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THE EFFECT OF SOME HEAVY METALS ACCUMULATION ON PHYSIOLOGICAL AND ANATOMICAL CHARACTERISTIC OF SOME *Potamogeton* L. PLANT

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Abstract- The degree of contamination by heavy metals (copper, silver) on some plants of genus *Potamogeton* L. has been studied. Plants were exposed to metal treatments of Cu and Ag for three weeks. Leaves and stems were harvested for studying anatomy and analyzing metal accumulation. Accumulation of Cu and Ag in all parts of the plant increased significantly with an increase in applied metal concentration Total chlorophyll content and biomass declined progressively with increasing concentrations of the heavy metal. In Ag the plant *P.crispus* more effective in the total protein reach 2.65%, while in *P. perfoliatus* was 6.32%, the Cu found the lower effect on total protein research to 7.34% in *P.crispus* at 15 mg/l concentration and 10.92% in *P. perfoliatus* compared with Ag treated.

Anatomical analyses of stem *Potamogeton* plants revealed several changes in the Leaves and stems of plants submitted to contaminated treatments. *Potamogeton* exhibits hydrophytic adaptations which include gradual changes in leaves and stem structure with an increase in metal concentration compared with the control treatment. The leaves of plants exposed to contamination presented modified anatomical characteristics. Exposure to heavy metals leads to a reduction in the size of blade thickness, number of conducting elements, reduced cell size of the epidermis and aerenchyma tissue. Stems undergo changes in size, shape and arrangement of cortical parenchyma cells, plants of the treatment with more contamination had widened cell spaces in the cortex of parenchyma cells, reduced in vascular bundles.

Keywords- Aquatic plants. Potamogeton, heavy metal, Chlorophyll, Protein, biomass, anatomy

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Introduction

The diversity in aquatic plants and the wide spread in water, in addition tolerance the changing environmental conditions, different species of plant families have used as evidence of the study of water pollution heavy elements. It is also becoming widely used in the field of purification of Biofilteration [1]. Many plants are capable of accumulating heavy metals (called hyperacumulators), the biological activity and physical structure of soil maintained by using technique is cheap [2], but accumulation and distribution of heavy metals in the plant depends on the plant species, bioavailability, pH, cation exchange capacity, dissolved oxygen, temperature, and secretion of roots [3].

Zeidler [4] reported that plant tolerance and heavy metal management is based on multiple physiological mechanisms. There were stated differences between concentrations in selected plant tissues. The presence of heavy metals disturbs the normal plant metabolism, but the response is determined by many factors: the internal concentration of the pollutant, the level of tolerance for the pollutant or its toxic derivatives, developmental (ontogenetic) stage, edaphic and climatic factors [5]. These effects manifest in decrease in germination, increase in seedling mortality, inhibition of growth rates and reduction in reproductive capability. For the essential micronutrients, there is usually a threshold concentration, below which no adverse effects are observed, but with elevation of levels above the threshold, monotonically increasing toxic impacts are evinced [6]. Takeda, et al [7] study focused on temperature and relationship with transportation of heavy metal in plants.

Among aquatic macrophytes pondweed *Potamogeton* L. (Potamogetonaceae) is a common wetland plant that grows widely in tropic and warm regions, mostly freshwater. Plants are sometimes annuals but are often perennial and typically produce rhizomes, all the leaves are submerged. There are some studies on some species of *Potamogeton* [8], there was a significant relationship between Cd concentration in samples of plants and water pH value. It has been found that the tissues of *Typha angustifolia* accumulate more heavy metals than the tissues of *Potamogeton pectinatus*. In the water *Potamogeton*, structures that use to absorb minerals, nutrients from water and soil and conduct these substances through the plant are greatly reduced, considerable absorption takes place through stem [9]. Jamnická, et al [10] relived that the highest values of heavy metals content were observed in submersed dissected and small linear-leaved species, e.g. *Batrachium penicillatum, Fontinalis antipyretica, B. aquatile* and *Potamogeton pusillus*. In submersed aquatic plants, higher heavy metals content was recorded than in floating leaf species when he studied the content of heavy metals - Zn, Cu, Pb and Cd in the phytomass of 21 aquatic plants growing mostly in runnig waters of Western and Central Slovakia. While studied *Potamogeton pectinatus* and *Potamogeton malaianus* by Peng, et al [11]. Heavy metals stress was studied in *Potamogeton pectinatous* and *Potamogeton crispus* were treated with 4 different metals of same concentration of 5 ppm for 5 day in aseptic condition in culture. They observed that *Potamogeton pectinatous* and *P. crispus* survive in treatment with heavy metal without visible damage. *Potamogeton pectinatous* was better adopted under metal stress in comparession to *Potamogeton crispus* [12]. Qinsong, et al [13] showed that 48-69% from heavy metal accumulation within tissue wall when exposure *Potamogeton crispus* to different concentration of cadmium (0, 20, 40, 60 & 80) uml/ L and has been studding the accumulation of nutrients, mineral photosynthesis and protein content.

