arsenic in *C. nigritanus* Tissues and Cells

This study determined the distribution of arsenic in plant roots, stem and leaves, precisely at the tissue and the cell level. For the analyses, samples of leaves, stems and roots of *C. nigritanus* were taken from the culture pot with the best yield of arsenic phytoremediation every month. Those samples were fixed for 24 h in 2.5% glutaraldehyde (pH 7.2). Then, they were rinsed two or more times with distilled water. A 2 mm cross-section of the samples (leaf, stem or root) was followed by dehydration in successive baths of 30 min of ethanol (from 70% - 100%). The samples were subsequently dried in the open air and fixed on pads placed on a plate carried in the metallizer to spray them with gold. The plate was finally mounted on the stage of Scanning Electron Microscopy with Energy dispersive X-ray spectroscopy (SEM-EDX) to perform arsenic weight (%) in the tissue and the cell.

2.2.6. Statistical Analysis

Statistical analysis of the data was performed with R software. The normality of the data distribution was verified with the Shapiro test. Parametric tests such as t test and ANOVA test were used to evaluate the differences between growth and biomass produced by the plant in the culture pots, concentrations of As in soils, transfer factor and bioaccumulation factors. Statistical significance was defined at the level of $p < 0.05$.

3. Results

3.1. Plant Growth

During the treatment, stem lengths of *Chrysopogon nigritanus* decreased in soil as the dose of arsenic applied increase (**Figure 1**). However, no significant differences were noted between contaminated soil at 50 mg As/kg (As 50) and the control (t test: $p > 0.05$). However, comparing growth of plants in the control and contaminated soil at 100 mg As/kg (As 100), they were significantly different, the same between the control and contaminated soil at 150 mg As/kg (As 150) (t test: $p < 0.05$). Furthermore, significant differences are shown between stem lengths of plants in As 50 and As 100 as well as between As 50 and As 150 (t test: $p < 0.05$). The order of *C. nigritanus* stem lengths at the end of the experiment is as follows: control (57.5 cm) > As 50 (48.5 cm) > As 100 (28 cm) > As 150 (25.25 cm).

3.2. Biomass Produced

Shoot and root biomasses harvested during the experiment are shown by **Figure 2**. It is noted that these biomasses have increased during the experimental and the shoot part has remained higher than that root in all the pots. But, these biomasses decrease with increasing concentration of arsenic applied in the soil.

From day 30 to day 180, shoot biomasses increased from 12.28 to 379.41 g, from 8.47 to 328.86 g, from 11.07 to 203.4 g and from 4.4 to 137.95 g respectively on uncontaminated (control) soil contaminated soil at 50 mg As/kg, 100 mg As/kg and 150 mg As/kg. As for root biomass, the values ranged from 5.44 to 115.00 g (control), from 2.46 to 97.00 g (As 50), from 2.24 to 51.25 g (As 100) and from 1.05 to 44.00 g (As 150). Shoot and root biomass of the control soil and contaminated soil at 50 mg As/kg were not significantly different (t test: $p > 0.05$). Considering the contaminated soil, it noted that shoot and root biomass values recorded in As 50 differ significantly from that As 100 and As 150 (t test: $p < 0.05$). On the other hand, no differences are observed between As 100 and As 150 (t test: $p > 0.05$). **Figure 3** shows a view of the plant biomass at the end of the experiment.

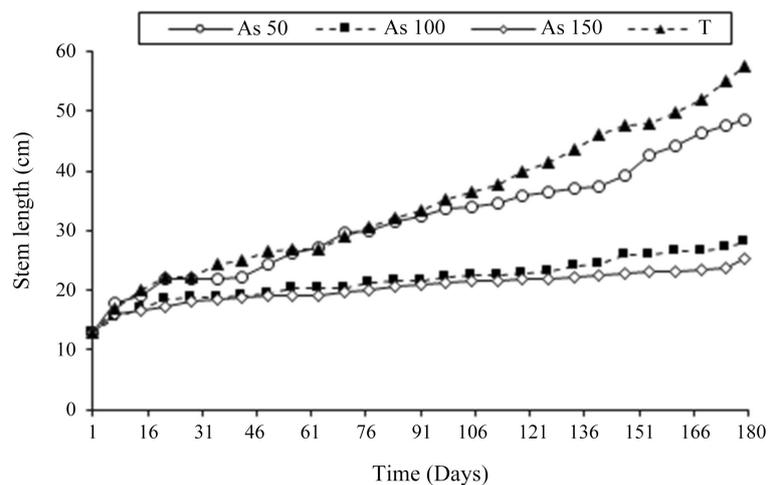


Figure 1. Stem length of *C. nigritanus* during the experiment; As 50: contaminated pot at 50 mg As/kg; As 100: contaminated pot at 100 mg As/kg; As 150: contaminated pot at 150 mg As/kg; T: control pot.

