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EFFECTS OF HEAVY METALS ON PHYSIOLOGICAL STATUS OF PLANTS

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Abstract

Plants efficiently remove contaminants from contaminated environments; however, when the contaminants accumulate in excess in plant tissues, they cause alterations in the vital growth processes of plants. The effects of four heavy metals, which were nickel, manganese, cobalt, and zinc on *Lemna* spp. and *Hydrilla verticillata* were investigated. The plants showed a decline in the growth, both chlorophyll and protein concentrations in plant tissues were reduced. Zinc and manganese were the most effective metals in reducing chlorophyll content in *Lemna* spp. and *Hydrilla verticillata* respectively, while the metals zinc and nickel were the most among the tested metals in reducing protein content in *Lemna* spp. and *Hydrilla verticillata* respectively. The effects of heavy metal concentrations and exposure period to heavy metals on plants' physiological status were significant, $P<0.05$. The plants can be used as an effective tool in removing contaminants from contaminated environments and choosing the plant species is based on the type of contaminant and its concentrations.

Key words: Heavy metals, protein content, chlorophyll content, *Lemna* spp. *Hydrilla verticillata.*

Introduction

Heavy metals are among the contaminants that pose serious environmental threats.

Beside the natural sources, heavy metals are released into the environment by anthropogenic activities, which are the major sources of heavy metals emission. The industrial activities such as mining, metal finishing, plating, lead smelting, ceramic, textile and glass industries constitute the major anthropogenic sources of heavy metals release to the environment (Keskinkan *et al.,* 2003). Heavy metals are emitted into the environment by various processes and pathways that contaminate air through metals extraction, combustion, and processing while water and soils are contaminated by heavy metals through runoff, leaks from metals storage sites and metals transportation (*Järup* 2003). Heavy metals emission in biologically-available forms by human actions leads to ecosystem alteration or impairment (Taylor *et al.,* 1989). Despite their known adverse health effects, heavy metals emissions to the environments continues, basically in the developing countries (*Järup* 2003). Due to their serious environmental threats and effects on human health, substantial attention has been paid to heavy metal removal methods. Several methods, such as, filtration, reverse osmosis, solvent extraction, electrochemical treatment, reduction, oxidation, precipitation, ion exchange, have been effectively implemented to remove heavy metals from contaminated environments (*Sternberg and Dorn* 2002). Most of these methods are expensive and inefficient in heavy metals removal. The use of aquatic plants to remediate the contaminated aquatic ecosystems is considered of an advantage over other methods of heavy metals removal due to its cost-effectiveness and environment-friendly (Srivastava *et al.,* 2006). Organic and inorganic environmental pollutants are degraded, metabolized, assimilated or detoxified into harmless forms by the green plants (Aisien *et al.,* 2010).

Aquatic macrophytes or hydrophytes are beneficial plants for aquatic environments since they have the potential to take up heavy metals from aquatic environments and accumulate them in their living tissues. Aquatic plants *such as Lemna minor*, *Pistia stratiotes, Eichhornia crassipes* and *Salvinia herzogii* have been effectively used to remove heavy metals from aquatic contaminated ecosystems (Lesage *et al.,* 2008). As inundated plants, they have the ability to take up heavy

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metals directly for the contaminated environments. *Lemna* spp. is a ubiquitous free-floating freshwater plant, and it provides foods and habitats for a variety of animals such as fish, invertebrates and aquatic birds. This plant has a number of important characteristics, such as the rapid reproduction cycle, the easy laboratory cultivation and the genetic diversity of different *Lemna* species, which makes it one of the appropriate plant species in toxicology studies (Kanoun-Boule *et al.,* 2009). *Hydrilla verticillata* (L.F. Royle) is a submerged native macrorphyte to warmer areas of Asia. This plant is well adapted to inhabit freshwater environment due to its specialized growth habit, high reproduction rate and physiological characteristics. *Hydrilla verticillata* causes serious economic hardship since it damage freshwater ecosystems, displace native species to aquatic environments and interferes with freshwater usage practices (*Langeland* 1996). Most studies have focused on the plants role in heavy metals removal, from contaminated environments. The objectives of this study were to determine the toxic effects of four heavy metals; nickel, manganese, cobalt and zinc on the physiological status of two aquatic plants, which were *Lemna* spp. and *Hydrilla verticillata*.