Aquatic plants vary in their ability to accumulate metal in their tissues such as the uptake of iron and copper by duckweed (*Lemna minor* L.) and water velvet (*Azolla pinnata* R. Br), the uptake rate of ions was highest on the uptake rate of copper [14]. *Typha latifolia* plants are tolerant to heavy metals toxicity, where at the same time their ability to accumulated metals in their tissue is substantial. The plants are supplied with all the necessary for their development micro and macronutrients both their ability to accumulate heavy metals as at the same time survive the toxicity is increased [15]. All the three aquatic plants: parrot feather (*Myriophylhum aquaticum*), creeping primrose (*Ludwigina palustris*), and water mint (*Mentha aquatic*) were able to remove Fe, Zn, Cu, and Hg from contaminated water [16]. The adsorption properties of *Myriophyllum spicatum* (Eurasian watermilfoil) for lead, zinc, and copper were studied by Keskinkan, et al [17] as well as on *Ceratophyllum demersum* [18]. Bioaccumulation pattern of some heavy metals was studied in plants by Ayari, et al [19] reported that Cu, Ni, Cr, Pb and Zn absorbed from plants increase level of risk of these elements for plants and food chain. Hamzah [20] the comparison of absorption by aquatic plants *Hydrilla verticillata* and Water hyacinth (*Eichhomia crassipes*) of malathion insecticide residues in water. Tajadod and Moogouei [21] reported that the plants can be able to survive in polluted environment (as applied method for filtrating sewage), is that they must have high performance in refining sewage, beside they not only absorb pollutants, but also show less toxic effects during process.

There are some anatomical changes can causes via heavy metal such as copper or cadmium applied alone or in combination caused reduction in root diameter, width and thickness of leaf midrib, diameter of xylem vessels of all seedling organs, parenchyma cell area in the stem, leaf midrib and pith and cortex of root, dimensions of stem vascular bundles, number of xylem arms in root, and frequency of stomata on abaxial leaf surface of *Sorghum bicolor* (L.) Moench [22]. On the other hand, reduction in root growth may be due to a decrease in cell division that led to increase the thickness of cell wall, and or a disorder in the activity and contents of phytohormones like auxin in the roots exposed to heavy metals [23]. Farzadfar and Zarinkamar [24] reported that heavy metals may cause anatomical changes in leaves, stems and roots. High absorption and accumulation of heavy metals in roots of different plants result in different anatomical changes, such as diameter of root, central vein, phloem and xylem of roots, size of precycle, epidermal and parenchyma cells were affected by experimental treatments were affected by an increased Cd stress. Copper, magnesium and ammonium sulfate have an inhibitory effect on stomatal parameters of wheat, the length of stomata is more sensitive to treatment with polluted water compared to other dorsal and ventral characteristics of wheat varieties seedlings [25].

The aim of the presented study was to determine plants of aquatic ecosystems may accumulate ions of toxic metals (Cu, Ag), as well as the present work aimed at study anatomy and doing cross-sections of plant organs of genus *Potamogeton* exposure with heavy metal for three weeks, which absorbed metal ions are accumulated.

Material and Methods

Experiments were conducted in College of Science, Department of Biology. The physicochemical properties were determined using standard methods of APHA [26]. Plants of *Potamogeton crispus* and *P. perfoliatus* and (approximately 100 g fresh weight) were individually treated with different concentrations of Ag and Cu for 2 and 7 days. Two sets of each experiment were kept in plastic beakers for each metal concentration and harvested after 7, 14 and 21 days. The harvested plants were washed thoroughly with distilled water, using $Ag(No_3)$ and $Cu(No_3)_2$ and prepared (5, 10, 15) mg/L.

The chlorophyll contents of fresh leaves were estimated by the method of Arnon [27] using 80% acetone. Prepared (5, 10, 15) concentration. 1 gm of the powdered plant material was weighed using a balance (Sartorius BL.210, Germany). The plant materials were ground with 20 ml from 80% acetone and filtered. Returned the ground by using 15 ml and 10 ml of 80% acetone and returned again. The chlorophyll concentration in mg g^{-1} of fresh leaves was estimated using Spectrophotometer aperture (645 and 660) nm. The chlorophyll concentration was calculated using the formula given by Duxbury and Yentsch [28].

The nitrogen of plant sample estimated by the method of Cresser & Parsons [29], and then protein content of leaf tissues was estimated by the method of Horwitz [30]. The biomass estimation of fresh weight, collected after three weeks of exposure in fresh weight / m^2.

For anatomical sections: permanent cross-section of stems and leaves were prepared. Leaf and stem sections were cut in middle, part of the midrib and stems. The plant parts were harvested after 7 days and cut into 10-15 cm pieces and fixed at least 48 hrs. in formalin-acetic acid alcohol (FAA) and preserved in 70% alcohol, then dehydrated in ethyl alcohol series, sectioned on a rotary microtome and stained in safranin and fast green and then mounted in Canada balsam on glass slides [31]. 100 sections of untreated and treated plant parts from each treatment were analyzed. The best five transverse sections were selected for study of anatomical characteristics. The sections were examined with Olympus CH4 light microscope and photographed with Digital camera type DCE-2 [32-35].

Statistical Analysis

All data were subjected to analysis of variance (ANOVA) and to determine the significance difference between treatments least significant difference (LSD) using the SPSSv.10 package.

Result and Discussion

Chlorophyll estimation

[Fig-1], [Fig-2] summarize the results for the effects of selected heavy metals (Cu, Ag) on total chlorophyll. Total chlorophyll content declined progressively with increasing concentrations of the heavy metal, the more effective in 15 mg/l concentration at the last week of experimental compared with control. In Ag the total chlorophyll of *P. crispus* was 15.122 mg/l in control treatment and began to decrease until reached to 6.557 mg/l at 15 mg/l concentration, and 7.043 mg/l in *P. perfoliatus* [Fig-1], as well as total chlorophyll content of leaves decreased with increasing Cu concentration, it decreased from 9.871 to 8.885 mg/l at 15 ppm concentration of Cu in *P. perfoliatus* and *P. crisps* respectively [Fig-2].

