Phytoremediation Potentiality of *Cyperus articulatus* L.

Hussein F. Farrag[1&2] and Manal Fawzy[3]

[1]Botany Department, Faculty of Science, Cairo University, Giza, Egypt.
[2]Biology Department, Faculty of Science, Taif University, Taif, KSA.
[3]Environmental Sciences Department, Faculty of Science, Alexandria University
hfarrag2012@hotmail.com

Abstract: The phytoremediation potentiality of *Cyperus articulatus* naturally growing in industrial wastewater was tested. Nine heavy metals were selected for this purpose; As, Cd, Cr, Cu, Fe, Hg, Mn, Ni and Pb. Metal concentrations were measured using the atomic absorption. The accumulation rates of the studied heavy metals showed considerable variations in the accumulation abilities according to the plant organ and type of heavy metals. Accumulation of iron recorded maximum values ranged between (105.5 and 900 µg/g d.wt.) in different plant organs of the studied species growing in wastewater, while minimum values were obtained for the accumulation of cadmium (0.9 to 1.95 µg/g d.wt.). In general the studied heavy metal accumulation can be arranged as follows: Fe > Cr > Cu > As > Mn > Pb > Hg > Ni > Cd. The "translocation factor" (TF) and "bioconcentration factor" (BCF) were calculated. TF was found to be less than 1 for all metal cases, which confirmed the significance of below-ground biomass as a heavy metal accumulator. The HPLC technique was used to prove and assess the effect of heavy metal accumulation in the different plant organs on the chemical components especially in the bellow-ground parts. The obtained results support the idea of using the study plant for phytoremediation in industrial wastewater.
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1. Introduction

Water pollution with heavy metals is one of the major hazards facing our environment and causes most serious environment problems throughout the world (Sundaramoorthy *et al*., 2010). The high concentration of dissolved heavy metals in drinking water may damage nerves, liver, bones, block functional groups of vital enzymes and are possible human carcinogens (Zhang *et al.*, 2012). Various industrial processes result in the generation of metal containing waste streams (Yadav *et al.*, 2012). The effluent discharging industries are distilleries, sugar mills, pulp and paper mills, detergent, chemical factories, textile dyeing industries, tanneries, electroplating, pharmaceuticals and dairy industries (Sundaramoorthy *et al*., 2010).

The removal of toxic heavy metals from industrial wastewater using conventional physico-chemical approaches such as adsorption (Dana and Sayari, 2012), oxidation - reduction and chemical precipitation, evaporation and membrane processes (Zhang *et al*., 2011). However, these techniques have certain disadvantages such as incomplete metal removal, high reagent and energy requirements and generation of toxic sludge that require disposal (Sekhar *et al*., 2003). In recent years, macrophyte-based wastewater treatment systems have several potential advantages compared to conventional treatment systems (Hafeznezami *et al.*, 2012). Phytoremediation is one of the best ecotechnologies in which the combined use of plants, soil amendments and agronomic practices are employed in order to remove the pollutants from the environment and decrease their toxicity (Salt *et al.,* 1998). The use of phytoremediators has been advocated because of rapid growth rates, achieving high levels of nutrient removal (Rahman and Hasegawa, 2011). Hyperaccumulators can tolerate, take up and translocate high levels of certain metals that would be toxic to most organisms. Many studies have been proposed for the use of phytoremediators to successfully improve the quality of contaminated waters and wastewaters for at least two decades (Zhang *et al*., 2010).

Moreover, proper selection of plant species for phytoremediation plays an important role in the development of remediation methods (Salt *et al.*, 1995). The search for indigenous plants, often better in terms of survival, growth and reproduction under metal-stressful field conditions may be an adequate approach to find plant species with metal resistance capabilities and even with the capacity to accumulate heavy metals at very high levels (Yoon *et al.*, 2006). Although there has been a continuing interest in this area, few studies have contributed to the evaluation of the phytoremediation potentiality of native species and the ability of these species to accumulate heavy metals in their belowground parts (roots) and the effect of these heavy metals on the chemical structure of different plant organs as compared to plants naturally growing in non polluted areas.

The aim of this study was to determine the accumulation of nine heavy metals, namely As, Cd, Cr, Cu, Fe, Hg, Mn, Ni and Pb in different plant organs of the native species *Cyperus articulatus*, naturally growing in industrial wastewater site and to relate that feature to the heavy metal content of the soil sediments and water. The present work evaluated the effect of heavy metal accumulation on the chemical structure of different plant organs using HPLC technique. The current study illustrated the potentiality of using *C. articulatus* as a phytoremediator.