Materials and Method

Two aquatic plant species, which were *Lemna* spp. and *Hydrilla verticillata*, were used to investigate heavy metal effects on their physiological status. The plants were brought from Al-Hilla River, Babylon Province, Iraq (GPS coordinates: 32.567145, 44.484861). Heavy metal salts; Nickel(II) chloride ($NiCl_2$), Manganese(II) chloride ($MnCl_2$), Cobalt(II) chloride $CoCl_2$, and Zinc chloride ($ZnCl_2$) were used from the Al-Qasim Green University. The experiment was conducted on a laboratory scale by taking 250 gm wet weight of each plant and adapted in 15 L plastic containers, containing 10 L of dechlorinated water. Five sets of microcosms were set in triplicates, which were untreated control, treated with $NiCl_2$, treated with $MnCl_2$, treated with $CoCl_2$, and treated with $ZnCl_2$ for both *Lemna* spp. and *Hydrilla verticillata*. Three concentrations; 10, 20, 30 ppm were used for each heavy metal salt. The experiment was terminated after a month and the samples of the two plants were taken at days 1, 10, 20, and 30 to determine heavy metal, protein, and chlorophyll concentrations in the tested plant tissues.

Protein concentration

Total protein amount in plant tissues was determined according to Bradford method (*Bradford* 1976). A half gram of fresh plant tissues was smashed by a ceramic mortar; then 1ml of phosphate regular solution and 5 ml of Bradford solution were added. The spectrophotometry was measured by spectrophotometer Sp-300 on the wave length 595 nm and the protein content was expressed as mg/g of plant tissues.

Chlorophyll concentration

The total chlorophyll concentration in both plant tissues was determined as described by (*Aminotand Rey* 2000). 0.15 gm of fresh tissues of each plant was smashed by a ceramic mortar after adding 2 mL of 80% acetone. The smashed plant tissues were filtered through a Watmann No. 1 filter paper, 0.45 µm. The extract was gathered, and the size was completed to 15 mL with 80% acetone. The absorbance was measured by spectrophotometer Sp-300 on 645 nm and 663 nm. The total chlorophyll content mg/g of plant tissues was calculated according to the following equation.

$$\text{Total chlorophyll (mg /g)} = [A_{645}(20.2) + A_{663}(8.02)]\ v/w \times 1000$$

Where:

A_{645} = the absorbance at 645 nm

A_{663} = the absorbance at 663 nm

W = plant tissues weight

Heavy metal concentration

After 30 days of growth, aquatic plants tissues were collected to determine heavy metals concentrations. The tissues of each plant were dried at 70^0C, grounded, sieved through a 40 mm mesh. After that, 0.5 g of the grounded and sieved plants tissues were placed in a Pyrex digestive tube. The samples were left for 16 hours after adding 5mL of HNO_3. Then the samples were digested at 100^0C for an hour. Later, 3mL of 70% perchloric acid $HClO_4$ was added for each sample and a reflux was done to samples for 30 minutes at 200^0C until getting a clear solution. The samples were centrifuged for 10 minutes at 2000 rpm to get rid of any suspended matter in the sample solution. Finally, The samples were filtered through 0.45 µm and the samples were completed to 50 mL with deionized water. Heavy metals concentrations in µg/g of plant tissues were measured by atomic spectrophotometer (WFX-110B) (*Antonijevicand Maric* 2008).

Data analysis

Heavy metals concentration and exposure period effects on chlorophyll concentration and protein concentration for both *Lemna* spp. and *Hydrilla verticillata* was analyzed by two-way ANOVA using IBM SPSS 22.0 software (SPSS Inc., Chicago, IL, USA).

Results and Discussion

Environmental contamination by heavy metals is considered one of the main environmental stresses for plants; however, plants are used to remove heavy metals from contaminated soil and water. Plants absorb heavy metals from the contaminated soil and/or water through their roots and translocate them to their shoots. The contaminants when accumulate in higher concentrations in plant tissues cause alterations in the vital growth processes of plants such asbiosynthesis of chlorophyll (Mocquot *et al.,* 1996), photosynthesis (Yruela *et al.,* 1996), transpiration (Richardson *et al.,* 1993) and cell membrane integrity (Sinha *et al.,* 1997). Exposure to 10, 20, 30 ppm of four heavy metals, which were nickel, cobalt, zinc and manganese have affected chlorophyll concentration in *Lemna* spp. and *Hydrilla verticillata*