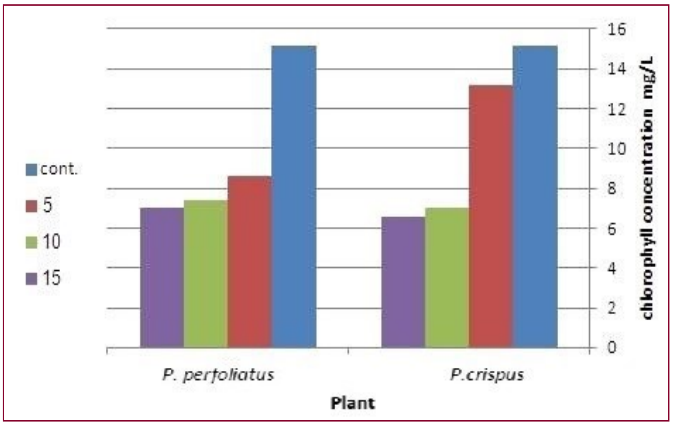

Fig. 1- Effect of different concentrations of Ag on total chlorophyll content (mg l $^{-1}$ f.wt.) on *P. crispus* and *P. perfoliatus* at exposures (days)

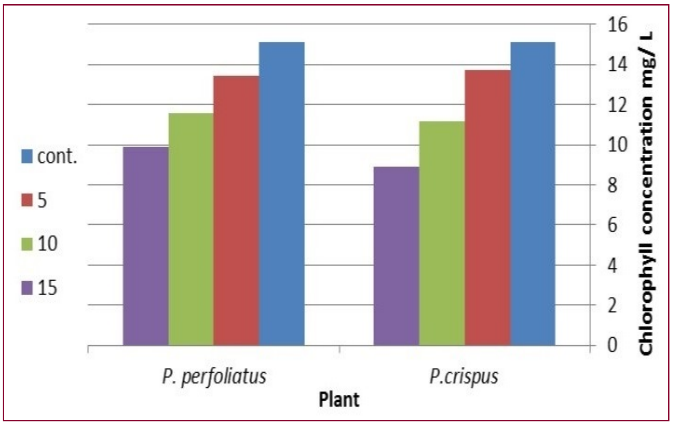

Fig. 2- Effect of different concentrations of Cu on total chlorophyll content (mg l $^{-1}$ f.wt.) in *P. crispus* and *P. perfoliatus* at exposures

Many researchers have reported decreased chlorophyll in several different plant species under the impact of heavy metals [12,15,36, 37]. Chlorophyll content is often measured in plants in order to assess the impact of environmental stress, as changes in pigment content are linked to visual symptoms of plant illness and photosynthetic productivity. Ag and Cu toxicity inhibit metabolic processes by inhibiting the action of enzymes for chlorophyll biosynthesis, and this may be the most important cause of inhibition and decreased chlorophyll content [38]. On the other hand ion toxicity affects photosynthesis by causing distortion of chloroplast ultrastructure, inhibiting synthesis of photosynthetic pigment in chlorophyll content and enzymes of the Calvin cycle [39].

Copper one of the heavy metal can be inhibit photosynthesis and reproductive processes, and ultimately plant growth becomes limited or impossible [19]. Increased contamination reduced the photosynthetic rate by approximately 50% in the treatment exposed to 15% of contamination [40]. On the other hand, the plants which are growing in polluted environmental conditions have anatomically and morphologically different chloroplasts than plants growing in natural environmentally conditions, the polluted leaves have lower chlorophyll content than control leaves due to chlorophyll in leaf mesophyll have irregular shape, with pockets or invaginations inside the organelles, both of them make the surfaces of chloroplasts larger, and result in an increase in the amount of substances exchanged between the chloroplasts and cytoplasm [41].

Uptake of heavy metal caused heterogeneity of damage in cells of a single tissue. It might represent a random distribution of heavy metal resistance, demonstrating phenotypic variability of cells within a tissue. But it might also be a hint towards an active stress response reaction of the plants and then these cells used for dumping heavy metals would die, but this would ensure the survival of the remaining cells, which were kept at low intracellular heavy metal concentrations [36]. Heavy metals inhibit metabolic processes by inhibiting the action of enzymes, and this may be the most important cause of inhibition. Decreased chlorophyll content associated with heavy metal stress may be the result of inhibition of the enzymes responsible for chlorophyll biosynthesis [38].

Protein Estimation

[Fig-3] shows the changes in the concentration of the total protein for effect of Cu and Ag in plants in 15 mg/l. In Ag the plant *P. crispus* more effective in the total protein reached 2.65%, while in *P. perfoliatus* was 6.32%, the Cu found the lower effect on total protein reach to 7.34% in *P. crispus* at 15 mg/l concentration and 10.92% in *P. perfoliatus* compared with Ag treated, Ag causing the greatest effect. Significant value found between different concentrations in (p<0.05). Our findings substantiate those of Hampp, et al [42], Satayakala & Jamil [43] on various aquatic plants. Ghani [44] in both the shoots and roots, the percent of nitrogen varied inversely with the amounts of metals added.