2. Materials and Methods

2.1. Plant samples and soil sediment preparations

Biomass of *C. articulatus* samples were collected from the industrial wastewater pond at Sadat City, located about 75 km west of Cairo, Egypt. Soil sediment samples collected from the same pond at 20cm depth. As a control, plant samples were taken from a nearby fresh water canal. The plant samples washed several times by deionized water to remove extraneous and salts then separated to individual organs of inflorescences, culms, rhizomes and roots. Plant samples then dried in an oven at 50 ^{o}C for 48 hrs, chopped and sieved. The particles with an average 0.5 mm were used for obtaining powdered plant material and then ready for measuring heavy metal concentrations.

2.2. Atomic Absorption and heavy metal analysis

One milliliter of sulphuric acid and 15 ml of double distilled water were added to a Kjeldahl flask containing 0.5 g of dried and powdered material was incubated overnight at 80 ^{o}C. After that, 5 ml of acid mixture (nitric acid and perchloric acid in the ratio of 3:1) was added and then digested. The digested material was cooled, made up to 50 ml and filtered through Whatmann No. 42 filter paper. The sample was aspirated to an Atomic Absorption Spectrophotometer with air/acetylene flame and the readings were taken for each heavy metal separately according to suitable wave length as described in APHA (1998) by using atomic absorption spectrophotometer (Perkin Elmer, Model No 200, USA). This analysis was carried out in the Environmental Sciences Department, Faculty of Science, Alexandria University, Egypt. The "translocation factor" (TF) for metals within the studied species was calculated. TF is expressed by the ratio of metal concentration in the above and below-ground biomass. Similarly, the "bioconcentration factor" (BCF) was calculated, which is the ratio of metal concentration in the root to soil sediments (Rezaei and Rezaei, 2006).

2.3. HPLC analysis

Exact 1gram of oil sample was taken from each organ of *C. articulatus* samples and socked separately in 50% acetonitrile (10 ml). After vortex the samples were filtered in micro filter (45 µm). Samples were examined using a HPLC system (Hp 1050) with a UV detector at 220 nm. The separation was accomplished with a ODS, C18 (5 µm. 4 x 250 mm) column. The mobile phase consisted of eluent acetonitrile / water (70/30 v/v) to (95/5 v/v) through 5 min. The flow rate was 1 ml /min. The column temperature was 27 ^{o}C with an injection volume of 10 µl. This analysis was carried out in the Biotechnology Unit of Plant Pathology Institute, Agriculture Research Center, Egypt.

2.4. Analysis of data

Data were analyzed by ANOVA test to determine the significant differences among the mean values at the $P< 0.05$ probability level using a "general linear model" procedure of the Statistical Analysis System (SAS) program (SAS Institute, 1985).

Figure 1. *Cyperus articulatus* after Keith Bradley. (Photo downloaded from: http://www.flickr.com/photos/35605280@N05/6348976834/in/photostream/)

3. Study plant

C.articulatus (Cyperaceae) is a tropical, perennial, rhizomatous, helophytic hydrophyte (Gordon-Gray *et al*., 2006). It is a medicinal plant used to treat many diseases (headaches, migraine, epilepsy, etc.) in some African countries and in Amazonia (Adjanohoun *et al*., 1984; Ngo Bum *et al*., 1996). *C.articulatus* often occurs in almost pure stands in tropical and warm temperate localities that provide permanent water. It is distinguished by its robust, leafless culms up to 1600 x 8 mm that are septate-noded and solid-pithed. In life the nodes are not always clearly defined externally but may be detected by gentle finger pressure or longitudinal sectioning. On drying, with shrinkage pith, the septa stand out markedly. Bracts of the brown, branched, terminal inflorescences are short (5-9 mm), stiff and sharp and never exceed primary ray length. The subterranean,

curved stoloniferous rhizomes are scale-covered when young and terminate, at intervals, in erect, emergent culms. Corm development at the rhizome joints is not usually evident apart from occasional slight enlargements and we found no reference to corm production in the literature. In areas with seasonal climate, fluctuating water levels and greater competition, the plant faces is modified. Culms remain tall, but are slender with fewer septa. Occasionally short, inconspicuous leaf blades terminate the exposed lower leaf sheaths (Gordon-Gray *et al.*, 2006). Bracts are longer (20-36 mm), becoming leaf-like, but seldom exceed the inflorescence (Fig. 1).