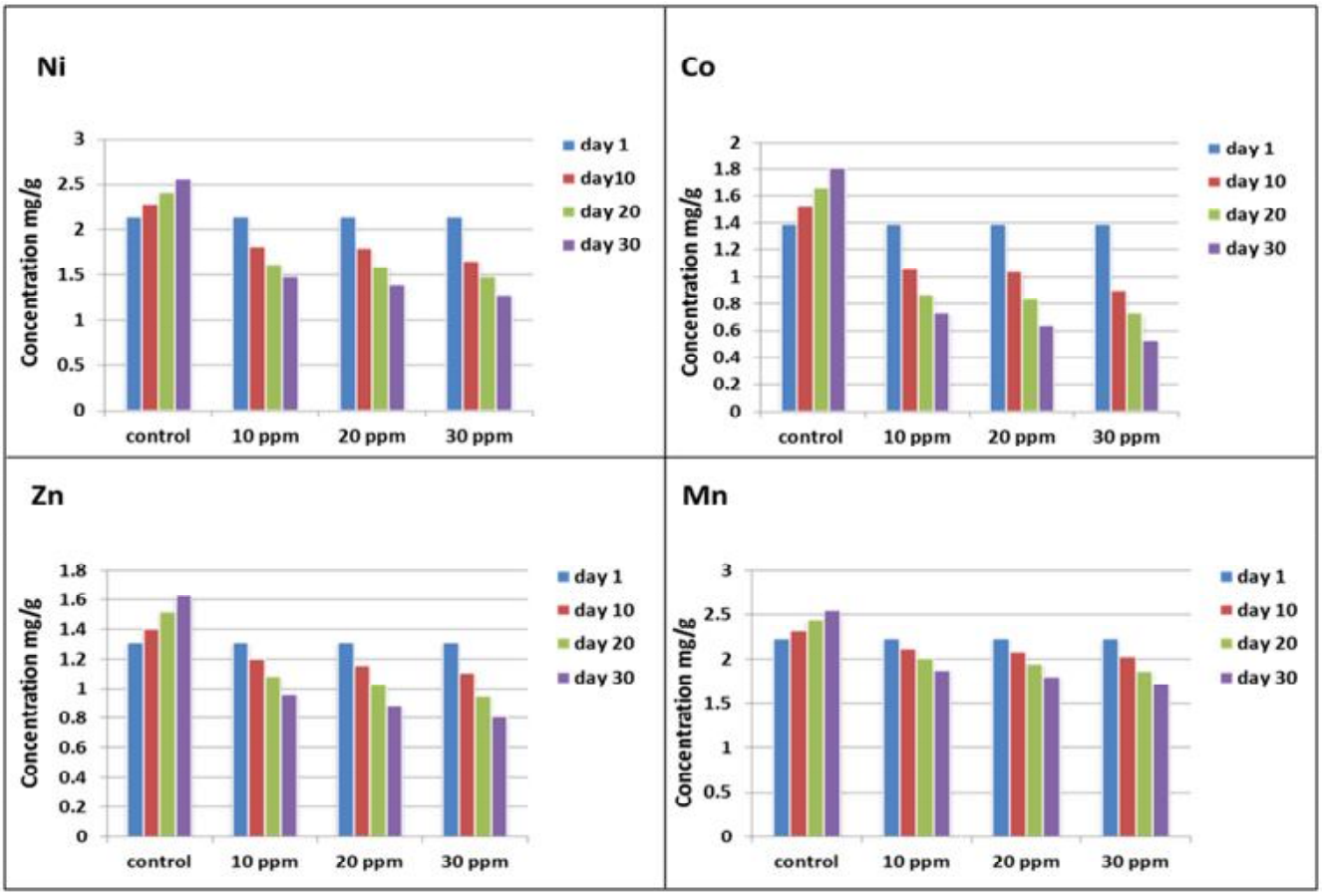

Fig. 1. Three different concentrations of nickel, cobalt, zinc, and manganese effects during four time periods on chlorophyll concentration in *Lemna* spp. tissues.

Table 1: Univariate analysis of variance for chlorophyll content in *Lemna* spp. tissues.

