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STUDY OF EFFECT OF HEAVY METALS ON PLANTS AND THEIR RECOVERY WITH BIOSTIMULANTS

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ABSTRACT

This paper examined the role of heavy metals like Chromium, Lead and Cadmium on factors affecting plant development like protein content. The objective of this paper was to bring into focus the role of biostimulants (organic sea weed and biozyme ratio 1:1) in aiding recovery from stress due to the presence of heavy metals in the soil on a cash crop Glycine max. The Folin-Ciocalteu method, also referred to as the Lowry method, was employed to estimate protein content in plants subjected to various conditions. Proline, an amino acid known for its role in enhancing stress tolerance, was also measured using the Ninhydrin test, providing insights into the plant's response to stress and recovery. The results demonstrated that exposure to heavy metals induced stress, as evidenced by a reduction in protein content. However, the application of biostimulants had a positive effect, leading to an increase in protein levels. Elevated proline content further confirmed that heavy metals caused stress, while biostimulants facilitated recovery, as reflected by the decrease in proline levels, indicating a reduction in stress.

KEYWORDS*: Effect of Heavy Metals on Plants.*

Article History

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INTRODUCTION

Biostimulant products are substances or microorganisms derived from natural resources used on plants or soils to enhance overall plant and soil health and productivity. They work by stimulating the plants' natural processes or enriching the soil microbiome, which results in a myriad of secondary benefits in the production system. In recent years, biostimulants have become an important tool for farmers to manage the productivity of their crops. Biostimulants are known to have various benefits for crop production. One product may induce one or more of the following effects: It could increase plant growth, pest and disease resistance, nutrient uptake efficiency, crop productivity, and quality of fresh produce. These benefits are not necessarily gained from a specific kind of biostimulant, and various products will have been proven to benefit plants in some of the ways mentioned. A secondary benefit of the use of biostimulants is improvement in soil health (as root growth is stimulated as is interaction with beneficial soil microbial organisms). Using biostimulants also supports the long-term sustainability of crop production and the preservation of natural resources. Interest in plant biostimulants has surged over the past decade, fuelled by researchers' and farmers' growing demand for eco-friendly solutions to boost crop performance. Biostimulants have been shown to alleviate heavy metal stress in plants through a variety of mechanisms, including enhancing plant nutrient uptake, improving antioxidant defence mechanisms, and increasing the activity of plant growthpromoting microorganisms in the soil. Seaweed extract is rich in bioactive compounds such as polysaccharides, amino acids, and plant growth hormones that can enhance plant growth and stress tolerance.

Soybean

Soybeans have become a staple part of the human diet because they are nutritionally excellent and contain various functional components that provide a health benefit beyond basic nutrition. Botanically known as *Glycine max*, soybean belongs to the leguminous family because of its ability to form nodules and fix nitrogen in the soil. Soybean is also a climate-resilient, low-cost crop with food security potential. Soybean is the most nutritionally rich crop as its dry seed contains the highest protein and oil content among grain legumes. Soybeans are also a good source of several vitamins and minerals, including vitamin K1, folate, thiamine, copper, manganese and phosphorus. Carbohydrates, protein and lipids in soybean contribute energy amounting to 475 kilocalories with fat contributing the largest proportion followed by proteins and carbohydrates. Essential amino acids in soybean include; histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. Soybean protein has potential to contribute towards meeting daily requirements of individuals particularly children aged 7–12 months followed by those aged 1–13 years with high protein requirements, as well as the physically active adolescents and adults. Soybean products therefore should be combined with other plant proteins such as those contained in whole cereals like maize to have a complete protein. As a good source of several vitamins and minerals, soybean could also contribute towards the age specific daily needs of minerals and Vitamins if well utilized. Soybean has unique characteristics as it can be made into a variety of products for income generation as well as for household food security. World-wide up to 85% of soybean produced is channelled into animal feed with the remaining being used for human consumption. Soy flour, weaning food formulations, soy-based soups, confectioneries, beverages and fermented soy products as well as extruded products have also beendocumented. Other known products are soy relishes, soy coffee, soy sausage and soy sprout as well as tempeh, soy sauce, soy candies and soy meat. Various studies have reported that abiotic stresses, such as heavy metal stress, severely threaten soybeans. The synergistic effect of heavy metal toxicity adversely influences soybean growth and metabolic processes.Moreover, soybean is not tolerant of heavy metal stress; hence, metal-induced stress significantly affects its growth from germination to production. The toxicity also enters the food chain and affects human health.There are reports of an increase in levels of toxic metals in the soil of soybean cultivating regions.Such toxic metal ions enter the plant cells by a similar uptake process as nutrient ions, competing for absorption and ultimately adversely affecting the metabolism to limit crop production. Cadmium (Cd) and mercury (Hg) were found to be highly hazardous toxic metals. Even at very low concentrations, Cd can severely change plant enzyme activities and ultimately cause stress. Another very potent toxic metal is mercury (Hg) which causes serious crop destruction in plants, alteration in RNA expression, DNA methylation, modifications in histones, and visible injuries and physiological disorders in humans. It is preferentially bound with ligands of sulphur and enters the cell through ionic channels. The consequence of heavy metals which altered physiological and biochemical traits in plants was also studied in this study. Exposure to Cd changed the expression pattern of various key genes involved in the regulatory mechanism of plants. Besides interacting directly to bring changes in metabolism and physiology, metals' stress influences the expression of certain genes, often to induce proper acclimation mechanisms and cellular reprogramming to minimize damage to the plants. Lead (Pb) contamination of agricultural soils, and subsequently of crops, has been widely reported. Soybean (*Glycine max* (L.) has been indicated as a plant that accumulates Pb, even in soils that do not exceed the maximum permissible levels. Lead toxicity symptoms in leaves (chlorosis and dark spots, and a decrease of biomass and leaf area, chlorophyll content, and photosynthetic efficiency), and an increase of the oxidative defence system were associated only with the highest Pb concentration.

