EPH - International Journal of Applied Science
ISSN (Online): 2208-2182
Volume 05 Issue 03-September-2019

DOI: https://doi.org/10.53555/eijas.v5i3.116

ACCUMULATION OF HEAVY METALS IN WASTEWATER, SOIL AND UPTAKE BY PLANT SPECIES WITH PHYTOREMEDIATION POTENT

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Abstract:-
The present study was to evaluate the concentrations of heavy metals (Cd, Cr and Cu) in soils, waste water and different plant organs; define which species and which plant organs exhibit the greatest accumulation and evaluate whether these species could be usefully employed in phytoremediation programs. Al- Marj plain is located in north eastern parts of Libya at the western edge of Al-Akhdar Mountain. Seven locations, distributed in polluted area. Data collected from heavy metal analysis demonstrated that the levels of the recorded metals in waste water were above the standard levels of USEPA, FAO and standard levels of Syrian Standard adopted by National Environmental Quality Standards for municipal and liquid industrial effluents in Libya. Heavy metals concentration in polluted soil samples were decreased in Sequence of: Cu>Cr>Cd. The thirteen species were subjected to suitable analysis to determine their capabilities for heavy metals accumulation as well as the metal allocation was also investigated. The results indicated that Cd could be accumulated in all plant organs (roots, stems and leaves). Generally, roots of some selected plants attained higher Cr and Cu, concentrations than other organs. The bioaccumulation factors of the investigated metals (BAF) increased in the sequence: Cu > Cr>Cd. According to the results Conium mculatum, Juncusrigidus,Uricadioica and Phragmitesaustralis could be used successfully in bio monitoring and phytoremediation programs for heavy metal contaminated soils.

Keywords:- *Heavy metal, accumulation, Phytoremediation, Polluted soils bioaccumulation factor.*

INTRODUCTION

In developing countries, fast-growing urban populations are demanding more fresh water and food, while generating greater volumes of wastewater. Due to the lack of comprehensive wastewater management, a major portion of the wastewater pollutes natural water bodies [1]. These polluted sources are used in and around the cities for agriculture and other purposes. Soil pollution, is a very important environmental problem and it has been attracting considerable attention in recent years. Deteriorating soil quality and decrease in vegetation abundance are grave consequences of open waste dumping which have resulted in growing public concern [2].In modern economies various types of activity including agriculture, industry and municipal produce a larger amount of wastes and new type of pollutants. Soil, air and water have traditionally been used as sites for disposal of all these wastes. Heavy metal contamination of soil is a major environmental problem that can reduce both the productivity of plant and safety of plant products as food and feed [3]. With the rise of global heavy metal contamination, the plants that can accumulate heavy metals might provide efficient and ecologically sound approaches to sequestration and removal of these pollutants [4]. Plants of a high metal bioaccumulation capacity and a good tolerance to high metal concentration over long periods of time could be efficient phytoremediator.

Since 30 years ago many places in Libya are subjected to untreated waste water discharge. Al-Marj plain is an example of such places. It has been impacted for many years by untreated industrial, agricultural and domestic waste waters through different discharges and drains [5].This Study aims to:1- Evaluate the concentrations of heavy metals (Cd, Cr andCu) in soils,waste water and different plant organs.2- Define which species and which plant organs exhibit the greatest accumulation and evaluate whether these species could be usefully employed in phytoremediation programs.

Materials and methods

Site characterization

The plant ,waste water and soil samples used in this study were collected from a known waste water contaminated site located in Al- Marj plain, located on the southern coast of the Mediterranean Sea in the province of Cyrenaica[6], Cyrenaica houses Al-Jabal AlKhdar (the Green Mountain),. The study area extends from Farzougha west to Al-Beyda city and bordered northeasterly by Batta and southeasterly by Gardis. It is penetrated by some agro - and populated areas [7].Astronomical site extends between latitudes 33º and 31ºN and longitudes 20.30º- 21.30ºE. It depends on ground water of 200-350m deep as the chief source of the potable water [5]. In Al-Jabal Al-Akhdar area precipitation is the main factor that determines vegetation cover and land use. Traditionally, extensive dry farming cultivation of wheat and barley and stock-herding with goats and sheep were the main activities in Al- Jabal AI-Akhdar region [8].our observation showed that the vegetation was few and non-compact.plant species collected were the most common/dominant species at the polluted area. A total of thirteen plants ,waste water and soils (at0-20 cmdepth from roots of each plant were taken from each site from where plant samples was rooted)were collected in March 2013 in triplicate from studied located for heavy metal determination and their scientific names and characteristic were determined .

