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Potential of *Canna indica* in Vertical Flow Constructed Wetlands for Heavy Metals and Nitrogen Removal from Algiers Refinery Wastewater

Katia Ghezali [1,*], Nourredine Bentahar [1], Narcis Barsan [2,*], Valentin Nedeff [2,3] and Emilian Moșneguțu [2]

[1] Hydrocarbon Technology Laboratory, University Mhamed Bougara of Boumerdes, Avenue of Independence, Boumerdes 35000, Algeria; n.bentahar@univ-boumerdes.dz

[2] Faculty of Engineering, Vasile Alexandri University of Bacau, Calea Marasesti, No. 157, 600115 Bacau, Romania; vnedeff@ub.ro (V.N.); emos@ub.ro (E.M.)

[3] Gheorghe Ionescu Sisesti, Academy of Agricultural and Forestry Sciences, 6 Marasti Blvd., 011464 Bucharest, Romania

* Correspondence: k.ghezali@univ-boumerdes.dz (K.G.); narcis.barsan@ub.ro (N.B.)

Abstract: Constructed wetlands (CWs) are important plant filters used for wastewater treatment. The behavior of the *Canna indica*-planted CWs in the face of a highly variable composition of industrial wastewater has yet to be understood. Here, we show the effectiveness of *Canna indica*-planted and unplanted vertical subsurface flow CWs for the treatment of Algiers petroleum refinery's effluent. The selected species was placed in the CWs containing light expanded clay aggregate (LECA) and sand as a substrate. The findings indicate that the planted constructed wetlands efficiently removed 85% of total suspended solids (TSS), 96.38% of total nitrogen (TN), 96.15% of nitrate nitrogen (NO_3^--N), 99.15% of ammonium nitrogen (NH_4^+-N), and 99.87% of nitrite nitrogen (NO_2^--N). The overall mean removal efficiencies for heavy metals in the vegetated CWs were considerably greater than those of the control. Concentrations of Cr, Cu, Fe, Pb, Zn, Al, Ni, and Cd were calculated in the roots, rhizomes, leaves, and stems of the plant; then, the bioaccumulation factor (BAF) and translocation factor (TF) were determined. An initial examination using scanning electron microscopy (SEM–EDX) was also included in the study. The analysis indicated that toxic elements were adsorbed on plant tissues, concentrated in the roots, and partially transported to the aerial parts. These results are useful for the design of CWs to treat industrial wastewater, enabling water of acceptable quality to be discharged into the environment, especially as a low maintenance and cost-effective technology in developing countries.

Keywords: constructed wetlands; *Canna indica*; industrial wastewater; heavy metals; bioaccumulation; translocation; phytoremediation

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1. Introduction

In recent years, researchers and ecologists have shown an increased interest in wastewater treatment, especially in industrial activities that release pollutants such as metals and organic compounds [1,2]. The presence of these toxic contaminants from industrial discharge in water can reduce crop yield and plant growth, and can also be harmful to aquatic organisms [3,4]. Oil refineries require large quantities of water. The quantity and properties of wastewater produced depend on the process configuration [5]. Petrochemical complexes and refineries generate polluted wastewater containing organic and inorganic matter, particularly benzene, styrene, toluene, naphthalene, xylene, oil and grease (O&G), and metals such as copper (Cu), lead (Pb), cadmium (Cd), chromium (Cr), iron (Fe), zinc (Zn), nickel (Ni), aluminum (Al), barium (Ba), and molybdenum (Mo) [6,7]. However, most conventional wastewater treatment plants are difficult, expensive, and far away from their optimum performance [8].

Constructed wetlands (CWs) have been well tested, to various degrees, as an attractive alternative for different types of industrial wastewater [9,10], which are usually treated by physical, chemical and biological treatment processes. CWs are very effective in the biosorption of heavy metals (HMs) from industrial wastewater [11–13]. Moreover, they can induce aerobic degradation of microorganisms and stimulate nitrifying bacteria [4,14]. Constructed wetlands do not require energy input; thus, their operation and maintenance costs are significantly lower than those of conventional treatment systems [15]. In addition, constructed wetlands allow the recycling of over 60% of influent water for other uses, especially irrigation [16]. Data from several studies suggest that vertical flow constructed wetlands (VFCWs) are suitable for different types of wastewater including refinery effluent [17–20]. Generally, vertical sub-surface flow CWs require intermittent pumping of wastewater, necessitate less land, and are highly efficient in eliminating organics and suspended solids [21,22]. Numerous factors, such as substrate material, macrophyte species, microorganism interactions in constructed wetlands [23,24], hydraulic retention time, and water depth [25] can also affect the remediation process of polluted effluents. One of the materials poorly documented, so far, is light expanded clay aggregate (LECA), a potential pollutant-purifying substrate, according to its reported efficiency of nitrogen adsorption [26] and removal of the emerging organic contaminants [27].