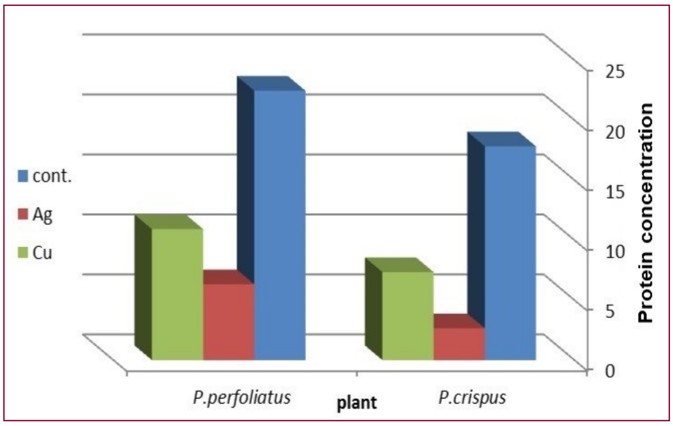

Fig. 3- Effect Ag and Cu on protein content in *P. crispus* and *P. perfoliatus* at 15 mg/l concentration

Cu and Ag Accumulation

Cu and Ag accumulation in plants of *Potamogeton* treated with different concentrations (0.0, 5, 10 and 15) mg/L resulted that the lower accumulated was (88.65) µg g^{-1} in fresh weight of Ag and in *Potamogeton perfoliatus* while in *P. crispus* accumulated more Cu was 123.46 µg g^{-1} of fresh weight [Fig-4]. The maximum absorption of Ag and Cu was found after three weeks of treatment in both the plants. All plants have the ability to accumulate heavy metals which are essential for their growth and development. These metals include Mg, Fe, Mn, Zn, Cu, Mo & Ni, and the heavy metal resistance and tolerance mechanisms have been suggested, especially for Cu, Zn, Ni & Cr, in plants growing on metalliferous soils [45].

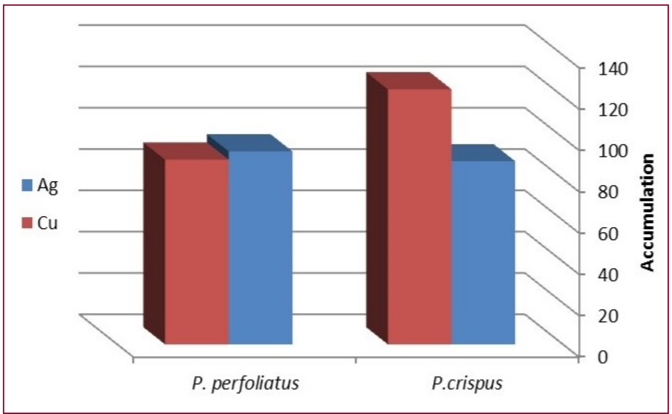

Fig. 4- Accumulation of Ag and Cu in *P. crispus* and *P. perfoliatus* tissue at three weeks of exposure

We have observed significant amount of Ag and Cu in plant tissue of *Potamogeton crispus* and *P. perfoliatus* possibly due to a constant contact of leaves and stems with water or the metal ions when enter roots, they are either stored in roots or translocations to shoots through xylem [39].

The metal ions enter roots, they are stored in roots or translocate to shoots through xylem, but in *Potamogeton* some of metal can absorbed via shoots or leaves possibly due to the leaves directly uptake heavy metal from water in addition to translocation from roots [39,46], in some plants involves binding toxic metals at cell walls of roots and leaves, or storing them in a vacuoles or complex them to certain organic acids or proteins and others plants make stable metal complexes in the root cells to prevent metal translocation from the roots to above-ground tissues [47].

Biomass Estimation

[Fig-5] showed the changes in the biomass in 15 mg/L for effected of Cu and Ag in plants.

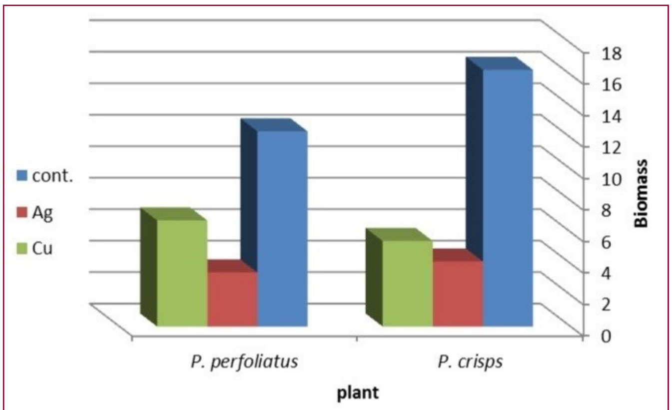

Fig. 5- Effect 15 mg/l of Ag and Cu on biomass of *P. crispus* and *P. perfoliatus* after three weeks of exposure.

The biomass decreased with Ag and Cu concentration, biomass in *P. perfoliatus* treated with Ag treated was 3.43 and 4.13 gm of *P. crispus*, in Cu treated was 6.74 gm in *P. perfoliatus* and 5.43 gm of fresh weight at 15 mg/l. The highest values of Ag and Cu content were observed in *P. crispus* this may due to the habit of plant such as it leaves oblong as observed earlier in aquatic plants [10] when studied the content of heavy metals Zn, Cu, Pb & Cd in the phytomass of 21 aquatic plants growing mostly in runnig waters of Western and Central Slovakia. Uptake and accumulation of metal can be cytotoxic in some plant species, Some metals such as Cu, Pd and Zn developing in contamination water causes decreased biomass production in *Potamogeton perfoliatus* and *P. pectinatus* [48]. Metal uptake by a living biomass needs two steps, one for binding between the elements' ion and the chemical receptor, and the second is accumulation by deposition [49].

