Eco-genetic study on water hyacinth, *Eichhornia crassipes* (Mart.) Solms, the world's most invasive aquatic plant

Tarek Abd El-Ghafar El-Shahawy*, Mahmoud Ahmed Khater, Faida Ahmed Sharara

(*Botany Department, National Research Centre, El Buhouth St., Postal Code 12622, Dokki, Cairo, Egypt*)

Abstract: Many aquatic plant species are regarded as problems disturb all interested and concerned entities in aquatic weed management. The water hyacinth, which is the world's most aggressive and destructive aquatic freshwater species, extends over vast areas of the world creating serious ecological, economic, and cultural problems. So far, water quality does not represent a dilemma against water hyacinth growth. The objective of the current work is to study the potential genetic differences between water hyacinth populations growing under different aquatic ecosystems in Egypt. Water and plant samples were collected from three different sites namely irrigation water, drainage water and sewage water at Al-Buhayrah Governorate, Damanhour District. The physicochemical properties and heavy metal contents of the opted water samples were estimated. Heavy metals in roots and shoots coupled with the patterns of genetic structure within each type were also evaluated. Poor quality of sewage water was prominent with relatively small concentrations of trace elements. Plants from different regions absorbed and accumulated heavy metals to varying degrees. Relatively high concentrations were estimated in sewage water plants compared with the plants from other sources. Root and shoot tissues of the same plant also exhibited various degrees of heavy metal accumulation. Overall, roots showed a high affinity for the different elements except for Zn^{2+}. The genetic variation between plants was also expected. DNA analysis of the plants using ISSR–PCR technique showed different genetic regions with an increasing number of molecular markers in sewage water plants. This fact surely indicates that water hyacinth has an innate ability to tolerate harsh growth conditions with high genetic potential which enables it to live sustainably.

Keywords: aquatic weeds, aquatic ecosystems, genetic variation, heavy metals, invasive plants, water quality

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1 Introduction

Water hyacinth, *Eichhornia crassipes*, is one of the world's most dangerous aquatic weeds that attack freshwater habitats. The plant has particular capabilities to be the master of aquatic ecosystems. It is categorized as a highly invasive weed hits many tropical, subtropical and worm areas of the world, causing serious ecological and economic, problems. Its physical presence is even considered a social challenge in many societies that depend upon fishing, sailing and recreation water activities as a sole source of income (Kateregga and Sterner, 2009; Villamagna and Murphy, 2010; Waithaka, 2013). Currently, water hyacinth is highlighted by IUCN's report as one of the "100 of the World's Worst Invasive Alien Species" around the world (Lowe et al., 2000).

Water hyacinth is a free floating annual plant. It is characterized by fast growth, with the opportunity to duplicate itself in as little as 12 days (Penfound and Earle, 1948; Perkins, 1973). It reproduces both vegetatively and by seeds. One plant can produce thousands of long-lived viable seeds (Barrett, 1980). According to the available information, water hyacinth's seeds can remain viable, without germination, for over 20 years (Matthews et al., 1977). Vegetative reproduction is the most common, but seeds play the most serious role in spreading and the infestation by the weed (Sullivan and Wood, 2012).

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***Corresponding author: Tarek Abd El-Ghafar El-Shahawy**, Botany Department, National Research Centre, Egypt. Email: el_shahawy4@yahoo.com

Water hyacinth spreads over a wide range of freshwater habitats worldwide. Wetlands, marshes, shallow ponds, sluggish flowing waters, stagnant water, large/small lakes, waterways, reservoirs and rivers are open settlements to the plant for growth and duplication (Jafari, 2010). Amazingly, it can grow in extremes of nutrient availability, pH level, temperature and toxic materials. The plant can also withstand the irregular fluctuations in water level and velocity (Gopal, 1987). Problems arising from water hyacinth growth are multifaceted. Water hyacinth grows in extensive thick mats that obstruct waterways and hinder the expeditious use of water. Indeed, it affects all water-based activities such as irrigation, fishing, navigation, quality of water, hydraulic and hydroelectric installations (Heuzé et al., 2015). The extensive growth of water hyacinth obscures light. The subsequent reductions in gaseous exchange with the air, water flow and oxygen available amount can be restricted or lethal to the associated organisms (Mironga et al., 2011; Mironga et al., 2012). The pervasive presence of water hyacinth has the potential to affect the biodiversity of ecosystems (Villamagna, 2009). The plant provides potential habitat for disease victors (of both human and animals) such as mosquitoes and the snails causing bilharzia (Mack and Smith, 2011). Furthermore, it refuges harmful insect pests, rodents and microbial agents that attach to the plant (Téllez et al., 2008).