Typha latifolia and *Phragmites australis* are well known hyper-accumulator emergent plants [28] that adapt to different environmental conditions. Similarly, *C. indica* is easy to propagate and grow fast, which makes it a preferred phytoremediation species and can attain high bioconcentration and translocation factors for heavy metals [29–33]. To date, previously published studies have demonstrated the potential of *C. indica* for the removal of many contaminants from domestic wastewater; however, few studies have been performed to examine the potential of its purification on a highly variable composition of industrial wastewater, mainly petroleum industry wastewater. Various substrate materials have been applied in CWs for wastewater treatment [34]. Light expanded clay aggregate (LECA) is a potential pollutant-purifying substrate based on its reported efficiency of nitrogen adsorption [26], and removal of emerging organic contaminants [27]. Therefore, the main objective of this study was to evaluate the effectiveness of combining *Canna indica* macrophyte and LECA substrate for treating wastewater contaminated by petrochemical wastes, using constructed wetlands as a promising, cost-effective, and eco-friendly technology.

In this study, the performance of vertical flow CWs, planted with *C. indica* and fixed onto LECA + sand substrate, on the removal of COD, TSS, TN, NO_3^-, NH_4^+, NO_2^-, TOC, TP, turbidity, and heavy metals (Cu, Ni, Pb, Zn, Al, Cr, Fe, and Cd) from refinery wastewater was assessed and compared to a system without vegetation. Heavy metals accumulation and translocation abilities in plant parts were also determined.

2. Materials and Methods

2.1. Experimental Setup

Cylindrical black plastic containers of 38 cm in height and 30 cm in diameter were used to build vertical flow constructed wetlands. To facilitate sampling and drainage operations, valves were placed at the center of the lower part of each wetland unit, and the bottom drains installed inside the large gravel layer ended to these outlets so as to completely collect the treated wastewater from the bottom of the constructed wetland. A perforated Polyvinyl Chloride (PVC) pipe was placed vertically to encourage rapid air penetration through the wetland bed and promote the activity of aerobic bacteria. The substrate was composed, from bottom to top, of 5 cm stones + 20 cm of light expanded clay aggregate (LECA) and 16–25 mm + 20 cm of coarse sand. Planting was carried out in spring 2021; two of the VFCWs were planted with *C. indica* obtained from a local supplier and one bed (unplanted) was used as a control. The systems were then placed in an air-conditioned greenhouse (Figure 1). The average temperature was maintained between 25–33 °C. Four plant shoots with roots and rhizomes were planted directly in the LECA and sand substrate. After transplantation, the CWs were fed with clean water during the one-month acclimatization period. To optimize biomass growth, N, P, and K liquid fertilizer amendments (Omex, King's Lynn, UK) were applied after transplantation. The microcosm wetlands received polluted refinery wastewater for three months.

The feeding strategy consisted of batch flow mode at the bed surface with hydraulic retention time (HRT) of 3 days and operational flow rate of 0.025 m^3/day. The wastewater inundated the entire surface of the wetland, percolated through the LECA and sand medium, and was retained in the bed, before being completely drained by gravity.

Figure 1. Vertical flow constructed wetland with growing *Canna indica* plants.

2.2. Sampling and Analysis

Samples of primary-treated refinery wastewater were taken from Algiers Refinery and collected into labelled polyethylene containers. The influent, control and VFCWs effluent liquid samples from the wetland units were collected twice per week (day 3 and 5) to determine the treatment effectiveness of the wetlands in reducing the levels of total suspended solids (TSS), chemical oxygen demand (COD), total organic carbon (TOC), total phosphorus (TP), total nitrogen (TN), nitrate-N (NO_3^--N), ammonium-N (NH_4^+-N), and nitrite-N (NO_2^--N). The pH and turbidity were also analyzed.

A UV–Vis spectrophotometer (HACH DR3900, USA) was used to determine COD, TOC, nitrogen compounds and TP. pH was measured using a WTW inolab-IDS Multi 9420 (Weilheim, Germany), and turbidity using a turbidimeter (WTW TURB 430 IR, Weilheim, Germany) instrument. The TSS was estimated using the photometric method. Concentrations of Pb, Cr, Fe, Cd, Al, Cu, Ni, and Zn in influent and effluent samples from all wetland units were measured after acidification with 2.5 mL of nitric acid (HNO_3). The plant samples were harvested, washed, and then separated into leaves, stems, rhizomes and roots. Scanning electron microscopy (SEM) was used to conduct morphological characterization and elemental analysis on a MIRA II LMU (TESCAN s.r.o., Brno, Czech Republic). Before analysis, subsamples of the plant parts were dried and coated with carbon. To assess the phytoextraction capacity of *C. indica*, roots, leaves, stems and rhizomes were dried in an oven to a consistent weight at 70 °C [12]. Dried tissues were weighed and powdered, and then 0.5 g of dry samples were extracted by acid digestion using 10 mL of concentrated HNO_3 at 100 °C, as described by [35]. After cooling and filtration, the solutions were diluted with deionized water to 50 mL, followed by analysis of all the metals of interest using ICP-MS (Agilent Technologies 7500 series, Weilheim, Germany).

