THE EFFECT OF SOME HEAVY METALS ACCUMULATION ON ANATOMICAL AND PHYSIOLOGICAL CHARACTERISTIC OF THE SUBMERGED MACROPHYTE VALLISNERIA PLANT

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Abstract— The degree of contamination by heavy metals (Hg and Pb) on *Vallisneria* plants has been studied. Plant was exposed to metal treatments of Hg and Pb for three weeks. Leaves were harvested for studying anatomy and analyzing metal accumulation. Accumulation of Hg and Pb in all parts of the plant increased significantly with an increase in applied metal concentration. Results showed the maximum heavy metal concentrations, in the root and minimum heavy metal concentrations in the leaves. Total chlorophyll content increasing concentrations of the heavy metal. In Hg was 5.50%, while in the Pb found the lower effect on total protein research to 4.22 % at 15 mg/l concentration.

Anatomical analyses of *Vallisneria* leaves revealed several changes in the leaves of plants submitted to contaminated treatments. 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. Leaves undergo changes in size, shape and arrangement of leaf blade and cortical parenchyma cells as well as reduced in vascular bundles.

Keywords— Aquatic plants, *Vallisneria*, heavy metal, chlorophyll, anatomy.

I. INTRODUCTION

Vallisneria spiralis L. (coiled vallisneria or tape grass) from Family: Hydrocharitaceae, it is an aquatic plant found in tropical and sub-tropical regions worldwide. *V. spiralis* is considered to be native to southern Europe, northern Africa, the Middle East, and southwest Asia. As well as, the species has spread into central, northern and northwestern Europe, France, Belgium, Netherlands, southern Britain, Poland and Russia[1,2,3, 4]. Pacific Island records of *V. spiralis*, in the Hawaiian Islands [5]and New Caledonia [6]. There are also geographically disjunctive introductions to the United Kingdom, North America, the West Indies and New Zealand. The use of the species as an aquarium plant, *V. spiralis* has a history of being applied indiscriminately to similar rosette plants from Asia, Australia, Europe and North America [7].

Vallisneria is a submerged perennial plant which is found naturally in the freshwater with fibrous roots. It is having horizontal runners and linear leaves.The plants dioecious with stems buried in mud; strap or tape shaped leaves arranged in a basal rosette, inflorescence axillary. Staminate plants, minute, numerous and imperfect flowers enclosed membranous spathe. Flowers with three sepals, slightly zygomorphic as well as pistillate flowers have scapes long, extended to spirally coil. Ovary inferior, unilocular; long, cylindrical, with mucilage-filled cavity containing numerous crowded ovules; fruit long, many seeds embedded in a gelatinous mass, enveloped by the persistent spathe; seed tapering, ellipsoidal [8, 9] For the environmental requirements *Vallisneria spiralis* tolerate low salinity and grows fastest in water temperatures of 25°C [10]. Rooted submerged plant species are considered to have value for phytoremediation because of their soil binding roots, rhizomes and stolons which help facilitate colonization by benthic algae, other microbes and invertebrates [11]. *Vallisneria spiralis* has been found to reduce cadmium, chromium and copper levels in water under laboratory conditions and removed heavy metals from wastewater [11, 12, 13]. Plants were effective in reducing COD, color and sodium content of effluents[14]. Heavy metal accumulation was studies in aquatic macrophytes used as biomonitors, in comparison with water and sediments for phytoremediation carried out by some workers. [15]studied heavy metals (Cd, Co, Cu, Ni, Pb and Zn) in sediments in roots, stems and leaves of seven species of aquatic plants *Vallisneria spiralis* L., *Ipomoea aquatica* Forsk, *Eichhornia crassipes*, (Mart.) Solms, *Typha angustata* Bory & Chaub, *Hydrilla verticillata* (L.f.) Royle and *Nelumbo nucifera* Gaerth.

Heavy metals are regarded as impactful, non-degradable toxic pollutants of natural water sources. Not only they persist within the environment but they also pose serious risks as far as population health is concerned since they can be accumulated by living organisms[16].

Phytoremediation is a new technology for the removal of toxic elements from the soils [17] It is used for reduce heavy metal pollution, include arsenic,

cadmium, chromium, copper, lead, nickel, and zinc, all of which pose risks for human health and the environment, this is often called phytoremediation [18]. It has the advantage of relatively low cost and wide public acceptance [19]. Plants can be used in different ways, Depending on plant species, metals may removed by two basic strategies: metal exclusion and metal accumulation[20, 21, 22] . Sometimes a contaminated site is simply passing through a process called phytostabilization. Plants would be necessary to find plants that could tolerate high levels of heavy metals.