Cadmium has different sources that include natural and anthropogenic sources triggered by human activities. Cadmium atmospheric emission includes; suspended particles of oxide, sulphide, sulphate, and chloride of cadmium, which are chemically stable and can stay for a prolonged period in the troposphere of about one to four weeks. Industry activities produce a huge amount of cadmium in both forms of particulate and soluble, though its effect is likely to be less in affecting soils directly. Its indirect effects through draining of waste into water sources and groundwater in soil impose high pollution and increased amount of cadmium in the soil.There are many sources that may contribute to increasedlevels of lead in residential soils including peeling, chalking, or activeremoval of lead-based paint; fallout from the discharge of community waste incinerators smelters, or foundries; dumping or burning of lead batteries and their casings; and emissionfallout from vehicles fuelled with leaded gasoline. Lead-based paint on exterior surfaces such as the walls of buildings, point source emitters such as smelters, batteries, or mine tailings,Leaded gasoline emissions from automobiles are three main sources of lead.Three thermodynamically stable Chromium forms, Cr (0), Cr (III), and Cr (VI), are used commercially and are present in the environment. Cr (0) is found almost exclusively in its metallic form, most commonly as a component of iron-based alloys such as stainless steel. Stainless steel contains up to 20% Cr by weight and is the highest volume product containing this metal. Incineration and emissions from cars create ambient pollution with small Cr (VI)- and Cr (III)-containing particles, which leads to low-level inhalation exposures by large segments of the general population and increases Cr levels in surface waters. The most serious cases of anthropogenic contamination of drinking water in the U.S. came from the discharges of toxic Cr (VI) by cooling towers. Other large-scale environmental pollution with Cr (VI) involved improper disposal of millions of tons of incompletely processed chromite ore. Cr (0)-containing products are generally highly resistant to corrosion; however, slow oxidation of Cr-containing alloys can result in the limited release of soluble Cr (III) into soil and water. Cr (VI) is the most mobile form of chromium in the environment's (VI) displays no ability to damage DNA directly and requires reductive activation for its genotoxic activity. Structural similarity of chromate ion to sulphate allows its easy entry through the general sulphate channels. Permissible level of Cd, Pb, Cr as per WHO guidelines is 0.002-0.5 mg/kg,0.3-10 mg/kg&0.002-0.2mg/kg respectively

Indication of Tests

The Lowry Protein Assay is a widely used method for quantifying the amount of protein in a sample. It was developed by Oliver H. Lowry and colleagues in 1951 and has since become one of the most widely used protein assays due to its high sensitivity, accuracy, and reproducibility. The Lowery protein assay involves the reaction of proteins in the sample with a mixture of reagents, including copper ions and a Folin-Ciocalteu reagent. The resulting colour change is then measured at a specific wavelength, allowing the protein concentration in the sample to be calculated. The significance of the Lowry Protein Assay lies in its ability to accurately and reliably quantify protein concentrations in a wide variety of sample types, including biological fluids, tissues, and purified protein samples. It is used extensively in research, clinical, and industrial applications. The most (if not the most) successful protocol for thesimultaneous quantification of chla and b is the one described byArnon in the middle of last century (Arnon, 1949). However, the equations proposed for chlmeasurement became the reason for its success. The useof Arnon’s method instead of more accurate methods is not trivial,and it leads to substantial and important errors, particularly for the calculation of the ratio chla :chlb. This parameter is of paramount importance as a robustindicator of significant processes, such as the circadian rhythms ofphotosynthesis, the light environment, in which a plant develops. Additionally, the chla :chlb ratio isalso a differentiating parameter among functional groups. Thus, worryingly, the use of Arnon's procedure couldlead to flawed conclusions regarding many different plantfunctional and evolutionary aspects. Overproduction of proline is a widespread response observed in plants experiencing various stresses, in particular osmotic stresses. The determination of this amino acid is therefore very useful to assess the physiological status and more generally to understand stress tolerance in plants. Here we describe a simple, fast and relatively harmless ninhydrin-based method, which is suitable for the high throughput determination of free proline content using a microplate reader. Ninhydrin (2,2- dihydroxyindane-1,3-dione, CAS number 485-47-2) is extensively used to assay amino acids.