Accumulation of rotting materials due to decaying vegetation may lead to low oxygen levels and poor water quality (Giraldo and Garzon, 2002). The exhaustion of oxygen through decomposing plant residues is well documented (Timmer and Wildone, 1966; Spellman and Stoudt, 2013). Mironga et al. (2011) reported that the plant itself did not release oxygen into water as do other vegetation and phytoplankton, causing a drop in dissolved oxygen concentrations. The most dangerous impact of water hyacinth plants is related to the loss of large water quantities through evapotranspiration (Lallana et al., 1987). Water loss from infested areas with water hyacinth may be two to ten times higher than that from open water surfaces (Penfound and Earle, 1948; Gopal, 1987; O’Brien, 1981; Singh and Gill, 1996). In a heavy invaded country like Sudan, water hyacinth results in an annual loss of 7 billion m^3 of water of the Nile River, which is approximately equal to one-tenth of the river’s gusher yield (Wolverton and MacDonald, 1979). In Egypt, the loss due to the infestation by water hyacinth plants was estimated at 3.45 billion m^3 annually because of the strict scrutiny in applying mechanical and manual control means (Abdel-Shafy and Aly, 2002; Abouziena et al., 2014). Air temperature, ambient relative humidity and wind speed are major factors influencing evapotranspiration from water hyacinth (Timmer and Wildone, 1966).

Water hyacinth control is fairly simple and quite challenging in the same time. Simplicity comes from the availability of a wide range of controlling options for use. The real challenge comes from being present in a sensitive environment *e.g.*, freshwater, which is a very important issue for the lives of people around the world. Chemical and mechanical methods are two of current most popular and versatile controlling tools (Charudattan et al., 1996). Great efforts have been forwarded to develop efficient elements for biological control during the last three decades (El-Wakil et al., 1989). Regardless of the benefits that could be achieved, each method/option has its own advantages and disadvantages. Chemical control by herbicide is widely used as effective and economic means, but caution is required as toxins are used on water. Regardless of the high cost and the environmental impacts that might arise, mechanical and manual methods are quite safe and effective options. Biological methods can be economically simple on the long term, but their implications on both the aquatic and natural terrestrial ecosystems should be regarded (Bisher and Bennet, 1985).

There were increased reports of water hyacinth in Nile Delta of Egypt. It was introduced into Egypt between 1879 and 1890 as an ornamental plant and soon became a highly environmental and cultural problem (Crafter et al., 1992; Osei-Agyemang, 2002; El-Morsy, 2004). Egypt has early adapted mechanical, manual, physical and biological methods as strategic options to eliminate water hyacinth, but the weed remained an intractable problem (Navarro and Phiri, 2000).

The main objective of the current work is to explore the unique ability of water hyacinth populations to germinate, grow and survive under different aquatic ecosystems, including extreme conditions, throughout Egypt. The focus of the study is to determine relevant genetic regions/genes that are associated with greater resistance and survival.

2 Materials and Methods

In the present study, we focused our investigation on three major aquatic habitats, irrigation water, agricultural drainage water and sewage water. Both water and plant samples were collected in the summer of 2015 from Al-Buhayrah Governorate, Damanhour District. The accompanying aquatic plant species in the three different sites were also counted.

2.1 Water sampling

Sampling was performed in waters infested with water hyacinth. Three sampling sites were selected, one location for irrigation water; one location for drainage water and one location for sewage water. Water samples were collected directly into pre-cleaned dark-glass containers (2 L/each); from three sampling point at each site; from an intermediate depth of 0.5 m below surface.

2.2 Plant samples

Plant samples of water hyacinth were collected from different points in the vicinity of the three aquatic milieus. Water hyacinth plants were carefully washed in tap water and then in distilled water to dispose of any organic/solid materials or zoo- and phytoplankton that may be associated with them. The plants were separated into shoots and roots followed by drying at 75℃ for 24 hr. Each fraction was thoroughly ground to fine particles in an electronic blender and then kept in dark brown bags until used in analysis.