Anatomical Studies

Transverse Sections of Leaves

The leaves of plants exposed to contamination presented modified anatomical characteristics [Fig-5] and [Table-1]. The epidermis of the adaxial and abaxial sides thickened as contamination, in *P. crispus* exposure with Ag the upper epidermis thickness of control 10.8 um while it was 12.9 um in *Potamogeton crispus* in 5 mg/l concentration and increased to reach 25.22 um in 15 mg/l concentration. In the lower epidermis reached to 66.65 um in 15 mg/l concentration [Table-1]. While in *P. perfoliatus* the upper epidermis thickness was 22.5 um in control and increased to 27.22 um in 15 mg/l [Table-2]. Our results found in Melo, et al [50], which have been reported that size variations in epidermal tissues in response to water pollution conditions. Increased thickness of the abaxial and adaxial, as caused by heavy metals, could be related to adsorption of metals in the cell walls, constituting an alternative pathway for allocation of these ions and preventing their translocation to photosynthetic tissues [40].

Exposure to heavy metals leads to a reduction of blade thickness to reached 15.21 um in *P. crispus* and 29.50 um in *P. perfoliatus* in 15 mg/l concentration in Ag metal [Table-1], [Table-2]. Exposure to heavy metals leads to a reduction in the thickness of mesophyll cells [51], could justify the thinned leaf blade observed in the treatments exposed to contamination. Conditional on anatomical plasticity, some species develop modified leaf tissues that allow better adaptability to different stress conditions [50].

The decrease in cellular size and intercellular spaces with an increase in metal concentration in Ag and Cu treated plants [Table-1], [Table-2]. The increase in a number of cells and area of endodermis could thus be a compensatory mechanism for the loss of the photosynthetic area due to a reduced leaf parenchyma. Also, considering its filtering function, the increase in the number of cells and area of the endodermis could be an adaptive measure to lower metal translocation to the chlorophyll parenchyma, preventing possible damage to the primary CO2 fixation system [40]. Reduced thickness of the leaf blade in *Potamogeton crispus* and *P. perfoliatus* is due to the reduced cell size of the epidermis and aerenchyma.

Deposition of metal ions in the cells was also observed highly reduced vascular bundles [Table-2], [Fig-1]. Reduction in the number of conducting elements of the xylem in response to heavy metals has been reported in literature as being an adaptive measure to secure water flow [52]. Directing the deposition of heavy metals to non-photosynthetic tissues could be a plant strategy to tolerate toxic levels of heavy metals, in a study with *Salix viminalis* cultivated in the presence of Cd, Vollenweider, et al [53] observed increased thickened walls of the collenchymas and pericycle, with higher concentrations of metal than the other tissues. Metal distribution among leaf tissues tends to occur as a means of minimizing its concentration in the chlorophyll parenchyma, preventing damage to the photosynthesis. An increased proportion of these tissues could also justify greater leaf turgor in the treatments exposed to contamination. Marsh plants accumulate toxic ions of the elements mainly in the epidermal layer and the cortex mesophyll [54].

Table 1- measurement of leaf blade in Potamogeton in micrometer

Species	Concentration	Blade thickness	Upper epidermis thickness	Lower epidermis thickness	Mid rib thickness
Potamogeton crispus Ag	Control	(45-48.60) 46.8	(9-14.40) 10.8	(18-27) 21.6	(91.43-212) 137.60
	5	(23.50-50.40) 34.4	(8.13-17.50) 12.9	(21.40-33.60) 27.20	(150.30-220.10) 190.80
	10	(18.10-37.40) 21.6	(12.90-17.20) 14.33	(17.20-31.50) 28.63	(228.12-236.50) 231.47
	15	(10-17.50) 15.21	(17-28) 25.22	(60.50-68.80) 66.65	(289-320) 305.33
Potamogeton crispus Cu	5	(40-49.50) 45.76	(6.32-14.40) 11.87	(30.40-33.60) 30.23	(120.10-250.30) 180.90
	10	(25.32-37.50) 35.60	(10-22) 15.54	(45.22-60.50) 58.13	(224.50-336.5) 264.39
	15	(12.32-20.20) 18.23	(15.20-20.30) 20.22	(48.34-78.20) 69.45	(250.90-390.10) 345.26

Table 2- measurement of leaf blade in Potamogeton in micrometer

Species	Concentration	Blade thickness	Upper epidermis thickness	Lower epidermis thickness	Mid rib thickness
Potamogeton crispus Ag	Control	(58-72) 61.28	(14.5-27) 21.59	(10.50-19) 15.32	(89.71-186.20) 107.50
	5	(34.40-43) 38.70	(22.9-27.2) 25.05	(8.60-17.20) 15.78	(150-250) 220.61
	10	(20.60-44.88) 32.16	(12.9-28.30) 25.21	(10.20-30) 25.21	(116-137) 227.56
	15	(20-37.42) 29.50	(17.5-30.40) 27.22	(56-75.03) 72.41	(140-350) 273.27
Potamogeton crispus Cu	5	(33-44.40) 46.28	(20.59-27.5) 24.95	(15.23-27.62) 19.75	(120-280.20) 210.72
	10	(20.20-45.80) 30.22	(15.69-35.50) 27.53	(15.23-37) 28.54	(116-137) 256.23
	15	(20-37.52) 27.50	(27.25-40.47) 37.22	(36-65.03) 62.65	(140-350) 360.23