In this study, the bioaccumulation of heavy metals such as Hg and Pb in the *Vallisneria spiralis* was analyzed. The specific goals were (1) to determine the heavy metal concentrations in the different organs (2) to determine the anatomical changes in leaves exposure on heavy metal concentrations.

II. MATERIALS AND METHODS

Experiments were conducted in College of Science, Department of Biology, Basrah University. The physicochemical properties were determined using standard methods of APHA [18].

Vallisneria spiralis was used at a rate of 100 gm fresh weight, individually treated with different concentrations (0.0, 1.0, 1.5 and 2.0 ppm) of Pb and Hg in the form of $PbSo_4$ and $HgSo_4$. Four replicates were prepared for each treatment. Two sets of each experiment were kept in plastic beakers for each metal concentration and harvested after 7, 14 and 21 days. The chlorophyll contents of fresh leaves were estimated by the method of Arnon [23] using 80% acetone. 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 [24]. The protein content of leaf tissues was estimated by the method of Horwitz [25]. The biomass estimation of fresh weight, collected after three weeks of exposure in fresh weight / m^2.

For anatomical sections: permanent cross-section of leaves was prepared. Leaf sections were cut in middle with part of the midrib. The plant parts were harvested after 14 days and cut into 10-15 cm pieces and fixed at least 48 hours in formalin-acetic acid- alcohol (FAA) and preserved in 70% alcohol, then dehydrated in ethyl alcohol series, sectioned on a rotary microtome then stained in Safranin and Fast green and then mounted in Canada balsam on glass slides [26]. 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 [27, 28, 29, 30] .

Statistical Analysis

All data were subjected to analysis of variance (ANOVA) and to determine the significance difference between treatments least significant difference (R.L.S.D) using the SPSS v.10 package.

III. RESULTS AND DISCUSSION

Chlorophyll estimation

Summarize the results for the effects of selected heavy metals (Pb, Hg) on total chlorophyll in [Fig-1]. Total chlorophyll content declined progressively with increasing concentrations of the heavy metal, the more effective in 2 ppm concentration at the last week of experimental compared with control. In Hg the total chlorophyll of *Vallisneria spiralis* was 15.32 mg/l in control treatment and began to decrease until reached to 5.30 mg/l in Hg treated at 2 ppm concentration, and 3.92 mg/l in Pb treated [Fig-1].

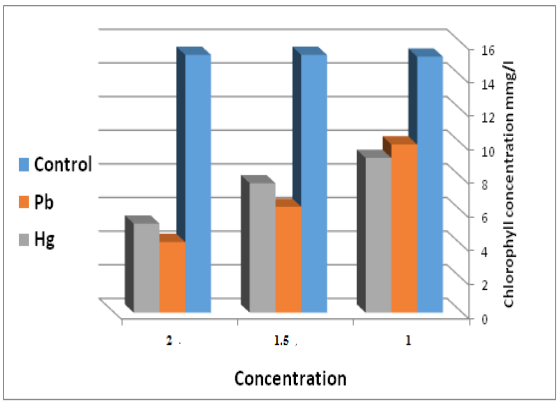
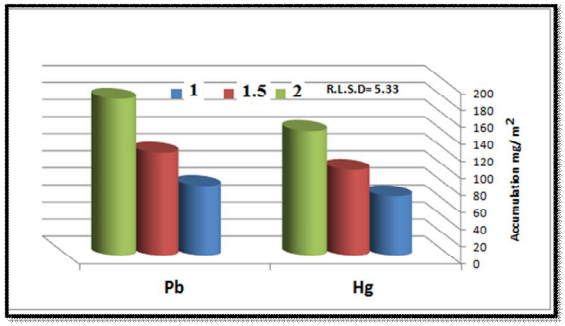


Figure- 1- Effect of different concentrations of Pb and Hg on total chlorophyll content (mg l^{-1} f.wt.) on *Vallisneria spiralis* at exposures (days).

decrease the chlorophyll content in plants reported from many researchers in several plant species under the impact of heavy metals [31,32, 33, 34,35]. This due to changes in pigment content are linked to visual symptoms of plant illness and photosynthetic productivity[36] or Inhibition of important enzymes and this may be the most important cause of inhibition and decreased chlorophyll content [36, 37, 38]. [4, 9,18] Heavy metals ions prevent uptake and transportation of other metal elements such as Mn, Zn and Fe by opposing effects and therefore cause the leaves lose the capacity of synthesis of chlorophyll [22, 21]. Our data recorded decrease in chlorophyll content. 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].