2.3. Calculations

The removal efficiency of the parameters (COD, TSS, TN, NH4$^+$-N, NO_3^--N and TOC) from the refinery wastewater and CWs effluent were calculated using the following equation:

$$\text{Removal } (\%) = \frac{C_{inf} - C_{eff}}{C_{inf}} * 100 \quad (1)$$

where $C_{\mathbf{inf}}$: refers to concentrations of contaminants in the wetland influent.

$C_{\mathbf{eff}}$: refers to pollutant concentrations in the outflow of *C. indica* and control VFCWs.

The bioaccumulation factor (BAF) of HMs in the *C. indica* plant was calculated as follows:

$$\text{BAF} = \frac{\text{Metal content in plants}}{\text{Metal concentration in the influent}} \quad (2)$$

where the metal concentrations in the influent were measured in mg/L and the metal content in the plant was measured in mg/kg dry weight.

The translocation factor (TF) is the quotient of the heavy metal concentration in the plant's aerial parts (stem and leaves) to its concentration in the belowground parts (roots and rhizomes), and was determined as follows:

$$\text{TF} = \frac{C_{\text{aerial part}}}{C_{\text{belowground part}}} * 100 \quad (3)$$

where $C_{\text{aerial part}}$ is the metal concentration in the aerial parts of the plant (mg/kg) while $C_{\text{belowground part}}$ is the metal concentration in the belowground parts of the plant (mg/kg).

3. Results and Discussion

3.1. Treatment Performance of Canna indica CWs

Table 1 summarizes the mean values of the physicochemical characteristics of the influent (settled Algiers refinery wastewater) and treated wastewater released by the VFCWs planted with *C. indica*. Results were compared with the effluent discharge guidelines provided by the World Bank Group (WBG) [36,37]. The pH of all the samples ranged between 6.6 and 7.5. Turbidity values were reduced from 52.26 in the influent to 5.47 NTU in the CWs experiment, and 8.56 NTU in the control. The influent COD was high, reflecting the characteristics of the industrial wastewater. The mean influent COD concentration was 330 mg/L. The effluents of the *C. indica*-planted wetlands varied between 81.8 mg/L and 89.8 mg/L; COD levels were decreased to below the 125 mg/L guideline fixed by WBG. However, the effluent mean COD concentration was slightly higher than that of the unplanted (68.5 mg/L). This could be explained by sufficient oxygen diffusion into the wetland cells in accordance with the wetland design, and thus aerobic degradation [17]. Moreover, the COD treatment performance of the LECA and sand system showed good effects due to the clay composition and porosity of the material, involving a high biological treatment. For the water quality parameters TSS, COD, TN, Nitrates-N, Ammonium-N and TOC, the removal efficiencies, considering the control and CW effluents, were calculated and are shown in Figure 2. The concentration of TSS in the outlet of the CWs was much lower than that in the control and influent, with an average removal rate of approximately 85%, due to sedimentation, filtration, bacterial decomposition and adsorption to the CW medium. Treatment of TOC in the unplanted control had a higher purifying efficiency (78.63%) than the planted CW (17.9%), owing to the release of Dissolved Organic Carbon (DOC) by submerged macrophytes associated with the process of photosynthesis [38]. Interestingly, the *C. indica* vertical flow CW effluent had a higher total phosphorus content than the unplanted control and influent. These results could be attributed to fertilizer addition during the acclimatization period, because the growth of the Canna vegetation and biomass production was observed in the constructed wetlands. Despite these unexpected proportions, the average TP values were below the threshold limit throughout the experimental period. These results are in agreement with [39] findings, observed for secondary refinery effluent in the researchers' study of the removal of petroleum contaminants in vertical subsurface flow planted with Typha latifolia, particularly with COD and TSS that had mean range removal efficiencies of 52–83% and 52–78%, compared to unplanted controls of 24–66 mg/L and 20–55 mg/L, respectively.

Table 1. Mean influent, effluent and control concentrations of the vertical CWs treating Algiers refinery wastewater.