4. Results and Discussion

The differences in the metal concentrations through different plant organs of *C.articulatus* growing in either wastewater or fresh water were demonstrated in Table 1. Obviously, metal concentrations of *C.articulatus* plant organs growing in wastewater attained relatively high values as compared to those growing in fresh water. As an example, the arsenic (As) concentrations of the measured plant samples growing in wastewater were found to be (258.5, 216.5, 256.5 and 147.2 µg/g d.wt.) for root, rhizome, culm and inflorescence; respectively and these values were greatly reduced into (55.26, 71.3, 67.05 and 74.1 µg/g d.wt.) for the corresponding plant organs growing in fresh water ($p<0.01$). These results if compared with arsenic content of sediments (254 µg/g d.wt.) and wastewater (10.01 µg/L.), it will confirm the phytoremediation potentiality of *C.articulatus* and that is in accordance with similar studies carried out by many authors for testing phytoremediation potentiality of other *Cyperus* species; Cradwell *et al.* (2002), proved the phytoremediation potentiality of *Cyperus eragrostis*, Deng *et al.* (2004) who proved the same potentiality for *Cyperus malaccensis* lam., and finally Marchand *et al.* (2010) and Soda *et al.* (2012) who tested the potentiality of *Cyperus alternifolius.* The concentration of the other metals in the present work follows the same trend (Table 1). Moreover, the metal accumulation rates by *C.articulatus* varied according to the type of heavy metal. Accumulation of iron recorded maximum values ranged between (105.5 and 900 µg/g d.wt.) in different plant organs of the studied species growing in wastewater, while minimum values were obtained for the accumulation of cadmium (0.9 to 1.95 µg/g d.wt.). The high concentration rates for some heavy metals in the present study might transport to the plant cells due to lack of selectivity in transmembrane (Soda *et al.*, 2012). Generally, the accumulation rates of *C.articulatus* toward the studied heavy metals can be arranged as follows: Fe > Cr > Cu > As > Mn > Pb > Hg > Ni > Cd. These results confirmed by the previous findings by Kropfelova *et al.*, (2009) and Marchand *et al.*, (2010). Moreover, Liu *et al.* (2010) concluded that wetland species show considerable variations in the metal uptake and accumulation abilities.

Phytoremediation process includes phytoextraction, phytostabilization, phytovolatilization, phytotransformation, and rhizofiltration (Table 2). Phytoremediators initially accumulate heavy metals to their roots through phosphate uptake pathway and then translocate to the above ground parts (Rahman and Hasegawa, 2011). The amount of heavy metal translocated from underground to aboveground parts of the studied species indicates the phytoremediation efficiency of the plant. However, more than 54 and 51 % of total arsenic and iron; respectively, accumulated into the plant were stored in roots. In accordance, Rahman and Hasegawa, 2011, mentioned that few plants have the ability to translocate high amount of arsenic and iron from roots to shoots. On the contrary, most phytoremediators have shown the highest ability to accumulate and translocate heavy metals from roots to shoots (Ma *et al.*, 2001). Accordingly, *C.articulatus* in the present work showed higher metal accumulation in the aboveground than below-ground plant parts concerning cadmium, mercury, manganese and lead. In addition, in order to study the metal mobility, the "translocation factor" (TF) and "bioconcentration factor" (BCF) were calculated and provided in Table 3. The TF for As, Cd, Cr, Cu, Fe, Hg, Mn, Ni and Pd was found to be 0.52, 0.15, 0.54, 0.44, 0.42, 0.95, 0.17, 0.08 and 0.71; respectively by plant samples growing in wastewater. In other words, TF was found to be less than 1 for all metal cases, which confirmed the significance of below-ground biomass as a heavy metal accumulator and that, is in accordance with results obtained by Yadav *et al.* (2012). It is to be noted here that, the translocation factor for heavy metals recorded in samples of *C. articulatus* growing in fresh water showed relatively higher values as compared to that of plant samples growing in wastewater. TF for cadmium was 0.15 and recorded in wastewater plant samples and this value greatly increased into 9.33 for fresh water plant samples (Table 3). The result indicated metal accumulation capability of *C.articulatus*. The present study revealed that different plant organs had different capability for removing and accumulating the heavy metals and this confirmed and coincide with obtained results by Wojciechowska and Waara (2011). On the other hand, BCF of most metal cases except iron and mercury was more than 1. This explained the special accumulation nature of *C.articulatus* and agrees with similar results carried out by Yadav *et al.* (2012) on the removal of heavy metals by *Cyperus alternifolius.* In addition, the

BFCs of metals in hyperaccumulators are greater by some order than those in non-hyperaccumulators. A specific hyperaccumulator can concentrate more than 100 ppm Cd, 1000 ppm Co, Cr, Cu or Pd; or 10000 ppm Zn or Ni (Reeves, 2003). Many researchers reported that metal uptake and accumulation by plants in wetlands depends on their initial concentration in the water (Soda *et al*., 2012). They concluded that the higher metal concentration in water leads to higher uptake by plants. This poses as a reason for higher metal concentration recorded in the different plant organs of the studied species.