Accumulation estimation

Uptake of Hg and Pb Plant showed maximum accumulation of Pb (mg/m^2 dry weight of the plant material) in the 2 ppm treatment , but accumulation level of Hg was comparatively lower [Figure 2]. Our results are in confirmation with that of [38,39].Roots of *Vallisneria spiralis* are efficient barriers to Hg and Pb translocation to the above ground plant parts. The uptake of metals by plant is coupled to a chemiosmotic process across the membrane of intact root cells. [39] suggested that Pb is unevenly distributed in roots, where different root and stem tissues act as barriers to apoplastic and symplastic Pb transport and hence Pb transport to shoot gets restricted.

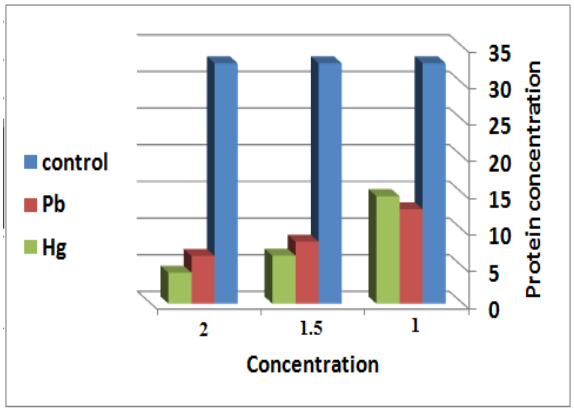


[Figure-2]- Effect of different concentrations of Pb and Hg on accumulation (mg m^2) on *Vallisneria spiralis* at exposures (days).

Estimation Protein

[Figure- 3] shows the changes in the concentration of the total protein for effect of Pb and Hg in plants in 2 ppm. In Hg the plant *Vallisneria* more effective in the total protein reached 4.02%, while in Pb was 5.25 % at 2 ppm concentration. Significant value found between different concentrations in (p <0.05). Our findings substantiate those of [40], Satayakala & Jamil [41] on various aquatic plants. Ghani [42] in both the shoots and roots, the percent of nitrogen varied inversely with the amounts of metals added Cd, as well as found more pronounced decrease in the protein content with heavy metal in *Lemna minor* [43]. Heavy metals have significant effects on chlorophyll and protein content in plants. These metals impede with synthesis of chlorophyll by inducing deficiency of Mg^{+2} ions [44, 45] .

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[Figure- 3] Effect Hg and Pb on protein content in *Vallisneria spiralis* at 2 ppm concentration.

Anatomical Studies

Transverse Sections of Leaves

In control treatment the leaf has aerenchyma tissue separated by 1-2 layers contains 1-2 cells, dorsal-ventral, consists of upper epidermis and aerenchyma tissue disposed under the lower epidermis[Figure- 4].

For the anatomical study, we used fresh material from *Vallisneria spiralis* harvested after 21 days from treatment. The leaf in control treatment has aerenchyma tissue separated by 1-2 layers as from cell to another, dorsal-ventral, consists of upper epidermis and aerenchyma tissue disposed under the lower epidermis. But 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,

Table 1- measurement of leaf blade in *Vallisneria spiralis* in micrometer

Vascular bundle thickness	Aernchyma thickness	Lower epidermis	Upper epidermis	Concentration (ppm)	Heavy metal
40.33	70.33	4.24	5.61	0	Control
32.31	65.53	7.43	7.43	1	Hg
30.22	41.88	11.33	8.55	1.5	
25.53	43.21	13.13	10.33	2	
40.10	51.33	7.66	5.82	1	Pb
34.90	48.51	7.90	6.98	1.5	
27.33	32.10	11.04	8.52	2	

in *Vallisneria spiralis* exposure with Hg the thickness of upper epidermis more in size compared with control treatment,it was in control 5.61 um,but in exposure treatment to high concentration increased reach to 10.33 and 8.52 um in Hg and Pb respectively in 2 ppm concentration. In the lower epidermis reached to 13.13 um and 11.04 in Hg and Pb respectively at 2 ppm concentration [Table-1]. Size variations in epidermal tissues in response to water pollution conditions. Increased thickness of the abaxial and adaxial, as