2.3 Water and plant analysis

The water samples were immediately analyzed for their physiochemical properties according to the Standard Methods for Examination of Water and Wastewater by APHA et al. (2012). Water quality parameters examined were pH, total dissolved solids, suspended solids, chemical oxygen demand (COD), phosphate (PO_4-P), sulfide (S^-), total hardness, calcium hardness, magnesium hardness, calcium, magnesium, alkalinity, carbonate, bicarbonate, hydroxide, chloride, oil and grease.

Heavy metal composition of the plants (at both shoot and root tissues; mg/kg Dr. Wt.) in conjunction with their amounts in water of the different areas (mg/L) was also determined using spectroscopy methods. Digestion of samples has been comprehensively addressed elsewhere (APHA et al., 2012). Trace metal (*e.g.*, Cu, Zn, Cd, Pb and Cr) analysis was conducted using a Flame Atomic Absorption Spectrometer (SpectrAA 220 FS, Agilent) equipped with hollow cathode lamps for the measured elements, a deuterium lamp for background correction and with flame atomization. The spectral lines used for trace determination were 213.8, 324.8, 228.8, 283.3 and 359.3 nm, for Cu, Zn, Cd, Pb and Cr, respectively. Standard solutions used in the calibration procedure were prepared by successive dilution of the standard stock solutions (Merck) with 1% HCl (Merck). The reagents were of analytical grade and all solutions were prepared using double distilled water (Hamilton - UK). All containers and glassware were cleaned by soaking in the 5 mol/L HNO_3 for at least 24 hours and rinsed three times with double distilled water prior to use.

Normal calibration curves were constructed with aqueous standards for every trace element. For each solution analyzed, the instrument software was programmed to give a value with a precision below 5% between readings, in five readings per replicate, to assure reproducibility of measurements. Blank solutions were prepared following the respective sample treatment and analyzed. Trace metal levels in blank solutions were always below the limit of detection of the analytical procedure for all elements. The limits of detection and limits of quantification for the measured elements were determined using the method of Reis et al. (2009).

Data were analyzed as a completely randomized block design using ANOVA table and $LSD_{0.05}$ test to compare between means.

2.4 Genomic DNA extraction

Healthy water hyacinth leaves were collected from the different sites of growth for DNA analysis. The leaves were processed separately, washed with running tap water (for 5 min.), then with distilled water and stored at –20℃ until use.

Total genomic DNA was extracted from 0.05 g leaf tissue of the different types of water hyacinth using the CTAB procedure (Doyle and Doyle, 1987). Quantitative analysis of the DNA (density of bands) was performed using 1% agarose gels in the presence of 0.5 mg/L ethidium bromide.

2.5 ISSR–PCR analysis

The following listed ISSR primers (Table 1) were used in the present study according to Sharama et al. (1995). Using these primer combinations, DNA amplifications were carried out in a total reaction volume of 15 μL containing 1 μL DNA (40 ng), 7.5 μL Master Mix (Gene Direx one PCR^{TM}), 1 μL template DNA and 1 μL primer.

Table 1 Primer sequences of ISSR molecular markers used for analysis of DNA fingerprinting in water hyacinth

Primer	Primer Sequence (5ˉ 3ˉ)
IS- 1	(CT)8 TG
IS- 2	(CT)8 AC
IS- 3	(CT)8 GC
IS- 4	(CA)6 AC
IS- 5	(CA)6 GT
IS- 6	(AC)8 YG
IS- 7	(GT)8 YG
IS- 8	CGC(GATA)4
IS- 9	(AGAC)4 GC
IS- 10	(GATA)4 GC

PCR amplification was performed in a Hybrid Cycler programmed to fulfill 35 cycles after an initial denaturation cycle for 5 min at 95 ℃. Each cycle consisted of a denaturation step at 95 ℃ for 1 min, an annealing step at 45 ℃ for 1 min, and an elongation step at 72 ℃ for 1 min. The primer extension segment was extended to 10 min at 72 ℃ in the final cycle. Agarose gel (1.2%) electrophoresis was used for separating the PCR products. Gels were photographed and scanned with Bio-Rad video densitometer Model 620, at a wavelength of 577.