Parameter	Influent	Canna VFCWs Effluent	Control	WBG *
pH	7.3 ± 0.27	6.68 ± 0.07	6.79 ± 0.2	6.0–9.0
Turbidity (NTU)	52.26 ± 28	5.47 ± 1.32	8.56 ± 3.3	30
COD (mg/L)	330 ± 4.24	85.8 ± 0.21	68.5 ± 0.42	125
TSS (mg/L)	253.3 ± 0.7	39.9 ± 0.42	173.3 ± 0.7	25
TOC (mg/L)	391 ± 6.36	321 ± 4.94	83.55 ± 2.65	/
TP (mg/L)	0.98 ± 0.4	1.5 ± 0.7	0.88 ± 0.4	2
TN (mg/L)	18.8 ± 0.14	0.68 ± 0.02	17.5 ± 0.28	10
Nitrates (mg/L)	7.28 ± 0.04	0.28 ± 0.01	7.04 ± 0.05	/
Ammonium (mg/L)	9.5 ± 0.14	0.08 ± 0.01	0.472 ± 0.01	/
Nitrite (mg/L)	1.18 ± 0.72	0.0015 ± 0.001	1.1 ± 0.17	/
Chrome total (mg/L)	2.33 ± 0.03	0.314 ± 0.004	2.27 ± 0.05	0.5
Copper (mg/L)	0.86 ± 0.03	0.079 ± 0.003	0.59 ± 0.05	0.5
Iron (mg/L)	2.28 ± 0.06	0.8 ± 0.08	1.3 ± 0.03	3
Lead (mg/L)	2.06 ± 0.03	0.48 ± 0.05	1.56 ± 0.11	0.1
Zinc (mg/L)	7.56 ± 0.09	0.26 ± 0.07	6.27 ± 0.02	/
Aluminum (mg/L)	1.92 ± 0.03	0.53 ± 0.01	1.33 ± 0.05	/
Nickel (mg/L)	1.03 ± 0.05	0.46 ± 0.007	1 ± 0.08	0.5
Cadmium (mg/L)	0.031 ± 0.003	0.002 ± 0.005	0.021 ± 0.003	/

* WBG: World Bank Group, Liquid Effluent Levels for Petroleum Refining Facilities.

Total nitrogen (TN) concentrations in the influent reached an average of 18.8 mg/L, and reduced to 0.68 mg/L in the effluent; as a result, the *C. indica* VFCWs were able to achieve high removal rates of nitrogen components (ammonium, nitrite, and nitrate). Indeed, the vegetated wetlands eliminated nearly 96% of NO_3^-, but the control wetland removed only 3.3%. Similarly, NO_2^- also decreased by 99% in the CW effluent compared with 6.8% in the control experiment. Meanwhile, 95% and 99% of the ammonium nitrogen disappeared from the control and Canna wetlands, respectively. These values were similar to those reported by [29] on their study of the removal of nutrients from wastewater by *C. indica* under different vertical flow constructed wetland conditions, where, under varied hydraulic loading rates, substrates, and vegetation planting methods, a longer operational period had the maximum removal rate for nutrients in the VFCWs. The roots of *C. indica* had a high sorption capacity for nitrogen nutrients, and the substrate material showed good adsorption of ammonium ions.

According to the results, *C. indica* appears to play an essential role in wastewater treatment. The removal of various organic substances and nutrients in treatment wetlands is thought to be the result of several mechanisms, such as sedimentation, direct plant uptake, NH_3 volatilization and degradation [40]. Moreover, drainage and ventilation pipes played a vital role in oxygen diffusion into the substrate while promoting mineralization and nitrification, an essential step in nitrogen removal.

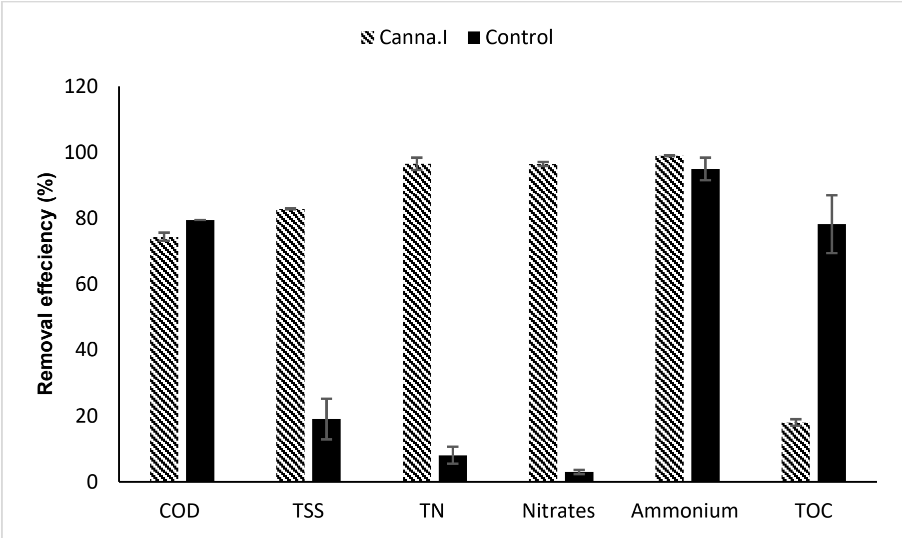

Figure 2. COD, TSS, TN, Nitrates, Ammonium, and TOC removal efficiency by *C. indica* VFCWs against unplanted control.