The HPLC analysis of *C.articulatus* rhizome growing in either fresh or wastewater were shown in figures 2 (a) and (b); respectively. Both chromatograms resulted in sharp, symmetric and well resolved peaks at a wavelength of 220 nm. Considering the HPLC chromatogram (a) and the library report given in Table (4) , there were seven well defined sharp peaks at retention times of 17.293, 10.948, 10.819, 8.493, 8.246, 7.647 and 6.249 representing (Bis(2-ethylhexyl) phthalate), (9,12-octadecadienoic acid), (Octadecanoic acid), (Hexadecanoic acid), (palmitic acid), (methyl ester) and (Methyl N-(1H-2-oxo-4-pyrimidinyl) aminoacetate); respectively. On the other hand, the HPLC chromatogram (b) and the library report given in Table (5), showed six sharp peaks at the following retention times; 17.282, 10.949, 10.819, 7.882, 7.659 and 4.698 for (di- (2-ethylhexyl) phthalate), (9,12-octadecadienoic acid), (Octadecanoic acid), (Hexadecanoic acid), (Palmitic acid methyl ester) and (Decanedioic acid); respectively. In other words, heavy metals changed the chemical components and causes disappearance of some components (e.g. Methyl N-(1H-2-oxo-4-pyrimidinyl) aminoacetate) and expression of the others (e.g. Decanedioic acid). The obtained results were confirmed by similar studies carried by Rani and Padmakumari (2012) on rhizomes of *Cyperus rotundus*. Moreover, the change in chemical components might be largely attributed to the internal degradation of complex organic and inorganic pollutants present inside plant tissues (Chandra *et al*., 2012). The HPLC chromatogram of inflorescence samples (Figure 2, c) and the given data in Table (6), showed only small weak peaks with exception of three small defined peaks at the following retention times; 17.294, 10.949 and 4.240. This might explained, as stated before, by the low metal accumulation rate of inflorescence as compared to other plant organs and that is in accordance with Sundaramoorthy *et al.* (2010).

Table 1. Heavy metal concentrations of *Cyperus articulatus* plant organs growing in wastewater and fresh water, soil sediments (µg/g d.wt.) and water samples (µg/L.). **Mean values** are given±*SD*. Results are means of three replicates.

Samples	Heavy metals								
	As	Cd	Cr	Cu	Fe	Hg	Mn	Ni	Pb
Inflorescence•	**147.2**$^{a\,b}$ **±4.0**	**1.55**$^{a\,b}$ **±0.05**	**151.5**$^{a\,b}$ **±3.5**	**138.5**$^{a\,b}$ **±11.50**	**434.0**$^{a\,c}$ **±16.0**	**6.95**^{a} **±0.25**	**35.7**$^{a\,b}$ **±1.70**	**1.15**^{a} **±0.15**	**35.95**$^{a\,b}$ **±1.75**
	74.1$^{b\,c}$ ±2.0	0.00 ±0.00	62.75$^{a\,b}$ ±2.7	79.33^{a} ±3.5	126.80^{a} ±6.4	2.04^{a} ±0.11	9.35^{a} ±2.10	0.00 ±0.00	3.43$^{b\,c}$ ±0.88
Culm•	**256.5**^{a} **±3.50**	**1.45**$^{a\,b}$ **±0.05**	**227.00**^{a} **±27**	**237.50**^{a} **±7.50**	**522.5**$^{a\,b}$ **±2.5**	**8.15**^{a} **±0.35**	**70.5**^{a} **±1.50**	**2.90**^{a} **±0.10**	**48.0**$^{a\,b}$ **±2.00**
	67.05^{b} ±1.70	0.11^{b} ±0.05	45.40^{b} ±4.6	54.64$^{a\,b}$ ±1.7	104.40$^{a\,d}$ ±4.5	2.11^{a} ±0.23	12.26^{a} ±0.30	0.00 ±0.00	3.75$^{b\,c}$ ±1.50
Rhizome•	**216.5**^{a} **±16.5**	**0.90**^{a} **±0.10**	**251.50**^{a} **±8.5**	**258.5**^{a} **±8.50**	**105.50**^{a} **±1.5**	**5.40**^{a} **±0.40**	**15.0**$^{a\,b}$ **±1.00**	**2.25**^{a} **±0.15**	**83.50**^{a} **±3.50**
	71.3$^{b\,c}$ ±5.75	1.60^{a} ±0.20	52.31$^{a\,b}$ ±6.8	89.86$^{a\,b}$ ±5.4	76.41^{a} ±8.3	1.05^{a} ±0.10	17.3$^{a\,b}$ ±1.20	0.63^{a} ±0.05	5.57^{b} ±1.96
Root•	**258.5**^{a} **±2.50**	**1.95**^{a} **±0.15**	**329.00**^{d} **±59**	**280.50**^{a} **±3.50**	**900.0**$^{b\,c}$ **±20.**	**8.35**^{a} **±0.25**	**20.5**$^{a\,b}$ **±1.50**	**3.45**^{a} **±0.15**	**5.40**^{b} **±0.40**
	55.26^{b} ±0.75	0.13^{b} ±0.05	82.2$^{a\,b}$ ±17.6	88.04$^{a\,b}$ ±0.5	270.55$^{a\,b}$ ±3.8	3.43^{a} ±0.35	11.66^{a} ±1.60	1.67^{a} ±0.13	0.38^{c} ±0.11
Sediments	254.0^{a} ±3.74	1.43$^{a\,b}$ ±0.54	257.66^{a} ±6.12	250.60^{a} ±5.70	5398.3^{c} ±82.1	4.26^{a} ±4.05	59.1^{a} ±13.10	20.36^{a} ±4.02	36.33^{a} ±18.66
Wastewater	10.01^{c} ±4.38	0.06^{b} ±0.02	9.05$^{b\,c}$ ±1.20	34.91^{b} ±14.5	124.80^{a} ±21.5	52.08^{a} ±17.37	1.30^{b} ±0.30	12.10^{a} ±1.71	25.58^{a} ±5.29
Fresh water	0.00 ±0.00	0.00 ±0.00	2.75^{c} ±0.25	0.00 ±0.00	7.70^{d} ±0.3	2.46^{a} ±0.38	0.00 ±0.00	0.00 ±0.00	6.83$^{b\,c}$ ±0.23
	**	*	**	*	**	n.s.	*	n.s.	**