Sampling

Soils as well as thirteen abundant and dominating native plants *(Eucalyptus leucoxylon, Nicotianaglauca, Amaranthusviridis, Conium mculatum, Pinushalepensis, Phragmitesaustralis, Ceratoniasiliqua, Juncusrigidus, Cupressussempervirensvar. horizontalis, Ficuscarica, Ricinuscommunis, Urticadioica* and *Foeniculumvulgare).* were collected from seven polluted locations in the study area for each soil sample heavy metals were measured.Plants sampling at least three whole plants of each species of current year were collected and then washed using tap water then distilled water to remove all the debris and other foreign particles, and then separated into stems, leaves and roots to identify the different accumulation capability and selectivity of each organ. The native plant species were identified according to [9]. For waste water samples were collected in polyethylene bottles About 1 liter waste water sample collected from each location in the study area. These samples was acidified immediately in the field with Nitric acid (1ml HNO_3/L) for heavy metals determinations.

Analytical techniques

Soil samples (a composite mixture) were air dried, then dried in a furnace at 120^0C for 48 h. After drying, samples were cleaned off from any stones and plant residues, grounded in a stainless steel soil grinder then, passed through 2 mm sieve.for heavy metal analysis,one gram of homogenized samples was digested using HNO_3-HF-H3BO_4 acids according to [10]. Plant materials were oven-dried to a constant mass into oven at 80^0C and grounded to a fine powder.in this way homogeneous samples were obtained for each plant organ. Approximately 0.2 gram of leaves, roots and stems powder were weighed and digested according to method described by[11].waste water samples were filtrated to remove suspended solids by glass fiber filters (Millipore type HA 0.45 um pore size, from Millipore Corporation, Bedford, Massachusetts 01730, USA) using a vacuum pump according to standard procedures[12] . About 1 liter water sample collected from each location in the study area. These samples was acidified immediately in the field with Nitric acid (1ml HNO_3/L) for heavy metals determinations. Soil, waste water and plant samples were analyzed for heavy metals by Perkin-Elmer 2380 atomic absorption spectrophotometer which calibrated with standard solutions containing known concentrations of each element. Standard solutions were prepared by diluting available high purity stock solutions (BDH).average values of three replicates were taken for each determination.The precision of analytical procedures was expressed as relative standard deviation which ranged from 510%/ was calculated from the standard deviation divided by the mean. Bioaccumulation factors (BAF) was calculated using the formula outlined by [13].

BAF = element concentration in plan rootst/ element concentration in waste water.

RESULTES AND DISCUSSION

Waste water, soil and plant analysis

Domestic waste water contain fairly high concentration of metals such as copper, iron, lead and zinc, which are derived from household products such as cleaning materials, toothpaste, cosmetics and human feces[14]. Also, there are additional quantities introduced (illegally into water canals) from industrial effluents. Most of the heavy metals are extremely toxic because of their solubility in water. In the present study, heavy metal concentrations in waste water samples were decreased in sequence of Cr> Cu> Cd. The same pattern was recorded for the studied metals in the polluted soil samples collected from the same area. Excessive accumulation of heavy metals in agricultural soils through waste water effluent, may not only result in soil contamination, but also affect food quality and safety [15]. Average Cd,Cr and Cu concentration of waste water in the seven polluted locations are given in(Table 1).

Cadmium (Cd) is one of the most toxic heavy metal and is considered non-essential for living organisms [16].Plant treated with higher concentrations of Cd usually become stunted in growth [17]. Data collected from the present study showed that waste water Cd level exceeded the upper limit of the [18 and 19](0.02,0.01and0.01 μgml^{-1}respectively), The maximum Cd waste water concentration was attained at location 4 (0.87 μgml^{-1}).