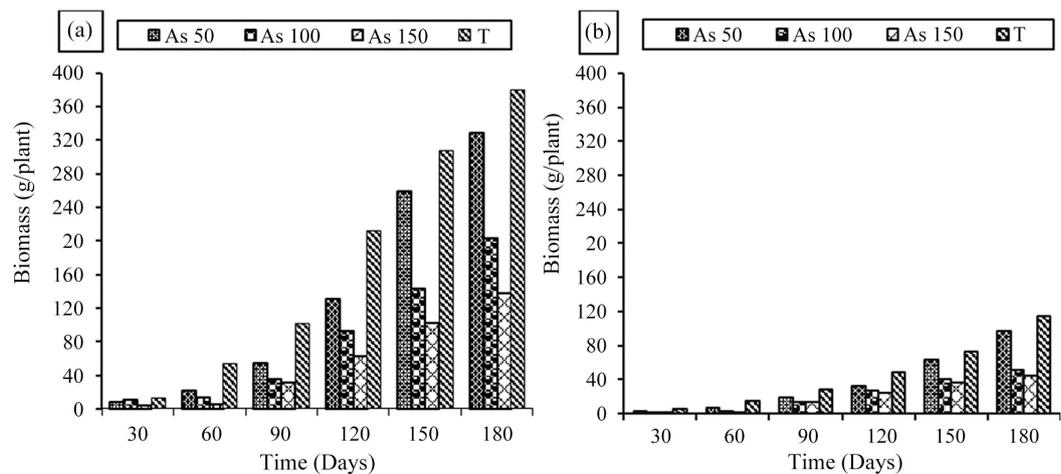


Figure 2. Fresh shoot (a) and root (b) biomasses produced by *Chrysopogon nigritanus* during the experiment; As 50: contaminated pot at 50 mg As/kg; As 100: contaminated pot at 100 mg As/kg; As 150: contaminated pot at 150 mg As/kg; T: control pot.



Figure 3. View of the plant biomass produced by *Chrysopogon nigritanus* at the end of the experiment; As 50: contaminated pot at 50 mg As/kg; As 100: contaminated pot at 100 mg As/kg; As 150: contaminated pot at 150 mg As/kg; T: control pot.

3.3. Arsenic Accumulation Potential of *Chrysopogon nigritanus*

As concentrations recorded in root biomass of *C. nigritanus* was higher than concentrations in shoot (**Table 2**). From day 30 to day 180, As concentrations in shoot biomass ranged from 0.91 - 11.64 mg/kg (As 50), 1.56 - 14.48 mg/kg (As 100) and 6.15 - 17.92 mg/kg (As 150). In the root, concentrations increased from 4.69 - 15.91 mg/kg (As 50), 5.42 - 30.28 mg/kg (As 100) and 10.85 - 41.32 mg/kg (As 150).

Table 3 shows the Bioaccumulation Factor (BF) and Transfer Factor (TF) of arsenic for *C. nigritanus*. BF remained higher in As 50 (0.11 - 0.53) than As 100 (0.07 - 0.40) and As 150 (0.11 - 0.34). FT was less than 1 [0.19 - 0.58 (As 50), 0.28 - 0.41 (As 100) and 0.40 - 0.62 (As 150)]. BF and FT values obtained from control soil and contaminated soil were not different (ANOVA test: $p > 0.05$).

3.4. Localization of Arsenic in the Tissues and Cells of *Chrysopogon nigritanus*

For this study, plant from soil contaminated at 50 mg As/kg was selected, whose removal efficiency and bioaccumulation factor remained the highest during the experiment. **Figure 4** shows the weight of As (%) recorded in the tissues and cells of roots, stems and leaves of *C. nigritanus*. In roots tissues, we noted higher weight of As in the rhizodermis and the pericycle. Furthermore, these weight of As decrease from the rhizodermis to the endodermis, as well as from the pericycle to the central cylinder. In stems tissues, As weights were higher in the conductive bundles than in the epidermis and cortex. In the leaves, arsenic was more retained in the conductive bundles during the first thirty (30) days. How-

ever, from day 60 until day 180, As was strongly accumulated in epidermis tissues. At the cellular, As remains essentially fixed to the intracellular compartment of the roots, stems and leaves.