3 Results

Water hyacinth was collected from different sites; irrigation water, drainage water, sewage water. Various types of aquatic vegetation were counted in the three different sites (Table 2). All sites were overwhelmingly dominated by water hyacinth plants with various degrees of infestation by other plant species. Regardless of water hyacinth, plants that were found in irrigation water were *Echinochloa stagnium*, *Panicum repens*, *Polygonum salicifolium* and *Typha elephantine*. Besides the excessive growth of water hyacinth, drainage water was heavily infested with *Phragmiyes australis* and *Limna gibba*. No vegetation was noted in sewage water except water hyacinth plants (Table 2). In sewage water plants, it has also been noted that root's growth and even color was substantially affected by the area of growth. Roots had a black color assembling to sewage color with a substantial reduction in growth and biomass of approximately more than half of normal (Data was not reported).

Table 2 The most commonly occurring aquatic plants with infestation levels in the three different sites of study

Area of collection	Plant species
Irrigation water	Water hyacinth [*Eichhornia crassipes* (Mart.) Solms]^3, barnyardgrass (*Echinochloa stagnium* (Retz) Beauv.)^2, torpedograss (*Panicum repens* L.)^2, knotweed (*Polygonum salicifolium* Brouss.)^1, and common cattail (*Typha elephantine* Roxb.)^3
Drainage water	Water hyacinth [*Eichhornia crassipes* (Mart.) Solms]^3, common reed (*Phragmiyes australis* Trin.)^1, and duckweed (*Limna gibba* L.)^2
Sewage water	Water hyacinth [*Eichhornia crassipes* (Mart.) Solms]^3

Note: 1 = mild infestation; 2= moderate infestation; 3= heavy infestation.

The data in Tables (3 and 4) show the physicochemical properties and heavy metal contents of water collected from three different sites. Obvious variation was observed between the three types of water regarding their physical and chemicals properties, including total dissolved solids, suspended solids, total hardness, calcium hardness, magnesium hardness, oil and grease, chemical oxygen demand as well as phosphate (PO_4-P), sulfide (S^-), calcium, magnesium, bicarbonate and chloride composition. Sewage water was superior to the other types in terms of all examined criteria. In sewage water, the values often reached twice those reported in irrigation and drainage waters. Exceptions include the pH, carbonate and hydroxide contents. Waters of the different sites were slightly alkaline with pH ranging from 7.4 to 7.7; meanwhile nothing was recorded on carbonates and hydroxides (Table 3).

Concerning water composition of heavy metals, the results did not show any significant differences between the three types of waters. Concentration of trace elements was even more limited within all types (Table 4).

Table 3 Physicochemical properties of the three different types of water

Parameters, mg L^{-1}	Source of water		
	Irrigation water	Drainage water	Sewage water
pH	7.7	7.7	7.4
Total dissolved solids	266.0	445.0	970.0
Suspended solids	8.0	24.0	162.0
Chemical oxygen demand*(COD)	28.0	30.0	420.0
Phosphate (PO_4-P)	0.015	0.062	2.62
Sulfide (S^-)	3.9	5.2	8.6
Total hardness	136.0	202.0	202.0
Calcium hardness	82.0	100.0	118.0
Magnesium hardness	54.0	102.0	84.0
Calcium	33.0	40.0	47.0
Magnesium	13.0	25.0	20.0
Alkalinity	336.0	440.0	760.0
Carbonate	0.0	0.0	0.0
Bicarbonate	336.0	440.0	760.0
Hydroxide	0.0	0.0	0.0
Chloride	26.0	44.0	124.0
Oil and grease	9.2	9.6	33.2

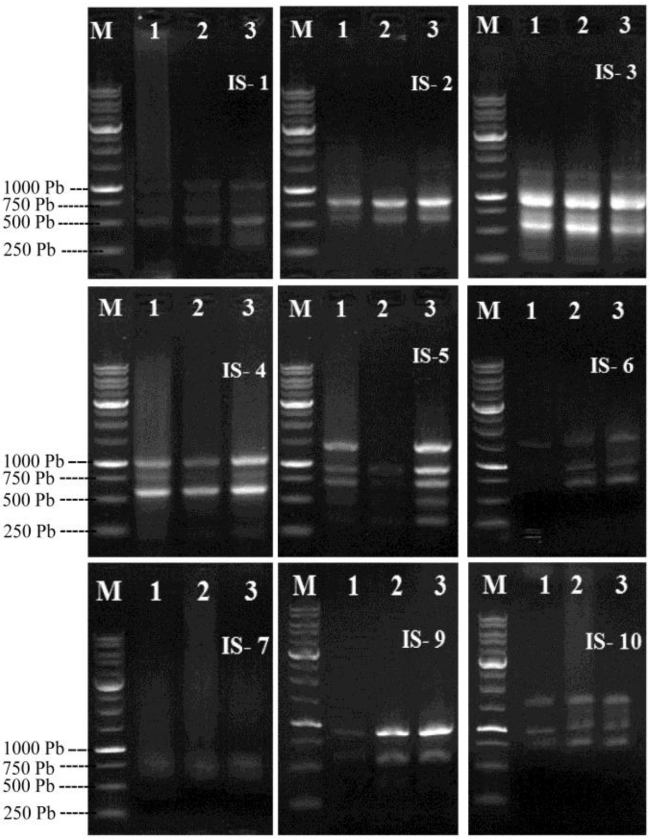
Note: * mg O_2/L.