MATERIALS AND METHODS

- Protein by Lowry's method Protein estimation was conducted following the method outlined by Lowry and Lopaz (1946), with Bovine serum albumin serving as the standard at a concentration of 1mg/ml. In the Lowry's method, the blue color developed results from the reduction of phosphomolybdic phosphotungstic components by amino acids like tyrosine and tryptophan found in the protein. Additionally, the color produced by the biuret reaction of the protein with alkaline cupric tartrate is measured. Various concentrations of the standard ranging from 0.1 to 1 mg/ml were prepared and adjusted to 1 mg/ml. Subsequently, 5ml of alkaline copper reagent was added, thoroughly mixed, and allowed to stand for 10 minutes at room temperature. Following this, 0.5 ml of diluted Folin's phenol reagent was incorporated and mixed well. The mixture underwent incubation for 30 minutes at room temperature, and the absorbance at 650 nm was measured spectrophotometrically. This process allowed for the estimation of protein concentrations in seaweed extracts.
- Estimation of Proline Content Proline content was determined following the method described by Edit Abraham et al. (2010) One gram of leaf material from both transgenic and wild-type (WT) control plants was ground in 20 ml of 3% sulfosalicylic acid, followed by centrifugation. To 2 ml of the resulting supernatant, 2 ml of glacial acetic acid and 2 ml of ninhydrin were added, and the mixture was boiled at 100°C for one hour. Afterward, the tubes were allowed to cool to room temperature, and 4 ml of toluene was added and thoroughly mixed. The chromophore (toluene) was then separated from the aqueous phase, and its absorbance was measured at 520 nm.

HISTOGRAMS

Estimation of Protein by Lowry's Method

Table 1

	Blank	Control	Stress	Recovery
O.D.	0.01	0.06	0.02	0.04
Δ O.D.	-	0.05	0.01	0.03

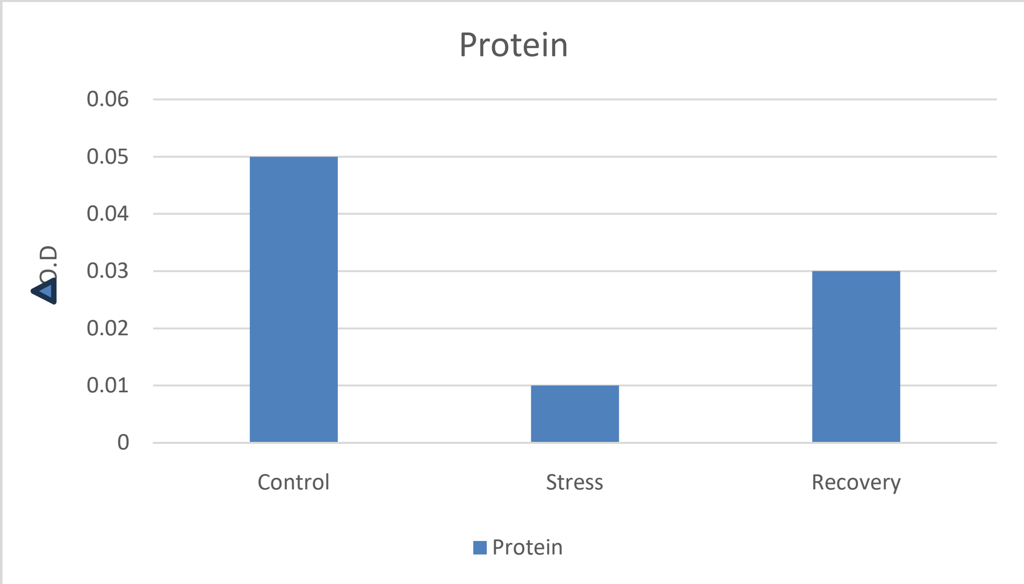

Figure 1

Result

- It was observed that the seeds under normal conditions showed highest concentration of protein.
- Heavy metals showed an adverse effect & concentration of protein fell drastically.
- Recovery with biostimulants resulted in an increase in concentration of protein.