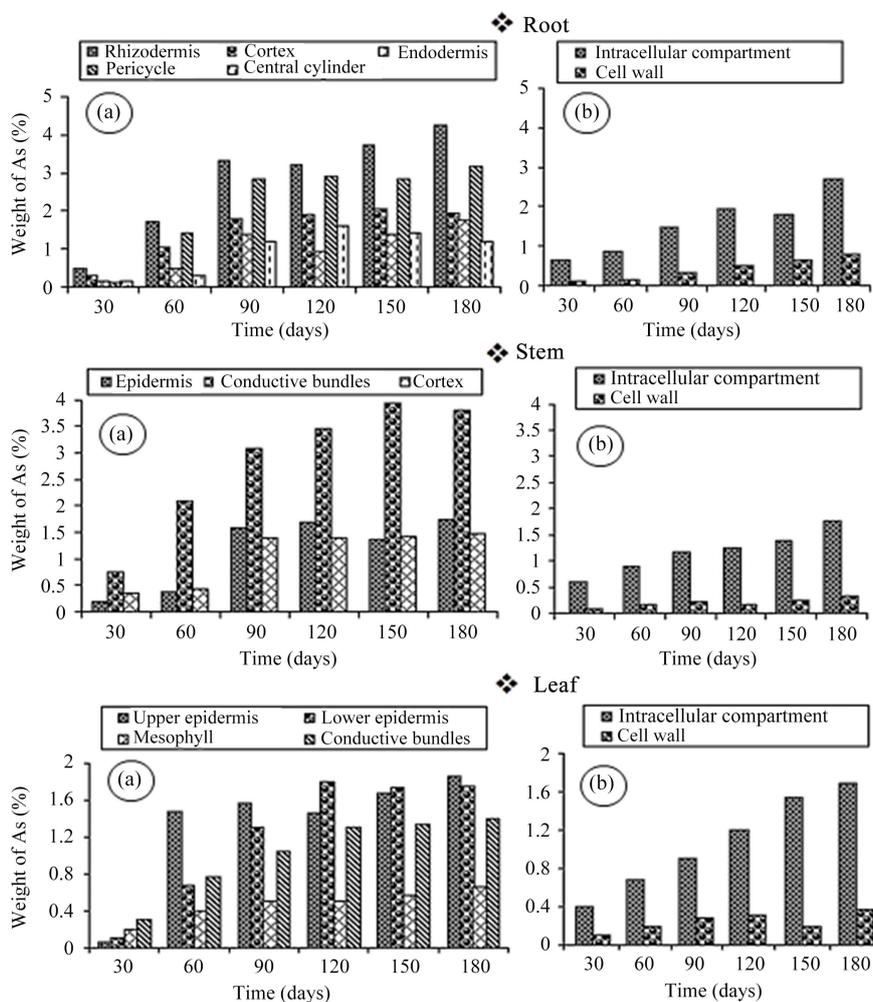


Figure 4. Weight of As (%) recorded in the tissues (a) and cells (b) of *C. nigritanus*.

Table 2. As concentrations (mg/kg) in *Chrysopogon nigritanus*, As 50: contaminated pot at 50 mg As/kg; As 100: contaminated pot at 100 mg As/kg; As 150: contaminated pot at 150 mg As/kg; D30: 30 days; D60: 60 days; D90: 90 days; D120: 120 days; D150: 150 days; D180: 180 days.

		D30	D60	D90	D120	D150	D180
As 50	Shoot	0.91	2.94	4.25	5.51	9.55	11.64
	Root	4.69	6.41	8.17	9.44	12.96	15.91
As 100	Shoot	1.56	4.91	6.89	7.34	10.48	14.48
	Root	5.43	12.86	17.04	20.03	24.10	30.28
As 150	Shoot	6.15	10	11.52	12.83	14.67	17.92
	Root	10.86	16.19	22.95	28.22	31.86	41.32

Table 3. Bioaccumulation Factor (BF) and Transfer Factor (TF) of arsenic; As 50: contaminated pot at 50 mg As/kg; As 100: contaminated pot at 100 mg As/kg; As 150: contaminated pot at 150 mg As/kg; D30: 30 days; D60: 60 days; D90: 90 days; D120: 120 days; D150: 150 days; D180: 180 days.

		D30	D60	D90	D120	D150	D180
BF	As 50	0.12	0.20	0.27	0.32	0.45	0.53
	As 100	0.08	0.19	0.25	0.29	0.34	0.40
	As 150	0.12	0.18	0.24	0.28	0.32	0.34
TF	As 50	0.19	0.46	0.52	0.58	0.58	0.57
	As 100	0.28	0.38	0.40	0.37	0.37	0.35
	As 150	0.57	0.62	0.50	0.40	0.40	0.39