Table 4 Heavy metals contents in water hyacinth plants collected from the different sources of water in comparison with their contents in water

Sample	Source of water	Organ	Heavy metal content					
			Unit	Pb	Cu	Cd	Cr	Zn
Water hyacinth	Irrigation water	Shoot	mg/kg	2.50	0.75	0.50	1.50	451.88
		Root	mg/kg	2.50	27.80	0.58	11.22	59.17
	Drainage water	Shoot	mg/kg	2.50	4.13	0.67	1.50	519.33
		Root	mg/kg	2.50	10.10	0.75	4.14	163.16
	Sewage water	Shoot	mg/kg	2.50	4.00	1.08	1.50	727.96
		Root	mg/kg	4.92	14.33	1.46	4.80	330.71
Water	Irrigation water		mg/L	0.10	0.03	0.02	0.06	0.01
	Drainage water		mg/L	0.10	0.03	0.02	0.06	0.01
	Sewage water		mg/L	0.16	0.03	0.02	0.06	0.03
$LSD_{0.05}$				0.22	0.15	0.18	0.76	9.12

The vegetation analysis disclosed a distinctive disproportion in heavy metal composition within plants as affected by the area of growth. Sewage water plants showed higher absorption capacity for heavy metals compared to the plants of irrigation and drainage waters, and a maximum affinity was for Zn. Overall, root tissues were significantly effective than shoot tissues in accumulating heavy metals. A different approach was noted with Zn. Shoot tissues recorded exceptionally high levels of Zn rather than root tissues. The shoot tissues were found to have a high zinc composition between 451.88 to 727.96 mg/kg compared to 59.17 to 330.71 mg/kg for aerial parts (Table 4).

Table 5 and Figure 1 show DNA fragments that were detected using ISSR primers in water hyacinth samples. Ninety bands were identified in this regard with 12 polymorphic bands have an average polymorphism percentage of 13.33%. For each primer, the number and molecular weight of detected bands varied from 7 to 13 bands and from 378.93 bp to 1959.32 bp, respectively. All types of water hyacinth expressed two monomorphic bands with molecular weights (848.41 bp and 521.80 bp). Yet, 4 bands with molecular weights (895.26 bp, 866.03 bp, 766.78 bp and 588.04 bp) were only detected in irrigation and drainage water plants. It was also pronounced that drainage and sewage water plants had the same bands with the molecular weights (1000.00 bp and 568.30 bp). However, irrigation and sewage water plants had the bands with the molecular weights (1959.32 bp, 1696.82 bp, 1043.60 bp, 703.49 bp, 307.79 bp and 243.66 bp).



Note: M, Molecular weight of DNA ladder; 1, irrigation water plants; 2, drainage water plants; 3, sewage water plants.

Figure 1 DNA fingerprint of water hyacinth plants generated by the 10 ISSR primers

Table 5 Molecular weight of DNA bands detected in water hyacinth plants collected from the different sources of water using ISSR technique

