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RESEARCH ARTICLE

EFFECT OF CHROMIUM AND CADMIUM TOXICITY ON BIOCHEMICAL PARAMETERS OF SPIRODELLA POLYRHIZA.

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Abstract

Chromium and cadmium the most toxic metals in the earth's crust can cause severe damage to plants like growth inhibition and even death. The purpose of this study was to evaluate the effect of different concentrations of Cr and Cd (0 ppm, 2 ppm, 4 ppm and 6 ppm) on the biochemical parameters (total protein, total lipid and total carbohydrate contents of free floating aquatic plant- *SpirodeLLapolyrhiza*. These parameters were calculated at the end of the experiment by colorimetric method. The results revealed that total protein content of the selected macrophytes was higher at low concentrations but decreased thereafter while the total lipid and total carbohydrate content decreased as the concentration of Cr and Cd increased in the external medium. Perusal of data revealed that the toxic effects of Cr and Cd are directly proportional to their concentrations.

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Introduction:-

Increasing anthropogenic activities are considered as leading causes of environmental deterioration and pollution. Among various environmental contaminants, heavy metals are of particular concern because of their potential toxic effect, non-biodegradable nature and ability to bio-accumulate in aquatic ecosystems (1). Heavy metals are potentially hazardous pollutants, and are different from other pollutants as they cannot be biologically degraded, but are only transformed from one oxidation state to another (2). Heavy metals like Zn, Fe, Cu, Mn are essential for plant growth and important constituents of many enzymes of metabolic importance. Other metals like Pb, Cd, As, Se, Cr and Al are biologically non-essential and toxic above certain threshold levels. Cr and Cd affect all elements of the food chain, from soil microorganisms to plants, animals and humans once they enter the natural environment (3).

Chromium (Cr)- the critical environmental contaminant found abundantly in the earth's crust adulterate the environment. Although, Cr is toxic but it has some nutritive importance as well (4). Among various valence states, Cr (III) and Cr (VI) are the highly stable forms of Cr (5). The toxicity of Cr (VI) compounds is higher than that of Cr (III) and mutagenicity is an exclusive characteristic of Cr (VI) compounds (6). Cr is phytotoxic either at all concentrations or above certain threshold levels (7). Cr accumulation in plants causes high toxicity in terms of reduction in growth and biomass accumulation and induces structural alterations.

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Cadmium (Cd) is the most fatal metal and is characterized by high stability and toxicity. The non-degradable property of Cd allows it to stay in circulation when it is released to the environment (8). The predetermined course of Cd is in water bodies when it is released from industrial wastewater treatment plants [9, 10]. The peculiar characteristics of Cd are that it is an odorless, silver-white, blue-tinged or grayish-white powder, having an atomic weight of 112.4 and other distinguishing features is the oxidation state of +2 in all its compounds. Cadmium leads to oxidative stress when it binds with essential respiratory enzymes [11]. Cadmium is a non-essential element and can be strongly phyto-toxic to aquatic organisms (12).

Few plant species are able to accumulate high amount of Cr and Cd without being damaged. Aquatic macrophytes, whether free-floating, submerged or emergent have been known to possess the ability to sequester heavy metals (13, 14). Such Cr-tolerant, hyperaccumulator plants are exploited for their bioremediation property.

The aim of this study was to determine Cr and Cd toxicity on biochemical parameters of a free floating aquatic macrophyte- *S. polyrhiza*.

Materials and Methods:-

Plant material:-

S. polyrhiza, commonly known as green duckweed, is a small, free floating perennial aquatic plant belonging to the Lemnaceae family. Duckweed species are used in water quality studies for monitoring heavy metals (15) and are considered as better alternative and have been recommended for wastewater treatment because they are more tolerant, easily harvestable and capable of rapid growth (16).

Culture media and plant sampling:-

Aquatic macrophyte was collected from Dal Lake and transferred to the laboratory in polyethylene bags, washed several times with tap and deionised water to remove adhering soil. Plants of similar size and height were selected and acclimatized to the growth conditions for a week prior to the experiment in clean water.

For preparation of culture media (hydroponics) lake water was used directly. Chemical composition of the lake water (Table 1) was analyzed before using it for preparation of culture media.

Table 1:-Chemical composition of lake water.

| Parameters | Results of chemical analysis |
|--|------------------------------|
| pH | 7.2 |
| Dissolved Oxygen mg/L | 7.9 |
| Total Hardness (as CaCO ₃), mg/L | 241 |
| Calcium content (Ca), mg/L | 182 |
| Magnesium (Mg), mg/L | 14.34 |
| Sodium (Na), mg/L | 8.0 |
| Potassium (K), mg/L | 1.5 |
| Chloride (Cl), mg/L | 4.1 |
| Total nitrogen, µg/L | 2720 |
| Total-phosphorus, µg/L | 240 |
| Cadmium (Cd), mg/L | *BDL |
| Chromium (Cr), mg/L | *BDL |

*BDL- Below Detection level

All parameters listed above were measured according to the Standard Methods for the Examination of Water and Wastewater as prescribed by American Public Health Association, 2005 methods.