Transverse Sections of Stems

The Ag and Cu in (5, 10, 15) mg/l concentration treated plants showed gradual changes in leaves and stem structure with an increase in metal concentration compared with the control treatment [Table-3], [Table-4] and [Fig-7], [Fig-8]. The mean of stem thickness increased as a result of acuminated of Cu and Ag metal in plants, it was 1061.20 um in *P. crispus* and 1167.50 um in *P. perfoliatus* when treated with Ag metal concentration at 15 mg/l concentration, as well as Cu metal showed increased in stem thickness [Table-3], [Table-4]. Anatomical analyses of stem *Potamogeton* plants revealed several changes in the stem of plants submitted to contaminated treatments. Stems undergo changes in size, shape and arrangement of cortical parenchyma cells [Fig-7], [Fig-8], the changes in cell shape and organization suggests heavy metal interference in the stem due to the ability of heavy metal disrupt the hormonal balance [40]. In stem control treatment the transverse section of the epidermal cells of *Potamogeton* are square-shaped or rectangular, uniseriate, While in the stem epidermis was increased in the treatment exposed to the highest contamination level concentration, *P. crispus* and *P. perfoliatus* was 19.16 & 20.66 um respectively in 15 mg/l of Ag metal, while in Cu was 19.27 um in *P. crispus* and 19.54 um in *P. perfoliatus*. The difference in structure and function of epidermis in hydrophytes as compared with that of plants growing in aerial habitat is outstanding. The epidermis is not protective in hydrophytes but absorbs gases and nutrients directly from water [9].

The most pronounced anatomical feature of *Potamogeton* species are presence of gas filled chambers and passages in stems and leaves. Air chambers are large, usually regular (circular to hexagonal) intercellular spaces extending through leaf and long distances through stem. These chambers provide a sort of internal atmosphere for the plant. In these spaces oxygen emitted during photosynthesis is apparently stored and used again in respiration. Carbondioxide from respiration is accumulated and used in photosynthesis. The cross partition of air chambers are called diaphragms, perhaps for preventing flooding. Air chambers also give buoyancy to the organs in which they occur. In the physiological sense, aerenchyma is applied to tissue with many large intercellular spaces which seem to be a constant feature of this species.

The cortex is composed of pseudohypodermis and aerenchyma tissue. pseudohypodermis was reduced in the treatments exposed to contamination compared with control treatment [Table-3], [Table-4] and [Fig-7], [Fig-8]. The aerenchyma lacunae are regular and divided transversally by uniseriate diaphragms. Plants of the treatment with more contamination had widened intercellular spaces in the cortical parenchyma caused by contamination [Fig-7], [Fig-8]. Metal Ag and Cu have ability to acuminated in intercellular space as this resulted occurrence of metals [Fig-7H], there are some metal such as Cd has been accumulated in cell walls and intercellular spaces of the cortical parenchyma of roots [40], metal adsorption in intercellular spaces is probably a strategy of accumulator species. Aerenchyma formation is one of the most important strategies of wetland plants acclimating to the waterlogged condition, not only facilitating internal gas phase oxygen transport within plants, but also improving potential for radial oxygen loss to the rhizophere [55], and also showed that relationship was found between metal tolerance in wetland plants and their radial oxygen loss and stem anatomy.

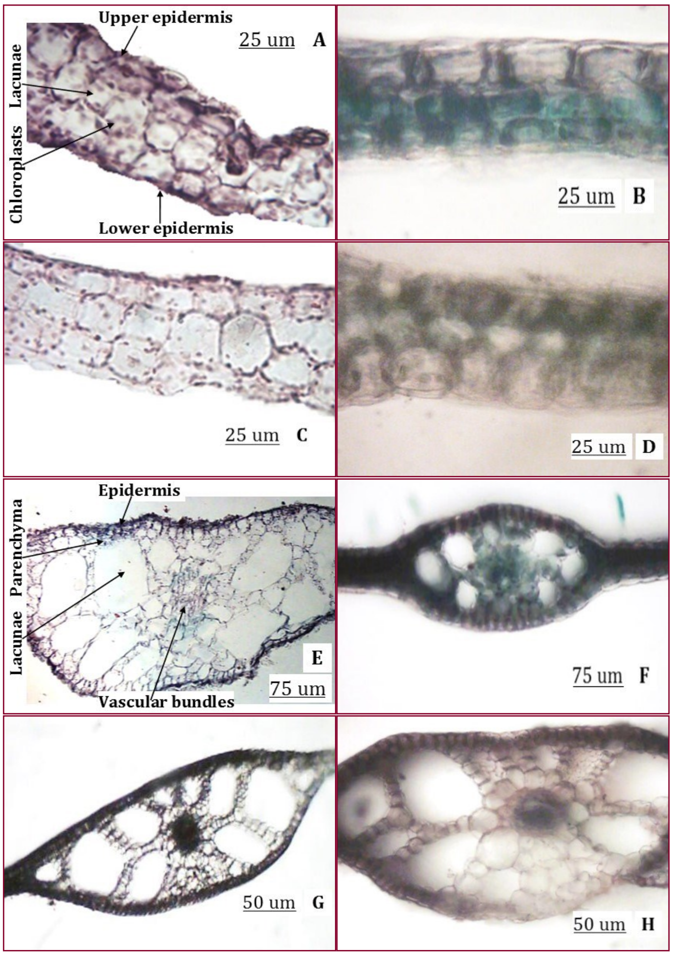

Fig. 6- Transverse section of Leaves and midrib of *Potamogeton*.

A- *P. crisps* control; **B-** *P.crispus* Ag 15 conc.; **C-** *P. Perfoliatus* control; **D-** *P. Perfoliatus* 10 conc. of Cu; **E-** *P. crispus* (midrib control); **F-** *P. crispus* 15 conc. with Ag treatment; **G-** *P. perfoliatus* Ag 10 conc.; **H-** *P. perfoliatus* Cu 10 conc.