Chromium (Cr) is one of the toxic metals widely distributed in nature. It has two forms found in the environment, trivalent and hexavalent. The latter form is considered to be the greatest threat because of its strong oxidizing ability as well as high solubility and availability to penetrate cell membranes [20]. Results obtained from this study, showed that Cr concentrations in waste water were higher than 0.73μgml^{-1} in all the investigated polluted locations .Thus exceeds the upper limit of [18and 19] (0.074, 0.1and 0.1gml^{-1} respectively). Copper (Cu) is a micronutrient for plants at very low concentration. However, excessive c

On centration of this metal are considered to be highly toxic. Mining, smelting and land application of fertilizers and sewage sludge together with the use of fungicides containing Cu and other human activities has led to wide spread soil contamination with Cu[21].Results obtained from this study, showed that Cu concentrations in waste water were higher than 0.40μgml^{-1} in all the investigated locations. Thus exceeds the upper limit of [18 and 19] (0.013 0.2 and 0.2 μgml^{-1} respectively).Cu speciation and toxicity in water may be influenced by a variety of physicochemical variables, particularly water hardness, alkalinity, pH and natural organic matter [22].

According to the results of this study, the native plants and soil can well present further information about the metal content of their environment. Average soil Cd, Cr and Cu concentration of the seven polluted locations are given in (Table 2). Average content of Cd in soils lies between 0. 06 - 1.1 μgg^{-1}d.w. [23]. The average soil Cd concentration recorded in the current study for the polluted locations was relatively high, 4.37μgg^{-1}d.w. Cd is considered as a heavy metal of most concern because it shows the greatest mobility in the soil environment [24] . The main anthropogenic sources of Cd are the amendment of agricultural soils with Cd contaminated bio-solids, phosphate fertilizers and industrial biproducts [25].In the current investigation, values of extractable Cd of all polluted soils samples were higher than limit values in soils (1-3 μgg^{-1}d.w) according to the Council of the European Communities[26]. except for locations 2 and 7 (2.95 and 2.66 μgg^{-1}d.w respectively).Sewage sludge, smelting operation and phosphate fertilizers are important sources of higher Cd concentration in the topsoil[23].Generally, the highest Cr concentration of soil samples collected from the polluted locations(46.70μgg^{-1}d.w) was recorded at location 3. This location is directly affected by the discharge of industrial effluents of a nearby textile and garments factories as well as municipal sewage sledges. While Cu concentration in the soils of the studied location lies within the accepted range (50-140 μgg^{1}d.w) of soil Cu content as reported by Council of the European Communities [26].However, the investigation native plants exhibited different element concentrations depending on plant organ and the sampling site.Results also indicated that Cd could be accumulated in all plant organs (root, stem and leaves). The distribution of Cd within plant organs is quite variable and clearly illustrates its rapid translocation from roots to shoots [23]. According to [27], Cd content of most plants did not exceed 1.9μgg^{-1} d.w. However, the Cd content recorded for all the studied species of the polluted locations exceeded this threshold and reached up to 22.78 μgg^{-1}d.w. *Eucalyptus leucoxylon* stem are given in Fig.1.

Results from the present study showed that the maximum Cr concentration in all the investigated plant species was always contained in the root system. The highest Cr concentration was attained by the roots of *Ceratoniasiliqua*at location 7 (395.43 μgg^{-1} d.w). This is in agreement with[28 and 29] .This could be because Cr is immobilized in the vacuoles of the root cells and showed less translocation, thus rendering it less toxic. According to [30] the toxic levels of Cr in plants range from 1 to 10 μgg^{-1} dry weight .Cr content of the weight .Cr content of the studied species could be arranged according to the pattern:

Amaranthusviridis>Coniummculatum>Phragmitesaustralis>Eucalyptusleucoxylon>Pinushalepensis>Foeniculumvulgare>Nicotianaglauca>Ficuscarica>Cupressussempervirensvar.horizontalis>Ricinuscommunis>Juncusrigidus>Urtica dioica>Ceratoniasi liqua were given in Fig.2

Generally, roots of most of the studied plants in the polluted locations attained higher Cu concentration than other organs with maximum value of 578.15 μgg^{-1}d.w attained by *Phragmitesaustralis* root. On the other hand, leaves and stems of both *Cupressussempervirens* and *Pinushalepensis* were found to accumulate considerable amounts of Cu (> 350 μgg^{-1}d.w)(Fig.3). This is agreement with [28].Cu concentrations in plant tissue above 10-30 μgg^{-1}d.w are regarded as poisonous [30]. Within roots, Cu is associated mainly with cell walls and is largely immobile. However, higher concentration of Cu in shoots (leaves and stems) are always in phases of intensive growth and at the luxury Cu supply level[31].High concentrations of Cu in the roots of *Phragmitesaustralis* with relatively high pH values in soil (> 7.0) may be attributed to the presence of plaque ,a metalrich rhizo -concentrations composed of iron hydroxides and other metals that are mobilized and precipitated on the root surface[32].