3.2. Heavy Metals Removal

The concentrations of HMs in influent, control, and effluent samples obtained from vertical constructed wetlands are listed in Table 1. Cr, Cu, Fe, Pb, Zn, Al, Ni, and Cd contents in effluent samples collected from the *C. indica*-planted CWs were lower than those in the control effluent. The mean influent Cr concentration was 2.33 mg/L, whereas the mean effluent Cr values for the *C. indica* and the unvegetated control vertical flow CWs were 0.314 mg/L and 2.27 mg/L, respectively. Ref. [41], in their studies, also found that the planted systems were more efficient than the unplanted systems. Overall, the metal concentrations in the treated effluents of the planted constructed wetlands were within the allowable WBG levels. The removal efficiencies of metals (Cr, Cu, Fe, Pb, Zn, Al, Ni, and Cd) from refinery wastewater, by both the *C. indica*-planted and unplanted control VFCWs, are shown in Figure 3. The plant showed substantial uptake of the heavy metals investigated. The metal removal order in the *C. indica* vertical CWs was Zn > Cd > Cu > Cr > Pb > Al > Fe > Ni.

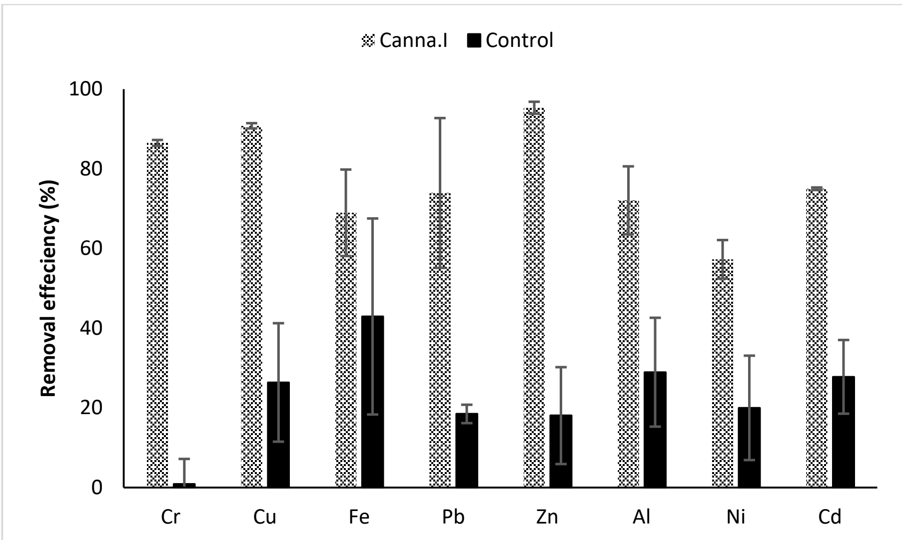

Figure 3. Heavy metals removal efficiency of *C. indica*-planted vertical CWs from refinery wastewater.

A comparison of the eight studied metals revealed that Zn had the highest removal efficiency (96.5%), followed by Cd (93.5%). The results agreed with the findings of [31], where Zn showed the highest removal in planted CWs (93.0% removal efficiency). In contrast, iron and nickel were the least removed metals, as confirmed by [42], for iron, and [43], for nickel. Ref. [44] reported that the presence of higher than toxic metal concentrations in some species suggests that, in addition to their exclusion methods, these wetland plants may have internal metal-detoxifying tolerance mechanisms. This can be attributed to the different type of wastewater, the heavy metal influent concentrations used, the CW design, or the macrophytes used [42].

3.3. Concentration of HMs in Canna indica Tissues

Table 2 presents the metal contents in *C. indica* tissues after remediating the Algiers refinery wastewater. The estimated concentrations of heavy metals in the various plant segments ranged from 10.5 to 710 mg/kg, 4.3 to 565 mg/kg, 4.8 to 475 mg/kg, and 10.5 to 600 mg/kg in roots, rhizomes, leaves, and stems, respectively. The roots retained the highest content of heavy metals, and the lowest was found in the leaves.

Table 2. Heavy metals concentrations in plant parts (mg/kg).

Element	Concentrations [mg/kg]			
	Roots	Rhizomes	Stems	Leaves
Al	102	29	36	26
Cr	450	615	250	70
Ni	131	29	23	24
Fe	645	480	170	46.5
Cu	520	325	290	240
Zn	710	565	600	475
Cd	10.5	4.3	10.5	4.8
Pb	222	36	51.5	61.5

3.4. Plant Potential for Bioaccumulation and Translocation of Metals

Figure 4a shows the bioaccumulation of metals for *C. indica* (grown in the experimental CWs), analyzed in the underground parts (roots and rhizomes) and compared with the values in the aboveground parts (stem and leaves). In most cases, the heavy metals content in the belowground biomass (mainly in root tissues) is usually higher than that of the aerial parts, with large variations observed for Fe, Cu, Cr, and Ni. The highest metal accumulation in Canna tissues was observed for Cu, which had the highest bioaccumulation factor, followed by Cd, whereas the lowest BAF was observed for Al. This may be because the accumulation depended on the physiochemical properties of HMs: Cu has higher relative binding strengths and accumulates more than other HMs, particularly when the absorption binding sites are limited [45]. The Cu, Cd, and Cr BAF in Canna roots were $\geq$300 times greater than the supplied influent, and 50–190 times the concentrations of the other HMs tested. The high accumulation of HMs in *C. indica* might be related to the design and operation mode of the present VFCWs. The aerobic environment created in RSHCWs could also promote the formation of oxides, hydroxides, and oxyhydroxides, which enable HM removal via complexation, as proposed by [32]. Metal ions can be retained in plant roots through other processes, such as adsorption. Metals can be mobilized from the aqueous phase in soluble or colloidal form, and then transported to the plant through the root membrane, involving circulation within the plant towards the shoots, thus increasing uptake of aboveground tissues. The degree and amount of metal translocation in the plant species is influenced by the metal type and the plant type. Ref. [46] reported that the quantity of heavy metals entering the CWs and their accumulation in the aboveground biomass are unrelated. This might be due to heavy metals settling in the anaerobic filter bed, rendering them inaccessible for plant absorption.