[Fig-7] (D-F) shows the cross section view of stem and treated *potamogeton* plants on comparing with control, the treated stems *P. crispus* showed an irregular epidermal and cortex regions through the number of air chambers increased. Deposition of metal ions in the cells was also observed along with highly reduced vascular bundles. The cross sectional view of stem *P. perfoliatus* [Fig-8] (D-F) showed increased number of air chambers together with stained aernchyma and vascular bundles which might be due to deposition of metals [Fig-8] (E-H), which agree with Gupta, et al [39]. [Fig-7], [Fig-8] and [Table-3], [Table-4] showed that reduced in vascular bundles compared to control plants reach to 301.43 um in Ag at 15 mg/l concentration and 210.77 um in *P. perfoliatus*, while in Cu was 276.23 um in *P. crispus* and 196.25 um in *P. perfoliatus* [Table-3], [Table-4]. Also the vascular bundles lost their shape in the stems of Ag and Cu treated plants [Fig-7], [Fig-8]. Reduction in the number and size of conducting elements of the xylem in response to heavy metals has been reported in literature [56].

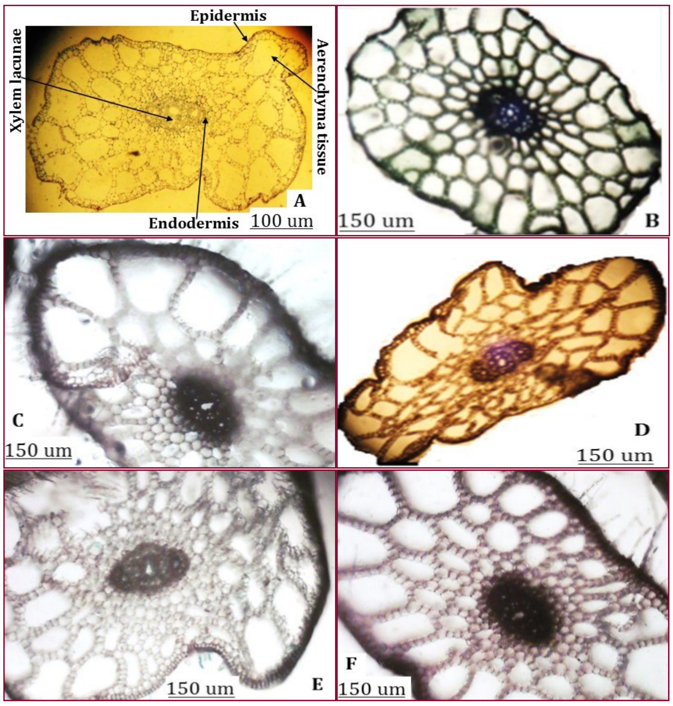

Fig. 7- Transverse section of stem of *Potamogeton crispus*.

A- control; **B-** *P. crispus* Ag 15 conc.; **C,D-** *P. crispus* Ag of 15 conc.; **E-** *P. crispus* of Ag in 10 conc.; **F-** *P. crispus* 15 conc. of Ag.

In control treatment the vascular bundles in *P. crispus* was lobed contain five vascular bundle in middle, one of them large [Fig-7], [Fig-8], while in *P. perfoliatus* the vascular cylinder is cylinder structure in transverse section, with four to six vascular bundles: two larger in the center and two smaller on each side, without pith formation. Both central and lateral bundles can exhibit fusion. Vascular bundle exhibit large lacunae of protoxylem, large phloem sieve elements and are partially surrounded by fiberss [Fig-7], [Fig-8](A). The tracheary elements of the xylem and cortical parenchyma of stems exposed to contaminated soil presented thicker walls than the control [Fig-7], [Fig-8]. The stems in contaminated treatments also presented a larger number of tracheary elements in the vascular cylinder. In *P. crispus* and *P. perfoliatus* the wall thickening of both xylem elements and accumulated the metal in vascular bundles [Fig-7](C-F), as well as thickening cortical parenchyma stem is another anatomical adaptation to heavy metals toxicity. Some authors suggest that in plant, the capacity to bind heavy metal in the cell wall has a protective action against the deleterious effect of heavy metals by reducing the amounts of cytosolic heavy metals [57,58]. This adaptation could also be associated to the capability to accumulate heavy metals by blocking ion reflux, particularly during periods of low or absent transpiration processes. On the other hand, reduction in root growth may be due to a decrease in cell division that led to increase the thickness of cell wall, and or a disorder in the activity and contents of phytohormones like auxin in the roots exposed to heavy metals [23].

Table 3- measurement of stems of Potamogeton in micrometer

Species	Concentration	Stem thickness	Epidermis thickness	Hypodermis thickness	Air chamber thickness	Vascular bundle thickness
Potamogeton crispus Ag	Control	(430-559) 505.25	(170-250.50) 180.50	(35.43-75) 61.34	(10-2.50) 5.40	(662-925.87) 814.50
	5	(450-475) 459.25	(150-300) 195.16	(37.50-75) 54.16	(5-10) 7.25	(645-989) 817.43
	10	(301-322.50) 310.66	(164.50-321.50) 243.76	(10-12.50) 11.43	(15-20) 17.25	(645-1419) 1032.50
	15	(299-320) 301.43	(250.5-351.80) 330.43	(5-7.50) 6.66	(12.5-27.50) 19.16	(1075-1290) 1061.20
Potamogeton crispus Cu	5	(330-355) 329.25	(140-270) 192.10	(27.50-62) 50.36	(5-11) 6.75	(540-829) 810.03
	10	(301-316.50) 292.44	(150.21-231.50) 210.86	(5-8.60) 6.33	(10.5-24.50) 16.16	(542-1121) 1020.55
	15	(210-289) 276.23	(230.50-391.80) 310.53	(10-12.50) 11.33	(17-24) 19.27	(975-1032) 985.65

Table 4- Measurement of stems of Potamogeton in micrometer.