Bioaccumulation in plants

Accumulation of metal ions varied greatly among plant species and uptake of an element by a plant is primarily depended on the plant species, its inherent controls and the soil quality [33]. Large number of factors control metal accumulation and bioavailability associated with soil and climatic conditions, plant genotype and agronomic management, including: active/passive transfer process, sequestration and speciation, redox states, the type of plant root system and the response of plants to elements in relation to seasonal cycles [34].The bioaccumulation factor (BAF) of the investigated metals, measured in comparison with the total content of metals in water, is a parameter used to describe the transfer of trace elements from polluted water to plant tissue. However, the bioaccumulation factors (BAF) increased in the sequence: Cu > Cr> Cd for most of the species in almost all location. Most of the plants under investigation had BAF>1 .Current results also reviled that *Conium mculatum* acquired the highest BAF value for Cd, Cu, (1,112.58-1,009.41) followed by *Juncusrigidus*. However, the latter species acquired the second highest BAF value for Cr and Cu (310.09872.37). *Phragmitesaustralis* is characterized by having the highest BAF value for Cr. *Urticadioica* acquired the highest BAF value for Cd (Table 3). Heavy metals tolerant species with high BAF can be used in phytoremediation programs of contaminated soils as these species retains metals in their roots and limit metal mobility from roots to shoots once absorbed by roots of plants [35].]According to the results collected from this investigation, the studied native plants and soil can well present further information about the metal content of their environment. Moreover, *Conium mculatum*, *Juncusrigidus*, *Phragmitesaustralis*and *Urticadioica*could be used successfully in bio-monitoring and phytoremediation programs for heavy metal contaminated soils.

Table. 1 .Average concentrations (mean± SD) of the investigated heavy metals in waste water samples (μgml^{-1}) collected from the studied polluted locations. Highest and lowest values are underlined.

Location	Cd	Cr	Cu
1	0.40 ± 0.02	0.97 ± 0.14	0.72 ± 0.02
2	0.69 ± 0.05	1.01 ± 0.06	0.53 ± 0.17
3	0.46 ± 0.05	0.81 ± 0.17	0.57 ± 0.05
4	0.87 ± 0.04	0.84 ± 0.06	0.43 ± 0.11
5	0.26 ± 0.08	0.73 ± 0.08	0.82 ± 0.04
6	0.79 ± 0.05	1.44 ± 0.02	0.64 ± 0.11
7	0.73 ± 0.10	0.78 ± 0.16	0.48 ± 0.10

Table 2. Average concentration (± SD) of the investigated heavy metals in the soil samples (μgg^{-1}d.w) collected from the polluted studied locations. Highest and lowest values are underlined.

Locations	Concentration (μgg^{-1}d.w)		
	Cd	Cr	Cu
1	5.06 ± 0.60	32.30 ± 0.67	67.24 ± 0.25
2	2.95 ± 0.66	41.28 ± 1.80	42.33 ± 0.66
3	4.78 ± 0.55	46.70 ± 1.40	71.85 ± 0.49
4	6.55 ± 0.44	32.81 ± 1.26	77.11 ± 0.02
5	5.35 ± 0.52	42.16 ± 0.60	88.27 ± 1.45
6	3.25 ± 0.43	31.90 ± 0.95	60.92 ± 0.92
7	2.66 ± 0.46	38.14 ± 0.88	53.07 ± 0.17

Table 3. Bio-accumulation factor (BAF) of Cd, Cr, Cu, in the selected species collected from the studied locations in the polluted site. Highest and lowest values are underlined.