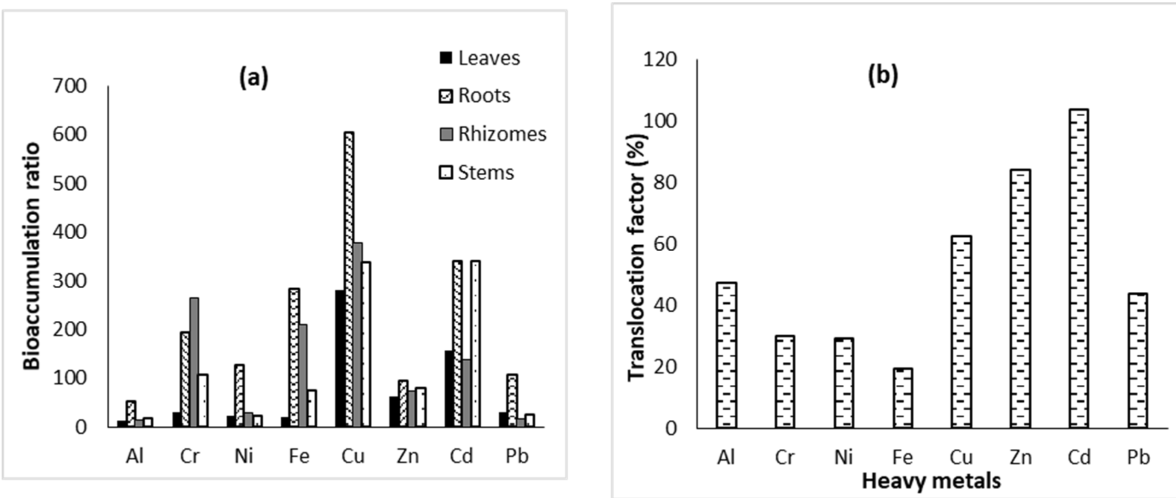

Figure 4. Variation in bioaccumulation factor (**a**) and translocation factor (**b**) of heavy metals in *Canna indica* tissues.

The translocation capacity of *C. indica* followed the order Cd > Zn > Cu > Al ≥ Pb > Cr > Ni > Fe, varying from 19.24 to 103.6% (Figure 4b). The data presented indicate that metals accumulated by the studied species were mostly retained in the root tissues, as shown by the general TF < 1. Thus, its exclusion tolerance makes this plant suitable for phytoimmobilization [47]. However, the Cd concentration in the aerial parts of *C. indica* was higher than that of the belowground parts, indicating the phytoextraction of Cd and good mobility from the roots to the shoots, corresponding to TF > 1. Zn was also easily taken up by the roots and transported to the shoots with a TF close to unity. Accumulation and translocation behaviors are characterized by high variability in most research on heavy metals removal by constructed wetlands for wastewater treatment.

3.5. SEM-EDX Results

The surface morphologies of various plant structures were studied using scanning electron microscopy. EDX analysis provides insight into the multiple mineral components of the plant to define the different processes of retention. This study is interested in determining the eventual location of the contaminants in the plant, and provides data on the bonds between the essential elements that will aid in understanding the mechanisms which affect the absorption of metals through the roots, and migration to the plant's upper sections.

Figure 5 reveals the four parts of *C. indica* tissues, at different levels of magnification. Image R-1 shows that the canna roots are composed of longitudinal and elongated fibers that make the surface porous, providing multiple sites for the adsorption process. When the magnification factor increased from 800× to 5000× (R-2), the internal areas of the fibrous root were distinguishable from the root hairs, and a hollow appearance emerged from the thickest roots. Microanalysis carried out in the root area (Table 3, R-a, R-b) indicated the presence of several elements, where high concentrations of Cd, Cr, Ca, Pb, P, Na, and Cl were detected. The highest iron and phosphorus content is observed in this tissue part.

The observations made on the rhizome show a high roughness, with irregularly shaped hollow cavities at 500× (Z-1), and a microphotograph at greater magnification 900× (Z-2). The observation points located on the tangled section of the rhizome correspond to the columns (Z-a and Z-b) in Table 3, showing significant quantities of Cl, S, Mg, and Co.