Tests of Between-Subjects Effects

Dependent Variable: concentration of chlorophyll

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	12.845[a]	15	.856	8.215	.000
Intercept	157.816	1	157.816	1513.902	.000
heavy metals	11.646	3	3.882	37.240	.000
days	1.075	3	.358	3.436	.024
heavy metals					
*days	.124	9	.014	.133	.999
Error	5.004	48	.104		
Total	175.665	64			
Corrected Total	17.849	63			

a. R Squared = .720 (Adjusted R Squared = .632)

Table 2: Univariate analysis of variance for chlorophyll content in *Hydrilla verticillata* tissues.

Tests of Between-Subjects Effects

Dependent Variable: Concentration of chlorophyll

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	8.294[a]	15	.553	6.911	.000
Intercept	231.002	1	231.002	2887.581	.000
heavy metals	7.448	3	2.483	31.035	.000
days	.752	3	.251	3.133	.034
heavy metals					
*days	.093	9	.010	.130	.999
Error	3.840	48	.080		
Total	243.136	64			
Corrected Total	12.133	63			

a. R Squared = .684 (Adjusted R Squared = .585)

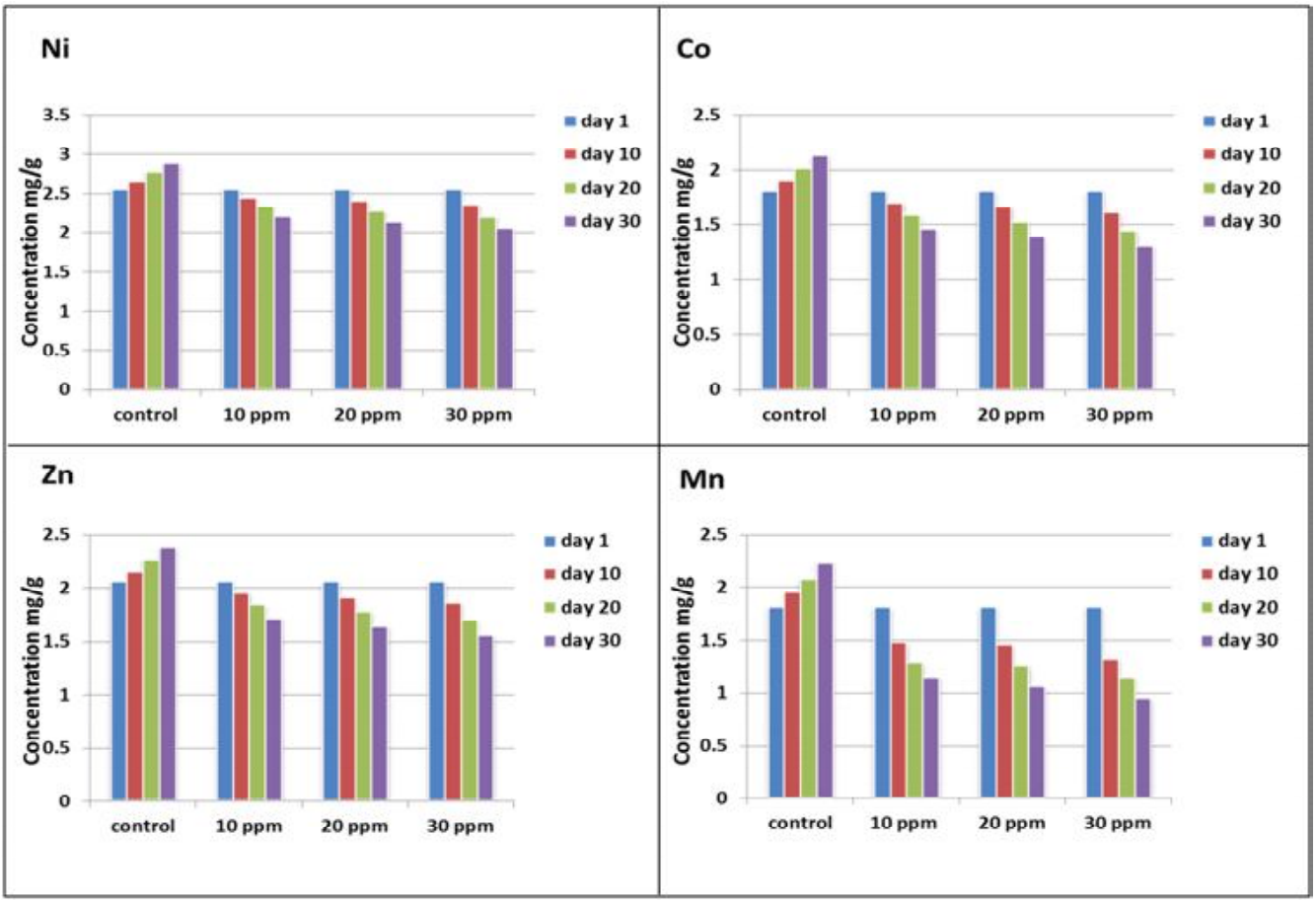

Fig. 2. Three different concentrations of nickel, cobalt, zinc, and manganese effects during four time periods on chlorophyll content in *Hydrilla verticillata* tissues.

Table 3: Univariate analysis of variance for protein content in *Lemna* spp.

Tests of Between-Subjects Effects

Dependent Variable: Concentration of protein

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	980.609[a]	15	65.374	2.081	.028
Intercept	22612.641	1	22612.641	719.885	.000
heavy metals	564.047	3	188.016	5.986	.001
days	412.422	3	137.474	4.377	.008
heavy metals					
*days	4.141	9	.460	.015	1.000