Primer	Molecular weight (bp)	Irrigation water	Drainage water	Sewage water
IS- 1	1062.71	-	+	-
	1041.38	-	-	+
	1020.48	+	-	-
	703.49	-	+	+
	674.1	+	-	-
	521.8	+	+	+
	331.58	-	-	+
	314.98	-	+	-
IS- 2	1554.92	-	-	+
	1176.08	+	-	-
	1041.38	-	-	+
	783.94	+	-	-
	766.78	-	-	+
	750	-	+	-
	568.3	+	-	+
	556.3	-	+	-
IS- 3	1554.92	+	-	-
	1500	-	+	-
	1440.4	-	-	+
	895.26	+	-	-
	875.66	-	+	-
	856.5	-	-	+
	544.56	+	-	-
	521.8	-	+	-
	500	-	-	+
	256.5	+	-	-
	243.66	-	+	+
IS- 4	1059.63	-	-	+
	1039.37	-	+	-
	1019.5	+	-	-
	801.48		-	+
	766.78	+	+	-
	600.08	-	-	+
	588.04	-	+	-
	564.67	+	-	-
	233.25	-	-	+
	222.72	-	+	-
	203.06	+	-	-
IS- 5	1415.58	+	-	-
	1335.92	-	-	+
	895.26	+	+	-
	875.66	-	-	+
	691.58	-	+	-
	677.7	-	-	+
	500	-	+	-
	488.58	+	-	-
	477.42	-	-	+
	307.79	-	+	+
	293.89	+	-	-
IS- 6	1840.19	-	-	+
	1732.05	-	+	-
	1595.38	+	-	-
	1119.22	-	-	+
	1000	-	+	-
	953.18	+	-	-
	786.84	-	-	+
	718.66	-	+	-
IS- 7	1959.32	-	+	+
	1000	+	-	+
	976.31	-	-	+
	953.18	-	+	-
	866.03	+	+	-
	845.51	-	-	+
	568.3	+	-	-
IS- 8	-	-	-	-
IS- 9	848.41	+	+	+
	600.08	-	-	+
	588.04	+	+	-
	378.93	-	+	-
IS- 10	1696.82	-	+	+
	1628.5	+	-	-
	1043.6	-	+	+
	940.22	+	-	-
	797.69	-	+	-
	781.47	+	-	-

The results in Table 6 also refer to the existence of several molecular markers in the three types of water hyacinth by applying ISSR technique. Numerous molecular markers were identified in drainage water plants amounted to 18. Meanwhile, the number elevated to 20 in irrigation water plants and to 22, with relatively higher molecular weights, in sewage water plants.

Table 6 Identified molecular markers of water hyacinth collected from the different sources of water

Molecular markers	Molecular weight, bp		
	Irrigation water	Drainage water	Irrigation water
1	1628.50	1732.05	1840.19
2	1595.38	1500.00	1554.92
3	1554.92	1062.71	1440.40
4	1415.58	1039.37	1335.92
5	1176.08	1000.00	1119.22
6	1020.48	953.18	1059.63
7	1019.50	875.66	1041.38
8	953.18	797.69	1041.38*
9	940.22	750.00	976.31
10	895.26	718.66	875.66
11	783.94	691.58	856.50
12	781.47	588.04	845.51
13	674.10	556.30	801.48
14	568.30	521.80	786.84
15	564.67	500.00	766.78
16	544.56	378.93	677.70
17	488.58	314.98	600.08
18	293.89	222.72	600.08**
19	256.50	-	500.00
20	203.06	-	477.42
21	-	-	331.58
22	-	-	233.25

Note: * Repeated with primers IS- 4 and IS- 9; ** Repeated with primers IS- 1 and IS- 2.

4 Discussion

The current investigation was carried out over different types of water with the aim of qualitative and quantitative analysis of the naturally occurring contaminants and to identify the relationship between patterns of genetic variation in water hyacinth plants and areas of growth.

The physicochemical properties of waters differed so widely across all studied criteria, with high values for sewage water. The physicochemical properties of irrigation water were the best compared with those of drainage and sewage waters. Analysis of heavy metal composition in the various types of water showed low average levels in Pb, Cu, Cd, Cr and Zn. Remarkably high amounts of heavy metals were found in both root and shoot tissues of water hyacinth plants. This confirms the fact supported by many researchers that the water hyacinths act like magnets, amazingly attract and accumulate heavy metals in their tissues. According to the relevant references, water hyacinth shows increased capacity to tolerate heavy metals with the ability to accumulate them in their parts, both above and beneath water surface (Memon et al., 2001; Prasad and Freitas, 2003). Thus, it is qualified for being used in areas experience a heavy metal pollution problem (Priya and Selvan, 2014; Rezania et al., 2015; Nasution et al., 2016). The phytoremediation of heavy metals by water hyacinth is strongly suggested to play a significant role in the future (Lone et al., 2008; Okunowo and Ogunkanmi, 2010; Swain et al., 2014).