Then the desired amounts of cadmium chloride (CdCl₂. 2H₂O) and potassium dichromate (K₂Cr₂O₇) salts were dissolved in distilled water separately to achieve the appropriate contamination levels of Cd and Cr respectively. Working metal and standard solutions with different concentrations of each metal (2.0, 4.0 and 6.0 ppm) were prepared by diluting the corresponding stock solution (1000 mgL⁻¹) using deionized water. After the adaptation period, acclimatized plant samples (25 g) were transplanted to the clear culture enclosures (diameter 8 cm, height 15 cm, volume 5L). These plants were grown in experimental enclosures known as treated enclosures containing metal (Cd and Cr) spiked lake water under natural conditions. A control setup was also established known as untreated

enclosures (where same amount of plant samples were cultured in lake water but without addition of contaminants). The experiment was conducted in triplicates (i.e. comprising of 3 containers for each concentration). The contaminated water was continuously aerated with an aquarium air pump. During the experimental period average temp was about ($25 \pm 2^{\circ}\text{C}$). The experiment continued for 28 days. At the end of the experimental period, the plant samples were harvested from each enclosure, washed three times with deionized water and analyzed for various biochemical parameters i.e total proteins, total carbohydrates and total lipids. Total carbohydrate content of the plant species was determined colorimetrically by phenol -sulphuric acid method (17) after extraction with ethanol, total protein content of plants was determined colorimetrically by Lowry's method (18) after the extraction of proteins with distilled water and total lipid content of the plants was determined colorimetrically by Sulphophospho-Vanillin Method (19) after extraction in buffer solution of pH-7. The results were expressed as percentage fresh weight of plants.

Results and Discussion:-

Aquatic macrophytic species cultured in experimental enclosures was compared for its biochemical parameters. Fresh plants were analyzed in order to get an insight into the variations in the biochemical parameters in different contaminants.

The results in the present study showed an increase in protein content at low concentrations but decreased with elevated heavy metal concentrations. The protein content showed a maximum increase of 30.34 % of the control at concentration of 4 ppm Cd and a maximum reduction of 19.72 % at 6.0 ppm Cr concentration (Fig 1).

The results obtained during the present study corroborate with the findings of 20, 21 and 22. The increase in total protein content under heavy metal stress might be related to the increase of the stress proteins synthesis such as enzymes involved in krebs cycle, glutathione and phytochelatin synthesis and also some heat shock proteins (23, 24, 25). However, reduction in total proteins might be due to degradation by proteases (22), structural and functional modifications by the denaturation and fragmentation of proteins due to toxic effects of reactive oxidative stress (26). The most decrease was observed in Cr treatments. Cr causes metabolic alterations in exposed plants which have been described in plants either by effect on enzymes or by its ability to generate reactive oxygen species, which may cause oxidative stress (27, 28)

Total lipid content in the present study decreased with increase in Cr and Cd concentration in the culture media. In *S. polyrrhiza*, the lipid content showed a maximum value of (2.41 ± 0.55) mg % in control and minimum value of 1.09 ± 0.05 mg % at 6.0 ppm Cd concentration. The greater reduction of 54.77% was found in case of Cd at 6 ppm concentration and in case of Cr the maximum reduction (50.62 %) was also registered at 6 ppm (Fig 2). Heavy metal toxicity to lipids might be due to oxidative damage (by generating reactive oxygen species- ROS) which can cause lipid peroxidation, membrane damage and leakage with fatty acid in membrane lipid bilayer (29, 30, 31). The results obtained in the present study are in line with works of (32, 33, 34, 35). Changes in the total lipids, reported to be an adaptive response to different environmental stresses, in order to restore optimum physical membrane properties (36, 37).

Total carbohydrate contents in the cultured macrophytes manifested a dose dependent relationship when exposed to different concentrations of heavy metals (Cr and Cd). Total carbohydrate content in *S. polyrrhiza* showed a maximum decrease from 16.28 mg % (control) to 4.29 mg % when exposed to 6.0 ppm Cr concentration and a minimum decrease (from 16.28 mg % to 13.64 mg %) at 2.0 ppm Cd concentration. The maximum reduction of 73.65 % was observed at 6.0 ppm Cr (Fig 3). Decrease in carbohydrate content might be attributed to the fact that during low photosynthetic production, the growth and development of plant is supported by stored carbon content in plant tissues (38), photosynthetic inhibition or stimulation of respiration rate (39). Our results corroborate with findings of (39, 40). Cr stress is one of the important factors that affect the photosynthesis, CO_2 fixation, electro transport, photophosphorylation and enzyme activities (41).