Locations	Species	Cd	Cr	Cu
1	*Amaranthusviridis*	29.40	293.67	329.21
	Conium mculatum	14.70	142.14	536.52
	Foeniculumvulgare	4.76	97.01	99.53
	Ricinuscommunis	5.21	76.83	122.33
2	*Conium mculatum*	19.50	138.62	598.45
	Eucalyptus leucoxylon	59.43	115.74	501.36
	Foeniculumvulgare	4.88	133.77	147.96
	Ricinuscommunis	7.62	83.64	121.10
3	*Amaranthusviridis*	29.83	230.49	702.20
	Conium mculatum	12.68	189.96	843.78
	Nicotianaglauca	24.66	73.10	58.44
	Pinushalepensis	10.12	108.51	620.64
4	*Conium mculatum*	11.71	306.73	590.17
	Eucalyptus leucoxylon	24.40	218.96	290.26
	Nicotianaglauca	22.96	158.71	41.84
	Pinushalepensis	14.48	180.35	454.13
5	*Conium mculatum*	1,112.58	259.27	345.96
	Ficuscarica	862.34	170.15	87.92
	Juncusrigidus	687.74	107.96	141.02
	Urticadioica	1,071.88	244.78	85.14
6	*Conium mculatum*	10.08	167.97	654.28
	*Cupressussempervirens*var. *horizontalis*	14.29	60.59	757.06
	Ceratoniasiliqua	15.05	103.40	331.04
	Phragmitesaustralis	15.58	172.51	508.26
7	*Ceratoniasiliqua*	16.29	190.88	441.39
	Conium mculatum	15.47	111.86	1,009.41
	Juncusrigidus	10.91	310.09	872.37
	Phragmitesaustralis	16.86	318.48	677.67

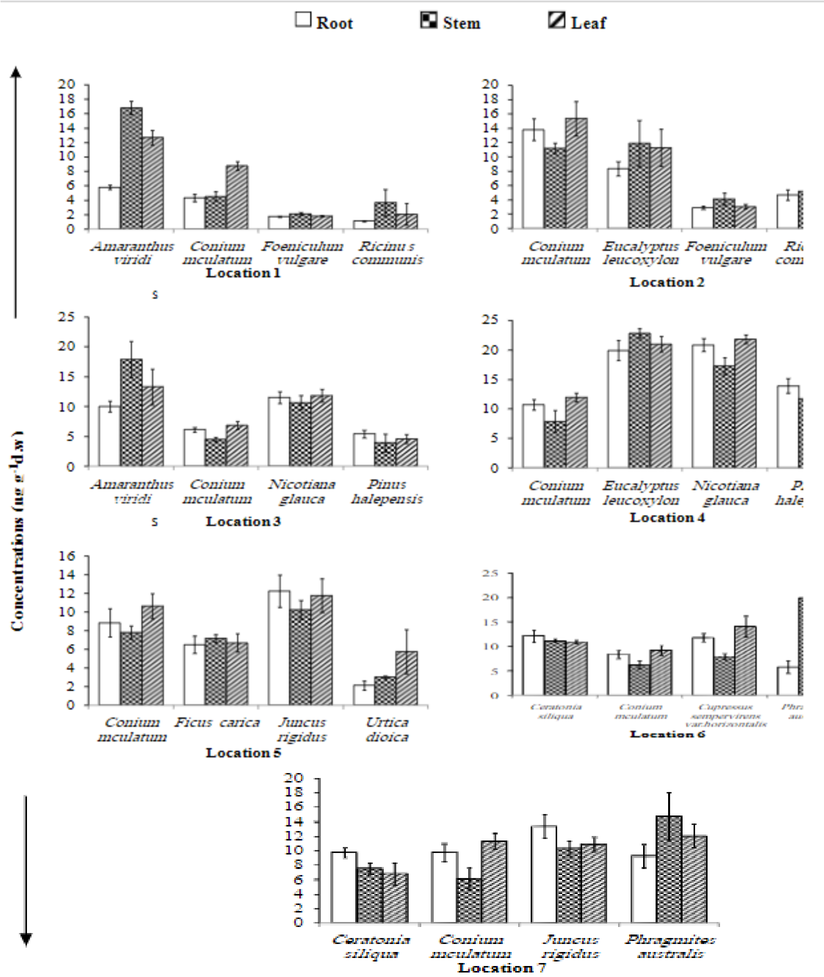
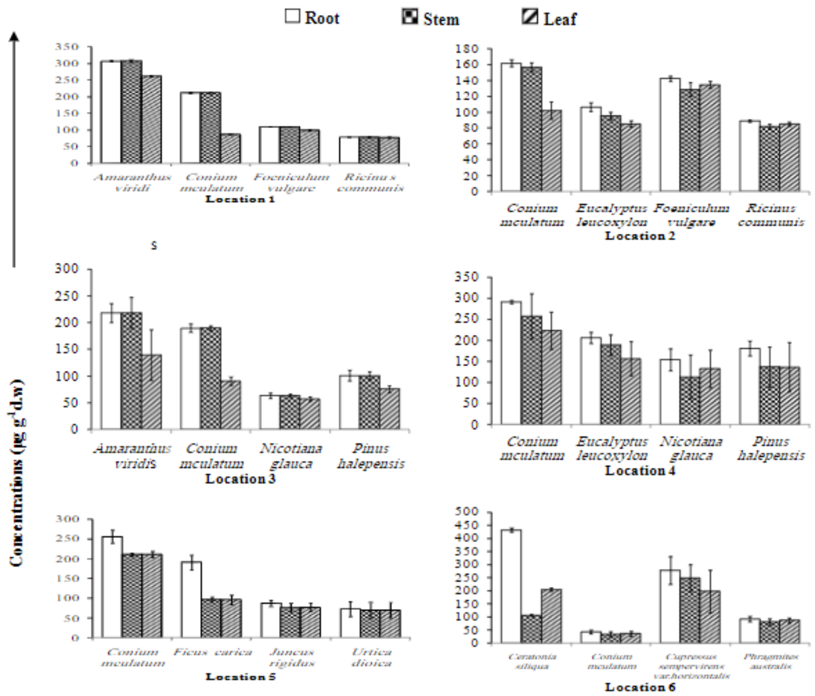