4. Discussion

The potential of *C. nigrifolius* to accumulate arsenic in polluted soil was investigated. The analysis of plant growth parameters (stem length, plant biomass) in pots showed a significant influence of the arsenic dose in the soil on the development of *C. nigrifolius*. In fact, if the plant grew of the dose of As used, the results indicate a significant reduction in stem length and plant biomass in polluted soils at 100 mg/kg and 150 mg/kg in As. Behind *et al.* [26], Tu and Ma [27] and Manirul *et al.* [28] made such observations respectively with *Brassica juncea*, *Pteris vittata L.* and *Oryza sativa* cultivated on soil contaminated with increasing doses of arsenic. This would be due to the phytotoxicity of arsenic at high concentrations in the soil. Various biological and biochemical phenomena resulting from the contact of plants with As may contribute to the inhibition of their growth. These include the disruption of nutrient uptake and disruption of essential physiological processes (photosynthesis, respiration) in plant development, and the replacement of essential ions of adenosine triphosphate (ATP) in plants [29]. However, up to 50 mg As/kg, the growth parameters of *C. nigrifolius* are of the same order as those of the control pot. This result could be explained by the fact that this concentration of 50 mg As/kg is well below the toxicity threshold (100 to 250 mg/kg) indicated by Truong [30].

In the shoot of *C. nigrifolius*, the concentrations of As obtained in the contaminated soils were lower than those determined in the root. In all the contaminated soils, a strong accumulation of As in the roots of plants was observed with transfer factors (FT) less than 1. These results corroborate those of Srisatit *et al.* [31] and Chiu *et al.* [32] who observed that vetiver accumulates arsenic mainly in the roots. Indeed, according to Raab *et al.* [33], with the exception of hyper-accumulative plants, 75% to 90% of the As absorbed by plants is concentrated in the roots. This observation was made on a set of 46 plant species. Furthermore, Zhao *et al.* [34] explain that by the reduction in this organ of arsenate ions to arsenite (thanks to arsenate reductase and the reducing power of glutathione) which are subsequently complexed with phytochelatin and sequestered in root

vacuoles [35]. This would justify the low rate of translocation of As to the aerial parts of plants observed in many plant species [36]. Concerning the bioaccumulation factors of As by *C. nigritanus*, these decreased with the increase in the dose of arsenic applied. This result could be explained by the fact that *C. nigritanus* would limit the accumulation of As when this pollutant is at high doses in the soil, due to its high toxicity.

In view of its superior soil arsenic removal efficiency and bioaccumulation factor compared to other pots, plant in the pot contaminated with 50 mg As/kg was studied for the location of arsenic in *C. nigritanus* tissues and cells. It shows that in roots, As is mainly stored in the rhizodermis and the pericycle. This trend was observed by Chao *et al.* [37] and Shi *et al.* [38] on *Arabidopsis thaliana* and *Oryza sativa* species. These authors attributed that to the presence of the HAC1 (High As content 1) gene in the rhizodermis and pericycle of the root which would favour the sequestration of arsenic in these tissues. At the stem, high presence of arsenic is observed in the conductive bundles. In fact, conductive bundle tissues, consisting of xylem and phloem, favour the translocation of trace elements. Indeed, xylem sap is the main means of transport of mineral ions from the roots to the aerial parts. The circulation of raw sap takes place by root growth and by foliar call during transpiration [39]. Regarding the distribution of arsenic in leaves, strong accumulation in the conductive bundles has been observed during the first thirty (30) days. Subsequently, the As moves into the epidermal cells. According to Lombi *et al.* [40], the sequestration of arsenic in the epidermis of leaves is a mechanism of detoxification of this pollutant in plants. On the whole, it can be seen that arsenic is concentrated mainly in the intracellular compartments of the roots, stems and leaves of *Chrysopogon nigritanus*. As most of this area is occupied by the vacuole, this would suggest that As is mainly contained in the vacuoles [40]. This attests to the role of the vacuole in maintaining cell homeostasis in the presence of an excess of As [41]. Indeed, thanks to protein transporters, arsenic is sequestered in vacuoles [42]. However, while intracellular compartments are the preferred arsenic storage areas, significant fractions of As have been recorded on the cell walls of different organs (stem, leaf, root) of the plant. According to Koch *et al.* [43], As would be bound to cell wall components such as cellulose, pectin and lignin.

5. Conclusion

The study showed the potential accumulation of arsenic by *Chrysopogon nigritanus*. Plant growth, bioaccumulation and transfer factors, as the location of As in tissues and cells of the plant have been determined. The growth of *C. nigritanus* decreases in soil with increasing arsenic concentrations applied. *C. nigritanus* accumulated more arsenic in the roots than in the shoots ($FT < 1$). Bioaccumulation factors decrease with increasing arsenic dose in soil. At the end of the experiment, values of the bioaccumulation factor were 0.53 (As 50), 0.40 (As 100) and 0.34 (As 150). As was essentially fixed to the intracellular compartment

of the roots, stems and leaves. In roots tissues, As was accumulated preferentially rhizodermis and the pericycle while in stems tissues arsenic highly in the conductive bundles. In the leaves, arsenic was more retained in the conductive bundles during the first thirty (30) days. However, from day 60 until day 180, As was strongly accumulated in epidermis tissues.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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