* $p \leq 0.05$, ** $p \leq 0.01$, n.s. = non significant.•Heavy metal concentrations of *C.articulatus* plant organs growing in wastewater (µg/g d.wt.) are given in bolded format.

Table 2. Different phytoremediation processes (Vamerali *et al.,* 2010).

Phytoextraction	In this process, plants uptake pollutants from soil and water, and translocate to and store in the harvestable biomass of the plants. Phytoextraction aims to remove pollutants from the contaminated sites. This process is usually observed in hyperaccumulating plants resistant to the pollutants.
Phytostabilization	Plants reduce mobility and phytoavailibility of contaminants in the environment. This process does not remove pollutants from contaminated sites but reduce mobility and excludes metals from plant uptake.
Phytovolatilization	Hyperaccumulating plants uptake pollutants from soil and water, and translocate to the aerial parts of the plants, and volatilize the pollutants in the air.
Phytotransformation	This process is one kind of plant's defense mechanism to the environmental pollutants. The hyperaccumulating plants modify, inactivate, degrade (phytodegradation), or immobilize (phytostabilization) the pollutants through their metabolism.
Rhizofiltration	Usually aquatic plants perform this process. The hyperacumulating aquaic plants adsorb and absorb pollutants from aquatic environments (water and wastewater).

Table 3. The translocation factor for metals within *C. articulatus* naturally growing in wastewater (TFW) and fresh water (TFF), the bioconcentration factor for metals within the plant in wastewater (BCFW) and fresh water (BCFF).

Metal	TFW	BCFW	TFF	BCFF
As	0.52	3.02	1.15	0.48
Cd	0.15	8.98	9.33	0.17
Cr	0.54	3.41	0.84	0.49
Cu	0.44	3.34	0.93	0.56
Fe	0.42	0.83	0.61	0.15
Hg	0.95	0.10	0.74	0.04
Mn	0.17	1.27	0.90	0.40
Ni	0.08	1.75	0.33	0.08
Pb	0.71	2.44	1.73	0.11

Table 4. Library report for the HPLC- Chromatogram of *C.articulatus* rhizome samples naturally growing in fresh water.