Species	Concentration	Stem thickness	Epidermis thickness	Hypodermis thickness	Air chamber thickness	Vascular bundle thickness
Potamogeton crispus Ag	Control	(250-356) 310.16	(117.50-210) 160.75	(10-32.32) 24.32	(2.50-7.50) 5.8	(312.50-575) 445.50
	5	(258-279.50) 265.87	(100-265.80) 89.661	(12.50-25) 17.52	(5-10) 7.5	(250-530) 403.53
	10	(98-330) 239.65	(107.50-225.80) 198.54	(15.61-19) 17.33	(6.5-10) 8.99	(336-1050) 825
	15	(98-352) 210.77	(215-349.65) 272.66	(4-8) 6.98	(17-21.67) 20.66	(756-1334) 1167.50
Potamogeton crispus Cu	5	(212-250.50) 236.42	(194.60-251.60) 231.66	(13.50-23) 15.12	(3-8) 5.75	(260-475) 321.63
	10	(88-298.50) 222.28	(225-384.15) 291.62	(5-9) 7.10	(6.50-10) 8.12	(321-950) 672.15
	15	(78-287) 196.25	(290.50-395.30) 379.56	(12.32-17) 15.88	(14-23.45) 19.54	(650-1123) 976.45

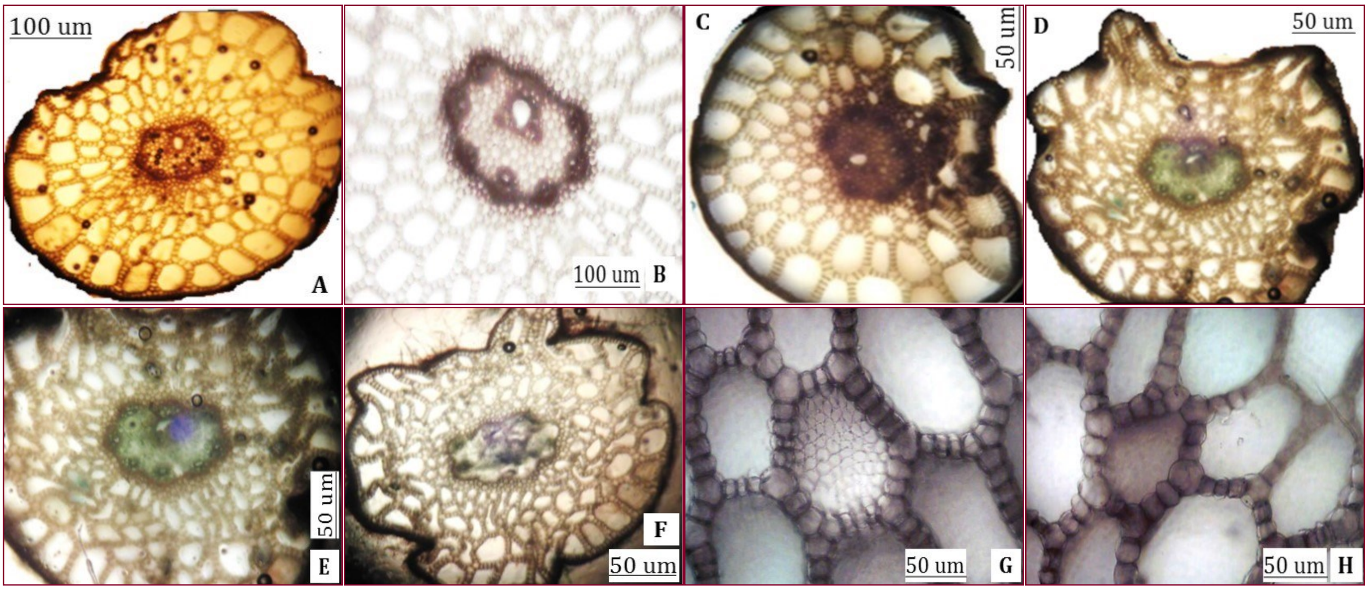

Fig. 8- Transverse section of stem of *Potamogeton perfoliatus*.

A- control (stem); **B-** Control (Vascular bundle); **C-** 10 + 15 Cu; **D,E-** Cu of 15 conc.; **F-** Cu of 15 conc.; **G-** control no stained; **H-** Ag of 15 conc. (stained air chamber with metal).

Conclusion

There was some inhibition in the plants growth noticed through reduction chlorophyll content, protein content and biomass recorded in the plants irrigated with the stronger of the higher concentration. In the same plants the larger heavy metals concentrations were also acuminated in plant tissue.

Aquatic plants vary in their ability to accumulate metal in their tissues. Both *P. crispus* and *P. perfoliatus* may be used as bioaccumulators because of their efficiency in metal absorption and abioindicators of Ag and Cu pollution due to their specific anatomical responses to the pollutant. The significant reduction in cell sizes in various plant organs may be due to arrested growth under stressed conditions as water contains heavy metals.

Conflict of Interest: None declared.

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