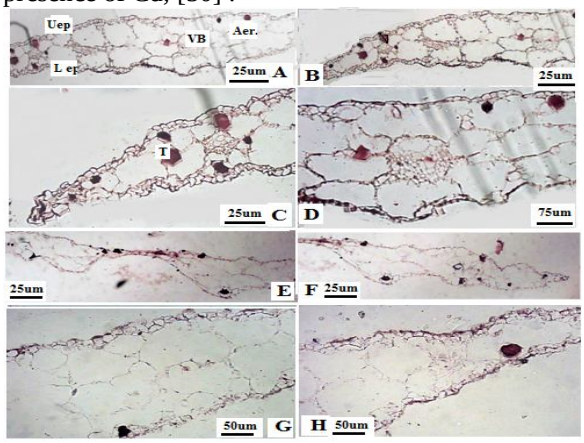
caused by heavy metals, could be related to adsorption of metals in the cell walls, constituting an alternative pathway for allocation of these ions and preventing their translocation to photosynthetic tissues [46, 47].

Exposure to heavy metals leads to a reduction of aernchyma thickness to reached 43.21 um in Hg treated and 32.10 um in Pb in 2 ppm concentration[Table-1].

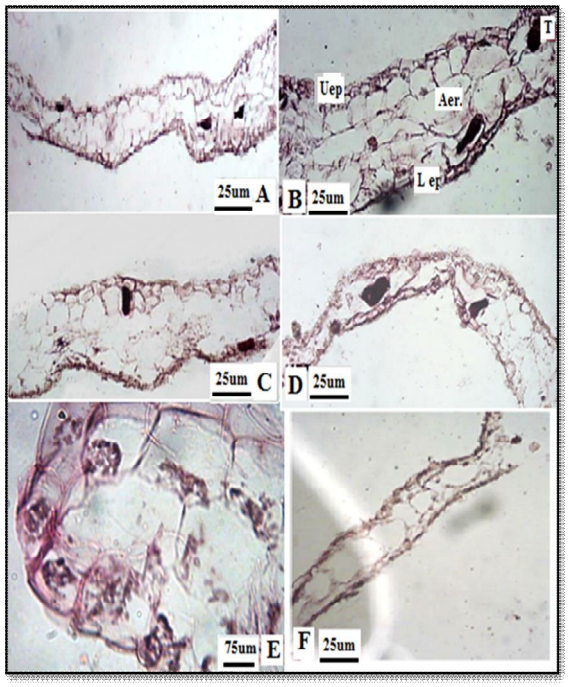
Exposure to heavy metals leads to a reduction in the thickness of mesophyll cells which agree with [48], could justify the thinned leaf blade observed in the treatments exposed to contamination. Conditional on anatomical plasticity, some species develop modified leaf tissues that allow better adaptability to different stress conditions [47].

Our results showed that decreased in cellular size and intercellular spaces with an increase in metal concentration in Hg and Pb treated plants [Table-1]. 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 CO_2 fixation system [46]. Reduced thickness of the leaf blade in *Vallisneria spiralis* 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-1], [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 [49]. Directing the deposition of heavy metals to nonphotosynthetic 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, [50] .



[Figure- 4] Transverse section of Leaves and midrib of *Vallisneria spiralis* . (A- B)control; C- control leaves ; D- control (midrib) E- Hg in 2 ppm conc. (reduced in leaf blade).; F- Hg in 2 ppm conc. Increased in cell size); G- Leaf blade Hg in 2 ppm conc. ; H- irregular cells Hg in 2 ppm conc. Uep:upper epidermis , Lep: Lower epidermis , Aer.: Aernchyma, VB:Vascular bundle, T: tannins.



[Figure- 5] Transverse section of leaves and midrib of *Vallisneria spiralis* . (A- reduced in leaf blade)B- increased in leaf blade under upper epidermis ; C- Pb in 2 ppm conc D- damage in tannin cells E- cytoplasm in aggregate inside cell in Pb in 2 ppm conc. : F- Pb in 2 ppm conc. Increased in cell size and reduced in leaf blade Uep:upper epidermis , Lep: Lower epidermis , Aer.: Aernchyma, VB:Vascular bundle, T: tannins.

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