Error	1507.750	48	31.411		
Total	25101.000	64			
Corrected Total	2488.359	63			

a. R Squared = .394 (Adjusted R Squared = .205)

Table 4: Univariate Analysis of Variance for protein content in *Hydrilla verticillata* tissues.

Tests of Between-Subjects Effects

Dependent Variable: Concentration of protein

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	631.438[a]	15	42.096	1.512	.139
Intercept	27142.562	1	27142.562	975.182	.000
heavy metals	387.813	3	129.271	4.644	.006
days	242.062	3	80.687	2.899	.045
heavy metals					
*days	1.563	9	.174	.006	1.000
Error	1336.000	48	27.833		
Total	29110.000	64			
Corrected Total	1967.438	63			

a. R Squared = .321 (Adjusted R Squared = .109)

tissues. Chlorophyll concentrations were measured during four times; 1, 10, 20 and 30 days for the two plants. The concentration of chlorophyll in *Lemna* spp. tissues decreases as the concentration of the four tested heavy metals increase in a timely-dependent way Fig. 1. At all the tested concentrations, heavy metals effect on lowering chlorophyll concentrations in *Lemna* spp. and *Hydrilla verticillata* tissues were significant in a timely-dependent manner ($P<0.05$; $r^2 = 0.720$ and 0.684 respectively) Tables 1 and 2. Out of the four tested metals, zinc had the most significant effects on reducing chlorophyll concentration in *Lemna* spp. tissues. At 30 ppm of zinc, the concentration of chlorophyll was reduced from 1.31 mg/gat day 1 to reach 0.81 mg/g at day 30 Fig. 1. (Bonnet *et al.,* 2000) found that the increase in concentration of zinc causes a decrease in magnesium, copper, potassium