Peak#	RT	Area %	Library/ ID
1	4.251	23.53	z,z-10,12-hexadecadien-1-ol acetate
2	5.473	1.03	Tertradecanoic acid (CAS) ss myristic acid ss neo-fat 14 ss Univol U 316S ss n- Tetradecoic acid
3	6.249	0.71	Methyl N-(1H-2-oxo-4-pyrimidinyl) aminoacetate ss Methyl 2-[(1H-2-oxo-4-pyrimidinyl) amino]acetate
4	6.401	2.70	3-Eicosene, (E)-(+)- bicycle [5.1.0] octan-2-one
5	7.647	7.33	Hexadecanoic acid, methyl ester ss palmitic acid, methyl ester
6	7.870	14.70	Hexadecanoic acid, methyl ester ss palmitic acid, methyl ester (CAS) ss methyl palmitate ss metholene 2216 ss palmitic acid, methyl ester
7	8.246	10.42	Hexadecanoic acid (CAS) ss palmitic acid ss palmitinic acid ss n-hexadecoic acid pentadecanecarboxylic acid ss prifrac 2960 ss coconut oil fatty acids ss cetylic acid ss emersol 140 ss emersol 143
8	8.493	2.95	Hexadecanoic acid (CAS) ss palmitic acid ss palmitinic acid ss n-hexadecoic acid ss pentadecanecarboxylic acid ss 1- pentadecanecarboxylic acid ss prifrac 2960 ss coconut oil fatty acids ss cetylic acid ss emersol 140 ss emersol 143
9	10.220	1.90	(R)- (-)-14-methyl-8-hexadecyn-1-ol
10	10.455	1.97	Cyclopentadecanone, 2-hydroxy-6-octadecenoic acid,methyl ester
11	10.549	1.12	Methyl dihydromalvalate
12	10.690	0.91	Octadecanoic acid, methyl ester
13	10.819	6.23	Octadecanoic acid, methyl ester
14	10.948	12.80	9,12-octadecadienoic acid (z,z)
15	11.371	2.02	Octadecanoic acid (CAS) ss stearic acid ss n-octadecanoic acid ss PD 185 ss NAA 173 ss VANICOL ss Kam 2000 ss Kam 2000 ss Neo-fat 18 ss stearic acid ss hystrene ss stearex beads ss Hystrene
16	13.698	0.82	9,12,15-octadecatrienoic acid, methyl ester, (z,z,z)
17	14.109	0.86	1H-indene, 5-butyl-6-hexyloctahydro- ss Bicyclo[4.3.0]nonane, 3butyl-4-hexyl
18	14.309	1.30	Barbituric acid, 5-allyl-5-(cyclohex-2-en-1-yl) ss thialbarbitone oxygen analogue
19	16.647	0.99	Ergost-25-ene-3,5,6,12-tetrol, (3.beta., 5.alpha., 6.beta., 12.beta.)- 1,E-8,Z-10-Tetradecatriene
20	16.976	1.01	1-Hexacosene
21	17.293	4.69	Bis(2-ethylhexyl) phthalate

*Compounds are listed in order of elution from the eluting column.

Table 5. Library report for the HPLC- Chromatogram of *C.articulatus* rhizome samples growing in wastewater.

Peak#	RT	Area %	Library/ ID
1	4.698	4.34	Decanedioic acid, dimethyl ester
2	6.448	0.86	Butyric acid, 2,3- dichloro- (CAS) ss 2,3- dichlorobutyric acid ss But anoic acid, 2,3-dichloro-Butanoic acid
3	7.659	7.89	Hexadecanoic acid, methyl ester (CAS) ss Methyl palmitate ss Methyl hexadecanote ss Methyl n-hexadeca noate ss Uniphat A60 ss Metholene 2216 ss Palmitic acid methyl ester ss Palmitic acid, methyl ester ss n-Hexadecanoic acid methyl ester ss Palmitic acid
4	7.882	27.39	Hexadecanoic acid, methyl ester (CAS) ss Methyl palmitate ss Methyl hexadecanoate ss Methyl n-hexadecanoate ss Uniphat A60 ss Metholene 2216 ss Palmitic acid methyl ester
5	8.246	6.09	n- hexadecanoic acid (CAS) ss Palmitic acid ss n- hexadecanoic acid ss Pentadecanecarboxylic acid ss Prifrac 2960 ss Coconut oil fatty acids
6	8.493	9.33	Cetylic acid ss Emersol 140 ss Emersol
7	10.232	2.55	9,12-octadecadienoic acid (z,z)-, methyl ester ss methyl cis-9, cis-12-octadecadienoate ss linoleic acid, methyl ester
8	10.455	2.55	9-octadecenoic acid (z)-, methyl ester (CAS) ss methl oleate ss methyl cis-9-octadecenoate ss oleic acid methyl ester ss emery oleic acid ester
9	10.561	3.33	9-octadecenoic acid, methyl ester (CAS) ss methyl octade-9-enoate ss methyl -9-octadecenoate ss methyl oleate
10	10.690	1.10	Heptadecanoic acid, 16-methyl-, methyl ester
11	10.819	15.97	Octadecanoic acid , methyl ester (CAS) ss methyl stearate ss Stearic acid methyl ester ss kemester 9718 ss Stearic acid methyl ester
12	10.949	9.35	9,12- octadecadienoic acid (z,z) methyl ester
13	11.477	1.43	Octadecanoic acid
14	13.910	1.31	Eicosanoic acid, methyl ester (CAS) ss Arachidic acid methyl ester ss methyl eicosanoate
15	16.964	1.84	Docosanoic acid, methyl ester (CAS) ss methyl behenate ss methyl docosanoate ss Behenic acid methyl ester
16	17.282	4.67	di- (2-ethylhexyl) phthalate