According to the current investigation, all heavy metals accumulated in greater concentrations in root tissues, except Zn^{2+}. Previous relevant research has suggested that some plants have highly evolved exclusion mechanisms to accumulate heavy metals in roots and frustrate transport to shoots (Iskandar, 2000; Gupta, 2013). The superiority of water hyacinth roots to accumulate heavy metals has been discussed in several studies (Mohamad and Abdul Latif, 2010; Vitória et al., 2010). Syuhaida et al. (2014) postulated that water hyacinth used some kind of rhizo-filtiration technique which accumulates contaminants in roots. The low accumulation level in the aerial parts might also refer to some physical parries in roots that act against transportation to stems and leaves (Lu et al., 2004; Fahr et al., 2013). Normally, the shoot-to-root metal-accumulation ratio varies from species to another (Tangahu et al., 2011; Amadi and Tanee, 2014), and the translocation process via plant tissues (from root-to-shoot) is governed by a gene expression (Clemens et al., 2002; Han et al., 2006).

The results also referred to a close correlation between heavy metal concentrations in water hyacinth and the chemical composition of the other components in waters. Based on the results of the current research, Pb uptake by roots of the plants growing in sewage was much higher than that by the plants growing in irrigation and drainage waters. Even though there was a high amount of phosphate in sewage water that might act against the solubility and bioavailability of Pb (Pinho and Ladeiro, 2012), an increasing amount of Pb was noted in roots of sewage water plants. Paradoxically, the data recorded lower amounts in shoot tissues. Regardless the potential ability of phosphate to react with Pb in the rhizosphere and make it unavailable for plant absorption, there may be a possibility to use phosphate by plants to cope with Pb detrimental effects. Laperche et al. (1997) noted that adding phosphates as soluble phosphate or as apatite worked well in elevating Pb and P contents in *Sorghum bicolor* L. treated plants, especially in root tissues. Practically, Pb was immobilized by P via formation of an insoluble pyromorphite-like mineral on root cell walls (Cao et al., 2003). Pb perception on plant cells has been reported earlier as a lead phosphate (Koeppe, 1977). Such chemical/plant behavior has been frequently found in diverse plant species (Sharma and Dubey, 2005; Fahr et al., 2013).

In general, plants have developed several defense mechanisms of elevated levels of heavy metals such as prevention from entering inside the plant via creating callose, detention in vacuoles, translocation and compartmentalization between the more tolerant parts (DalCorso et al., 2013; Fahr et al., 2013). Under heavy metal stress, a number of antioxidative enzymes can be induced in water hyacinth plants [*e.g.*, superoxide dismutase, catalase, ascorbate peroxidase and peroxidase (Malar et al., 2014)] as a result of increasing the expression of genes in various tissues (Bücker-Neto et al., 2017).

Examination of the possible genetic variation disclosed that there is strongly convincing evidence regarding the effects on genetic diversity due to the area of growth. Considerable number of bands was detected by each primer using PCR technique. The annealing temperature of 45 ℃ for 1 min with 35 cycles allow the primers to perfectly bind to the template DNA producing the above-mentioned high number of detected bands. Typically, these conditions have been considered the best in this regard (Ahmad et al., 2008).

Pronounced genetic variations were noted among water hyacinth plants; a plurality of molecular markers was clearly recognizable. A wide variety of double-stranded DNA fragments, that were not present in irrigation and drainage water plants, was detected in sewage water plants. This evidence refers clearly to the genetic variation between water hyacinth plants as affected by the area of growth. Primarily, water hyacinth is characterized, among other plant species, by the low genetic diversity because the plant reproduces mainly by offspring that are genetically identical to each other and to the mother plant (Eckert, 1999; Carter and Sytsma, 2001). Such genetic uniformity is thought to play a significant role in the invasive spread of water hyacinth populations (Zhang et al., 2010). In integration with the results that we have found, water hyacinth has a unique ability to regulate and maintain proper growth levels with the use of genes providing resistance to unfavorable growth conditions which add a complexity in its management. This hypothetical inference comes in conformity with field observation results. Water hyacinth was the only species that was found in sewage water. However, in irrigation and drainage waters, multiple species were found accompanying the water hyacinth.

It is concluded that water hyacinth is an exceptional plant. The superior adaptability of this plant to both adverse and unfavorable growth conditions puts it in the ranks of the plants with phytoextraction properties that can be relied upon in the future in the remediation of heavy metal-polluted waters.

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