Fig.1.Average concentration of Cd in different organs of the selected plant species collected from the polluted studied locations together with standard deviation (SD) bars.



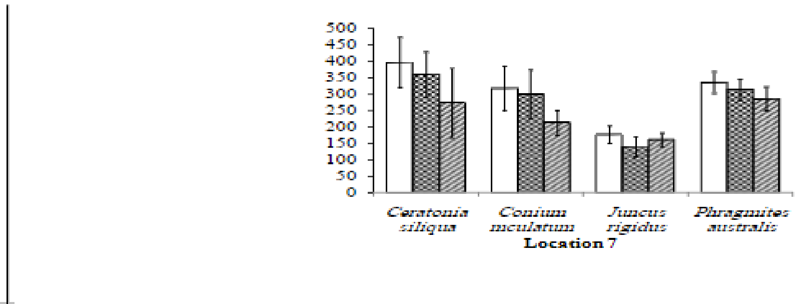


Fig. 2. Average concentration of Cr in different organs of the selected plant species collected from the polluted studied locations together with standard deviation (SD) bars.

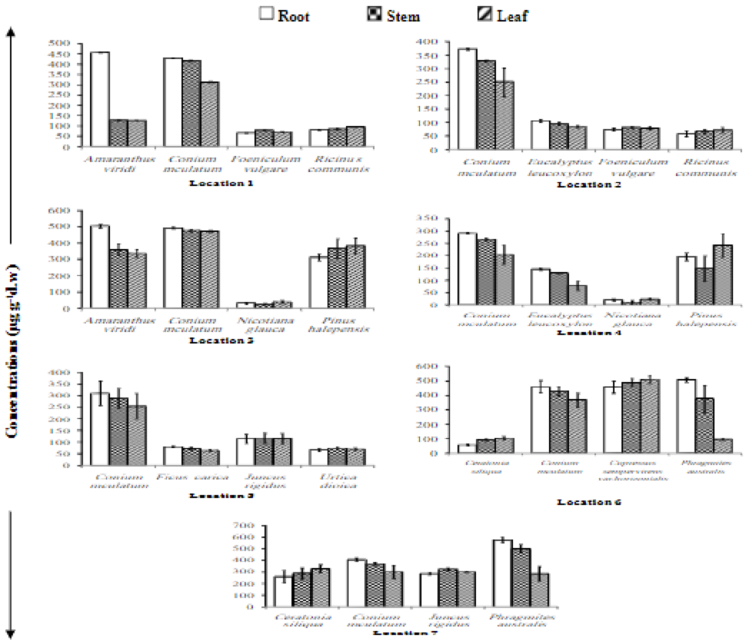


Fig.3.Average concentration of Cu in different organs of the selected plant species collected from the polluted studied locations together with standard deviation (SD) bars

Recommendation:

Serious actions to be taken to halt pollution by industrial and domestic waste water in AlMarj plain,and toothe *Conium mculatum*, *Phragmitesaustralis*, *Juncusrigidu* sand *Urticadioica* could be successfully used in biomonitoring and phytoremediation programs of polluted soils.

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