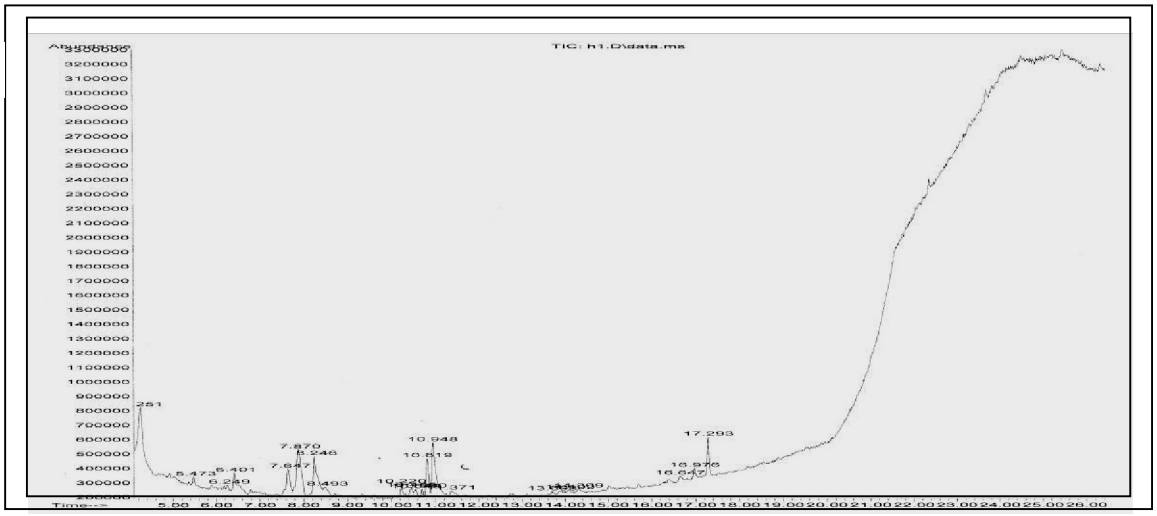
*Compounds are listed in order of elution from the eluting column.

Table 6. Library report for the HPLC- Chromatogram of *C.articulatus* inflorescence samples growing in wastewater.

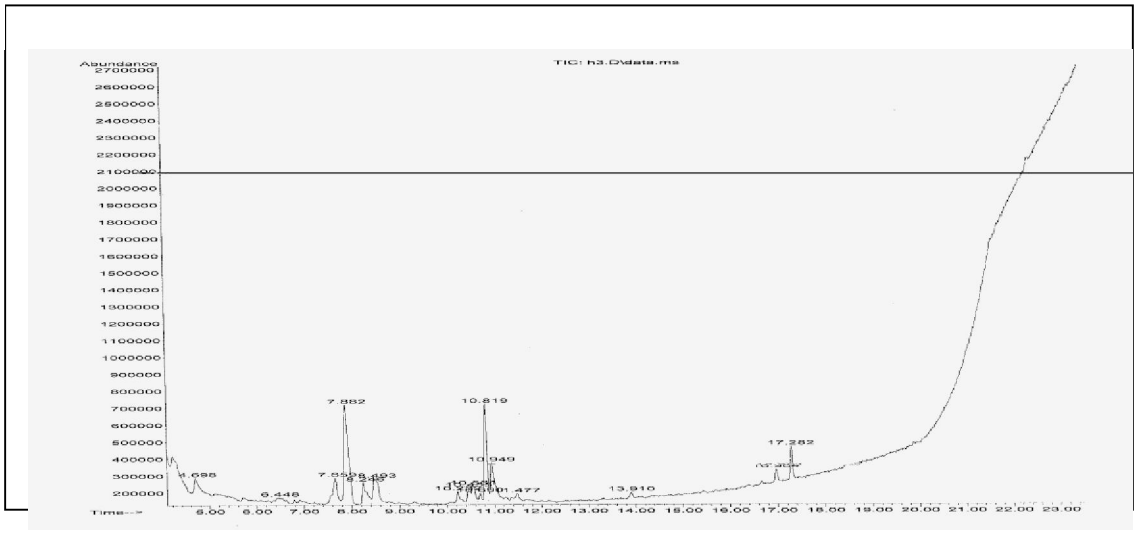
Peak#	RT	Area %	Library/ ID
1	4.240	80.72	(7R,8S)-cis-anti-cis-7,8-Epoxytricyclo[7.3.0.0(2,6)] dodecane
2	8.223	2.70	n-Hexadecanoic acid
3	8.317	2.64	Curan-17-oic acid,2,16-didehydro-19-hydroxy-, methyl ester, (20.xi.)-ss Echitamidine
4	9.292	3.55	Bis (5,5,5-trifluoro-4-oxopentan-2-n-propylene) amine ss 2-pentanone,4,4'-[iminobis(3,1-propanediylitrilo)] bis [1,1,1-trifluoro- (CAS)]
5	10.949	3.40	9,12-octadecadienoic acid (z,z-CAS) ss Linoleic acid ss Linoleic ss Unifac 6550 ss Linolic acid ss Telfairic acid ss Grape seed oil ss Polylin No. 515 ss Cis,cis-Linoleic acid ss 9,12-octadecadienoic acid ss cis-9,cis-12-octadecadienoic acid
6	11.689	3.15	N-Benzyl Phthalimide ss 1H-Isoindole-1,3 (2H)-dione, 2-(phenylmethyl)- (CAS) ss Phthalimide, N-benzyl silane
7	17.294	3.85	1-(Methylpropyl)-4-(1',1',2'-trichloro-3'-ethylallyl)benzene