A fragment of a *C. indica* stem observed at 370× (S-1), and a detail at 920× (S-2), correspond to a fibrous and rough area, with linear structures forming longitudinal tissues, which coincide with a high concentration of Cr, Co, Cu, and F (Table 4, S-a and S-b). The zinc and copper contents reached significant values in the first section, as well as Cl. These values are consistent with the translocation factor calculated above.

The L-1 image shows an overall view of the leaf section at a magnification of 500×. The image highlights numerous rectangular cells that form a longitudinal lattice, and the holes are clearly differentiated in an enlarged area (L-2). Points L-a and L-b in Table 4 are characterized by high amounts of essential elements, such as Na and K. The results reveal that the leaves represent the lowest amount of elements, and hence, low intracellular translocation.

In general, a metal's accumulation is determined by its absorption capacity and intracellular binding sites. Tissue and cell specific differences, as well as intercellular transfer, complicate the situation. Mobilization and absorption from the medium, compartmentation and sequestration within the root, loading and transit efficiency, distribution between metal sinks, sequestration, and storage in aerial parts cells are all thought to influence metal accumulation rates in plants. A metal must be captured by root cells. Metals are initially bound by the cell wall, which is a low affinity and low selectivity ion exchanger. The uptake is subsequently mediated and driven across the plasma membrane by transport mechanisms and intracellular high-affinity binding sites [48]. The membrane potential, which is negative on the interior of the plasma membrane in root epidermal cells [49], is a powerful driving factor for heavy metal uptake. The retained elements on root surfaces subsequently translocated into stems by crossing the membrane of root cells and sequestered in leaf cell vacuoles.

Table 3. Results of quantitative analysis in roots and rhizomes (Weight %).

Element	R-a	R-b	Z-a	Z-b
Aluminum	0.28	0.22	0.01	0.01
Arsenic	0.01	0.01	0.01	0.01
Cadmium	0.49	0.01	0.05	0.08
Calcium	2.09	4.02	0.75	1.23
Carbon	58.47	53.14	56.09	58.12
Chlorine	1.76	1.41	0.95	2.03
Chromium	0.02	0.22	0.06	0.03
Cobalt	nd	nd	0.01	0.22
Cooper	0.12	0.4	0.12	0.07
Fluorine	nd	nd	0.38	nd
Iron	1.94	1.75	0.05	0.31
Lead	0.01	0.27	0.01	0.01
Magnesium	1.63	0.73	2.97	0.78
Mercury	nd	0.2	0.01	0.01
Nickel	0.04	0.17	0.07	0.04
Nitrogen	5.76	6.89	nd	3.66
Oxygen	22.36	26.39	29.74	28.49
Phosphorus	0.2	0.58	0.15	0.16
Potassium	0.46	0.33	0.76	0.33
Silicon	0.53	0.5	0.17	0.15
Sodium	3.47	2.16	6.59	4.08
Sulphur	0.35	0.25	1.02	0.17
Vanadium	0.01	0.14	0.03	0.01
Zinc	0.01	0.22	0.01	0.01

nd: not detected.