Estimation of Proline

Table 2

	Blank	Control	Stress	Recovery
O.D.	0.03	0.07	0.16	0.08
ΔO.D.	-	0.04	0.13	0.05

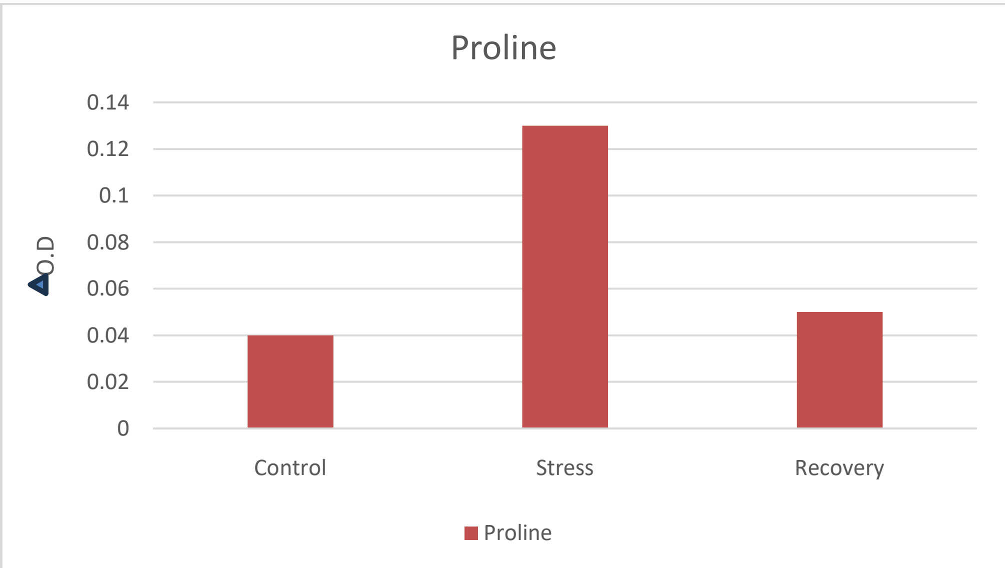

Figure 2

Result

- Proline an indication of stress rose and reached the highest concentration with seeds treated with heavy metals.
- The fall in proline concentration reaffirmed the role of biostimulants in recovery.

COMBINED DATA

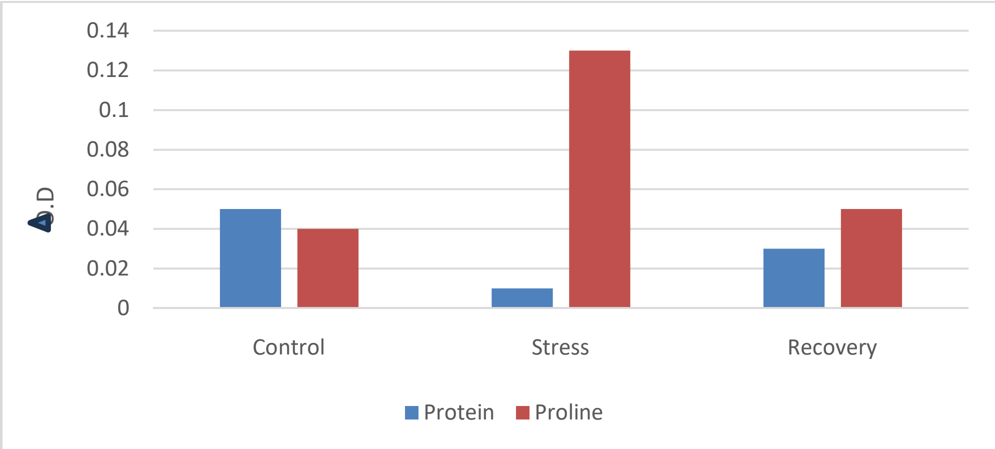

Figure 3

DISCUSSION

The two factors show a clear role of Biostimulants in recovery due to heavy metals stress. While other factors need to be further researched upon, the use of Biostimulants shows a positive effect on plant recovery and organic and natural Biostimulants maybe used for better crop yield Though the effect of organic and chemical biostimulants may vary it needs to be researched further taking into account more factors in order to confirm the result.

Protein and proline content are taken as factors because of their importance in affecting plant growth and health. They give a clear indication of the effects of stress and recovery.

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IMAGES

Apparatus

Colorimeter

Garden soil Proline Test tubes

Petri plates with soyabean Petriplates without Soyabeans

Protein Test Tubes

Soaked Soyabeans