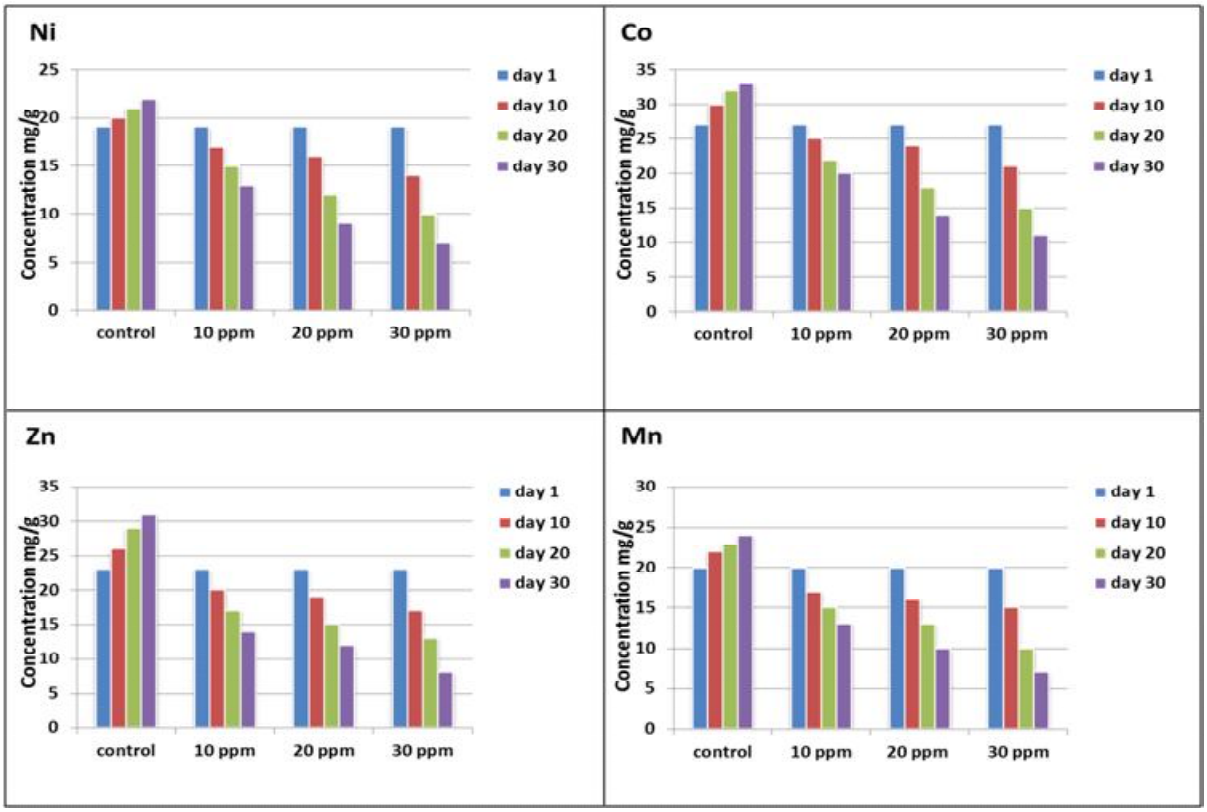

Fig. 3. Three different concentrations of nickel, cobalt, zinc, and manganese effects during four time periods on protein content in *Lemna* spp. tissues.