*Compounds are listed in order of elution from the eluting column.

(a)



(b)



(c)

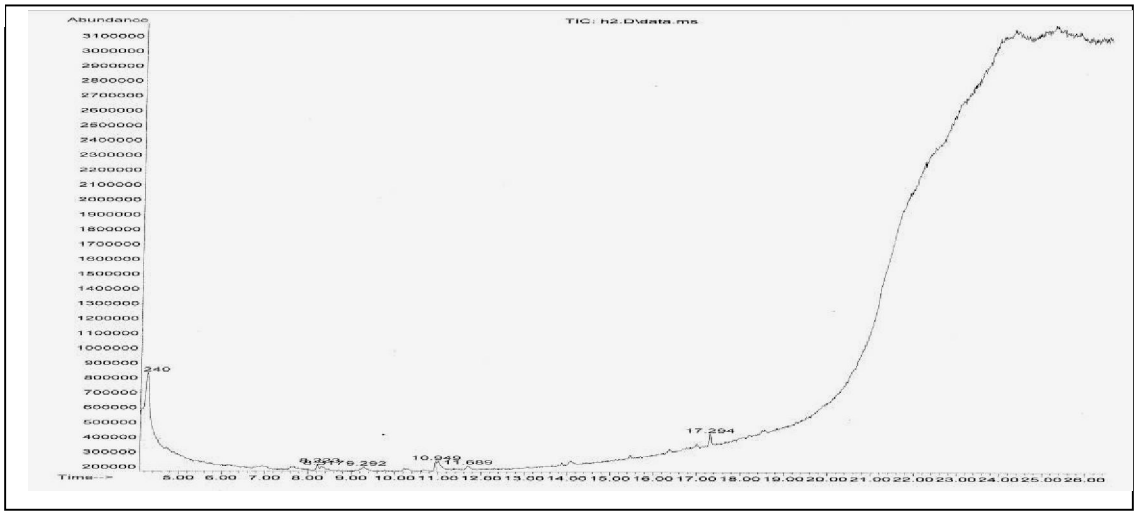


Figure 2. HPLC –Chromatograms of *C.articulatus* rhizome samples naturally growing in fresh water canal (a), naturally growing in wastewater (b) and inflorescence samples naturally growing in wastewater (c) at 220 nm.

5. Conclusion

It can be concluded that *Cyperus articulatus* has the potentiality for phytoremediation of different heavy metals *e.g.* As, Cd, Cr, Cu, Fe, Hg, Mn, Ni and Pb in wastewater. The species show considerable variations in the metal uptake and accumulation abilities according to the plant organ and the type of heavy metal. Heavy metal accumulation in the different plant organs changed their chemical components especially in the bellow-ground parts. Extra investigations on the remobilization and biomineralisation mechanisms of heavy metals are required.

Corresponding author

Hussein F. Farrag[1&2]
[1]Botany Department, Faculty of Science, Cairo University, Giza, Egypt.
[2]Biology Department, Faculty of Science, Taif University, Taif, KSA.
hfarrag2012@hotmail.com

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