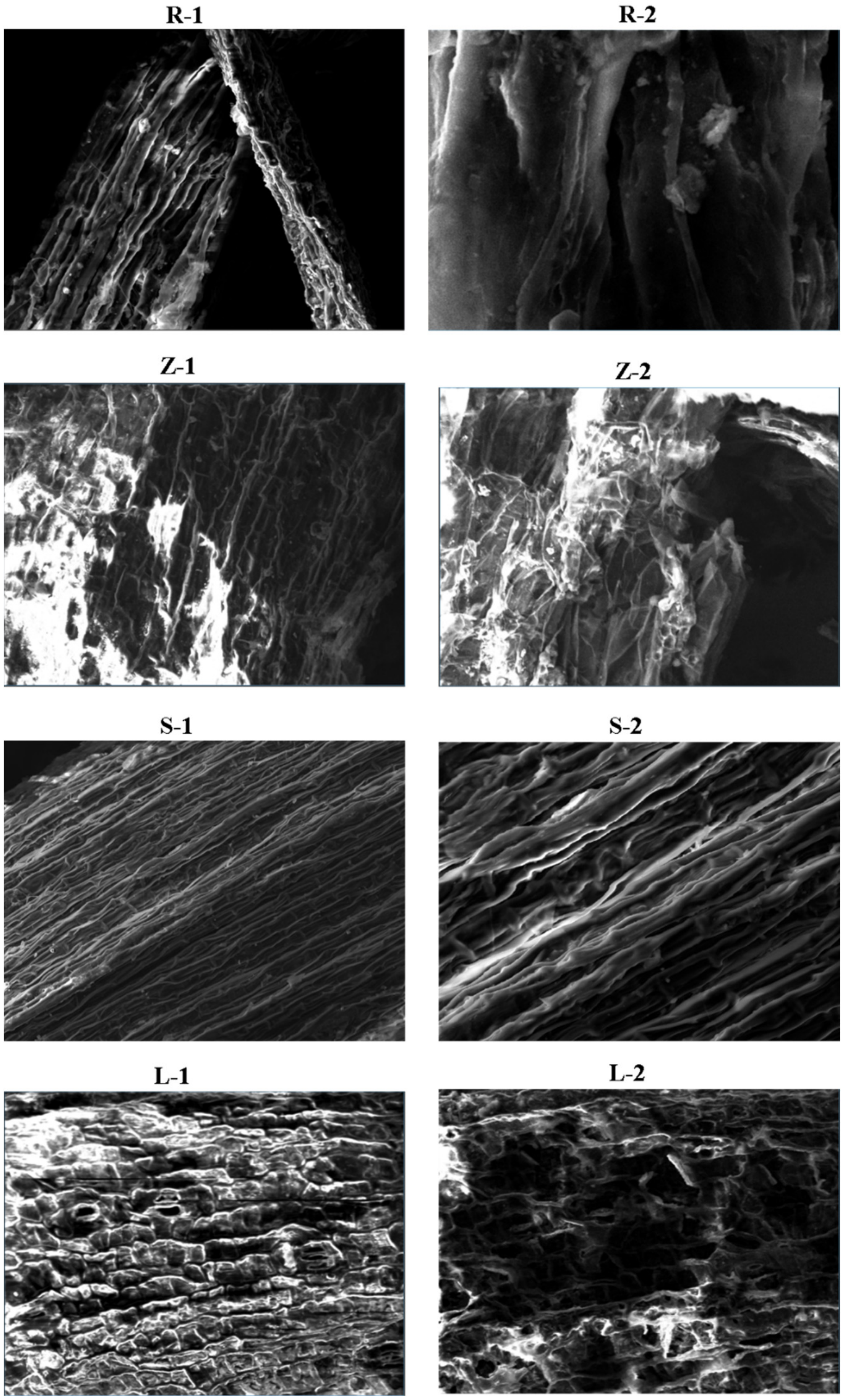

Figure 5. Images of the scanning electron microscopy (SEM) analyses of *Canna indica* root (R-1, R-2), rhizome (Z-1, Z-2), stem (S-1, S-2) and leaf (L-1, L-2).

Table 4. Results of quantitative analysis in stems and leaves (Weight %).

Element	S-a	S-b	L-a	L-b
Aluminum	0.28	0.23	0.11	0.08
Arsenic	0.45	0.12	0.01	0.01
Cadmium	0.01	0.01	0.01	0.01
Calcium	nd	0.37	nd	nd
Carbon	64.8	65.14	68.33	66.92
Chlorine	2.43	2.74	0.76	0.58
Chromium	0.31	0.21	0.01	0.01
Cobalt	0.32	0.24	0.05	0.14
Copper	0.56	0.28	0.01	0.01
Fluorine	0.12	0.33	0.89	1.01
Iron	0.43	0.28	0.01	0.01
Lead	0.01	0.01	0.01	0.01
Magnesium	0.01	0.01	0.18	0.15
Mercury	0.11	0.01	0.01	0.01
Nickel	nd	0.17	0.01	0.01
Nitrogen	0.27	3.67	4.55	4.56
Oxygen	24.08	22.88	22.21	24.16
Phosphorus	0.01	0.01	0.13	0.06
Potassium	0.77	0.98	1.16	0.82
Silicon	0.01	0.01	0.11	0.07
Sodium	1.05	1.92	1.43	1.36
Sulphur	0.01	0.01	0.02	0.01
Vanadium	0.15	0.13	0.01	0.01
Zinc	0.6	0.23	0.01	0.01

nd: not detected.

4. Conclusions

This project was undertaken in order to design an experimental system of intermittently flooded vertical flow CWs planted with *C. indica* in the presence of sufficient oxygen, and evaluate the ability of CWs to eliminate organic contaminants, nutrients, and heavy metals from primary refinery wastewater. The results of this investigation show that the *C. indica*-planted CWs reduced heavy metals and nutrients more effectively than the organic contaminants in the CW effluent. The most obvious finding from this study is that the remedial capacity for total nitrogen by the tested CWs was very high, with a removal rate higher than 96%. Fertilizer application enhanced plant growth, but simultaneously increased the phosphorus content that was maintained in the rhizosphere. Therefore, it appears that the CWs removed nitrogen and its derivatives more efficiently than TP. On the other hand, heavy metals were absorbed by the plant roots, rhizomes, and stems, with the roots delivering the finest results. Following binding to electrostatic exchange sites, *C. indica* was efficient in the accumulation of heavy metals in its tissues, demonstrating a high tolerance towards the toxicity of heavy metals. These findings suggest that, in general, metal accumulation and the rate of translocation depend on the metal type and tissue bodies. The main strength of this study is that *C. indica* is a good candidate plant species for removing nitrogen compounds and heavy metals that can be used in constructed wetlands for the treatment of refineries and/or other industrial wastewaters, especially as a cost-effective and low maintenance eco-technology in developing countries.

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