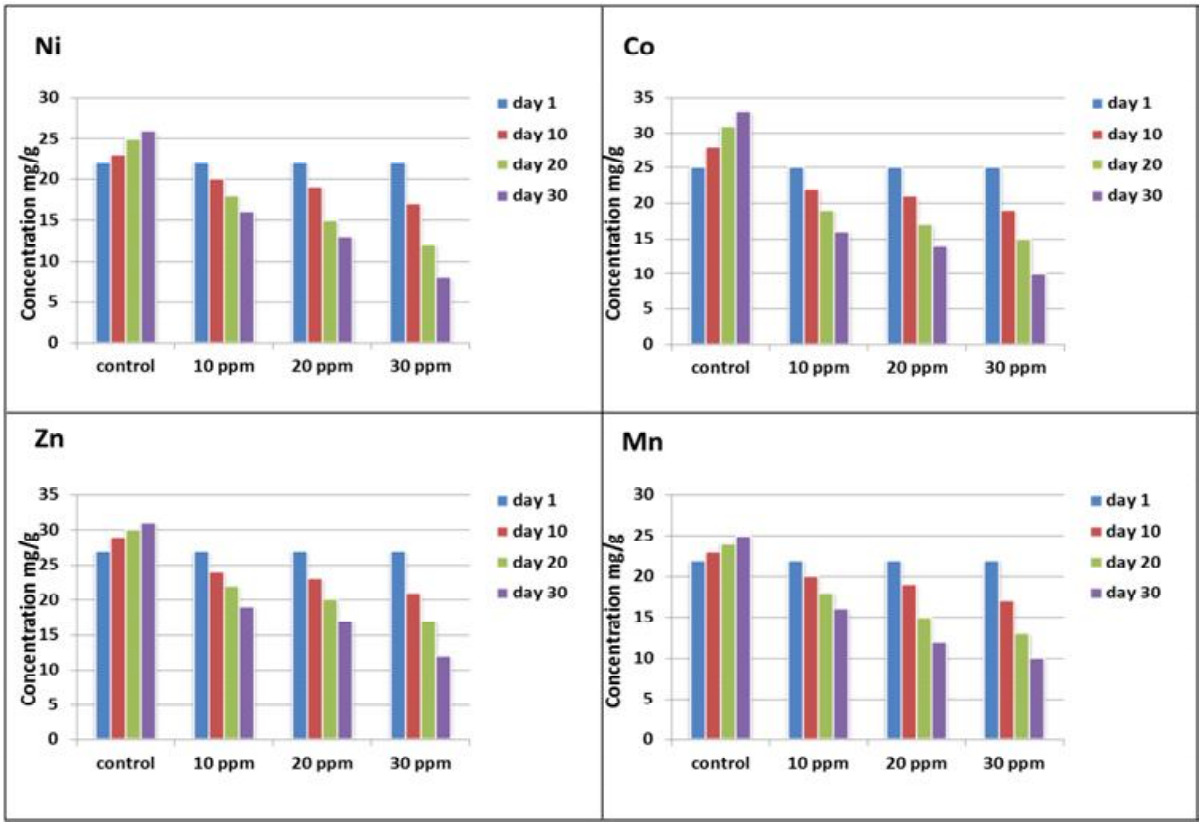

Fig. 4. Three different concentrations of nickel, cobalt, zinc, and manganese effects during four time periods on protein content in *Hydrilla verticillata* tissues.

and calcium contents in plant leaves and consequently the quantum yield of electron flow through photosystem and the efficiency of photosynthesis energy conversion are reduced. Among the tested metals, manganese had the most significant effects on reducing chlorophyll in *Hydrilla verticillata* tissues Fig. 2. After 30 days exposure to 30 ppm of manganese, the concentration of chlorophyll was reduced from 1.91 mg/g to 0.95 mg/g Fig. 2. Heavy metals at high concentrations reduce the uptake of iron and its translocation to plant leaves, and consequently increasing accumulation of metals in plant tissues, hindering plant growth and inducing noticeable symptoms of plant toxicity. (Pandey and Sharma, 2002) found that exposure of cabbage plants to 500 mM of each nickel, cadmium, and cobalt caused a decline in chlorophyll content and an inhibition the activities of Fe-dependent enzymes; peroxidase and catalase, proposing a reduction in Fe availability for biosynthesis.

Protein concentration in both plants tissues was reduced after exposure to heavy metals. The concentration of protein in both plants decreases as the concentration of heavy metals increases in a timely-dependent way Fig. 3 and 4. After 30 days of exposure, the concentration 30 ppm of all the tested metals was the most affected concentration in reducing the concentration of protein in the tissues of both tested plants Fig. 3 and 4. Both exposure period to heavy metals and heavy metal concentrations impact on lowering protein concentration in *Lemna* spp. and *Hydrilla verticillata* tissues were significant ($P<0.05$; $r^2 = 0.394$ and 0.321 respectively) Tables 3 and 4. Of the tested heavy metals, zinc and manganese were the most affected metals in reducing protein concentration in *Lemna* spp. tissues. Protein concentration was reduced from 23 mg/g to 8 mg/g, and from 20 mg/g to 7 mg/g after 30 day exposure to zinc and manganese respectively Fig. 3. The high concentrations of zinc in plant tissues have been reported to cause negative effects on enzymatic activities associated with metabolisms and mineral nutrition (Bonnet *et al.,* 2000). The most affected heavy metal in reducing protein content in tissues of *Hydrilla verticillata* was nickel Fig. 4. Many interconnected physiological and molecular mechanisms determine the sensitivity of plants to heavy metals. The mechanisms includes 1) uptake and buildup of heavy metals through binding to cell wall constituents and extracellular exudates, 2) efflux of metals from extracellular environments to cytoplasm and extra-nuclear compartments such as vacuoles, 3) increase complexity of metal ions inside plant cells by various compounds in the cell such as amino acids, organic acids, metallothioneins and phytochelatins, 4) induction of anti-oxidative enzymes and buildup of osmoprotectans and osmolytes, and 5) modification and/or modification plant metabolism (Cho *et al.,* 2003).

Conclusion and Recommendations

Heavy metals negatively affect the vital growth processes, and the effects increase as the concentration of contaminant increase in a timely-dependent way. Zinc and manganese were the most effected metals in lowering chlorophyll content in *Lemna* spp. and *Hydrilla verticillata* respectively, while the metals zinc and nickel significantly reduced protein content in *Lemna* spp. and *Hydrilla verticillata* respectively. Plants are an effective biological tool in removing contaminants from heavily contaminated environments, and the selection process of plants species is based on the contaminant type and its concentration in the environment.

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