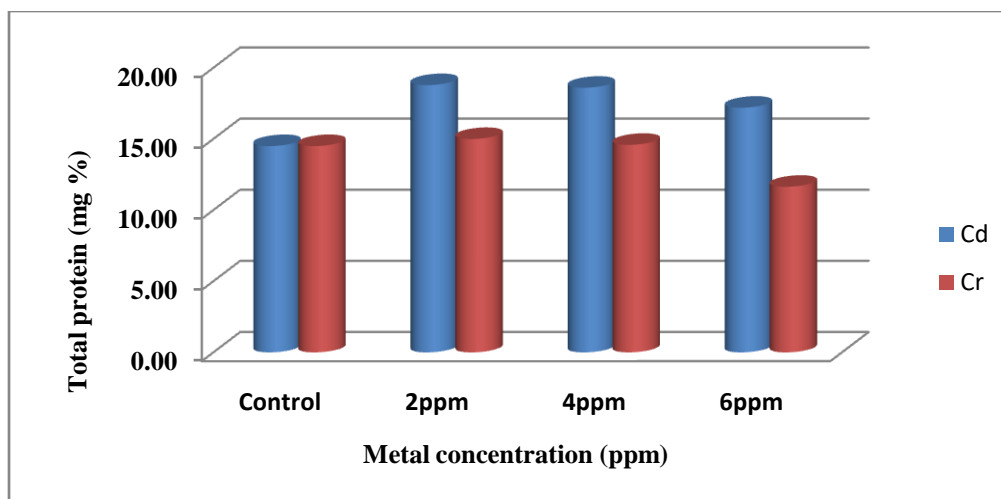


Fig 1:-Cd and Cr toxicity on total protein content of *S. polyrhiza*.

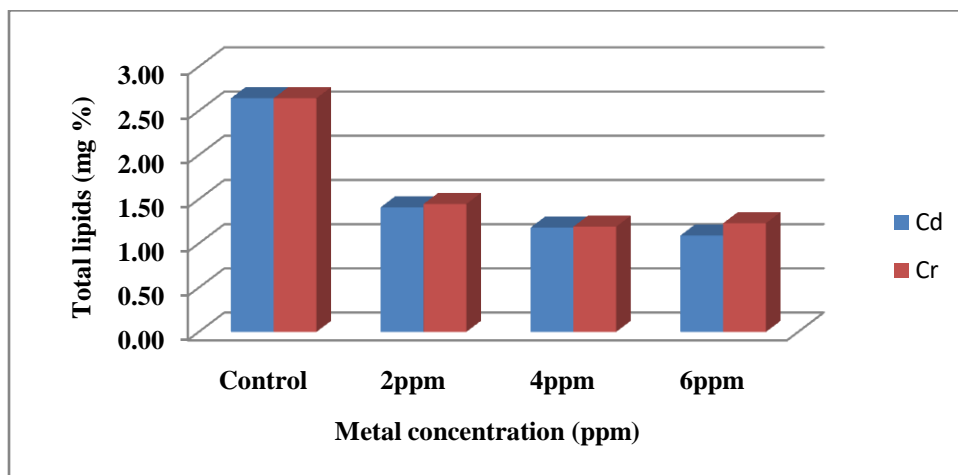


Fig 2:-Cd and Cr toxicity on total lipid content of *S. polyrhiza*.

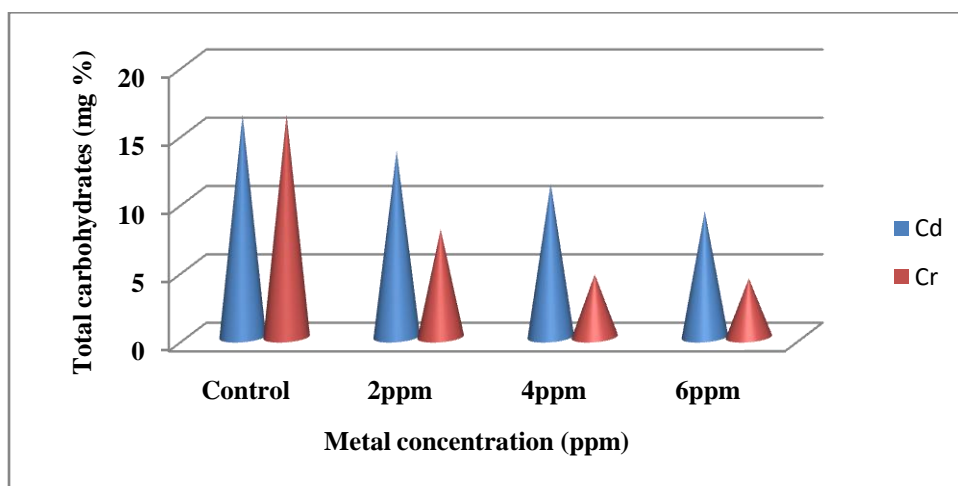


Fig 3:-Cd and Cr toxicity on total carbohydrate content of *S. polyrhiza*.

Conclusion:-

From the above discussion, it has been found that Cr and Cd effect all the studied parameters especially at the higher concentrations but Cr is found more toxic than Cd that induces toxicity in plants at biochemical levels. Perusal of the data revealed that the impact of Cr and Cd toxicity in *S. polyrhiza* are directly proportional to their concentrations in the culture media.

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