INVESTIGATION OF USAGE POSSIBILITIES OF NATURAL WETLAND PLANTS FOR THE TREATMENT OF NANO-SIZE HEAVY METAL POLLUTION

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Abstract - In this study, Brassica sp. Which is known as a natural wetland vegetation in the country and which is found mostly in natural wetlands of our country. (Brassica sp.) which was prepared by using AgNO3 (Silver Nitrate) chemistry at different concentrations. It has been determined at what concentrations the plant can tolerate the Ag heavy metal and how the different concentrations used affect the stress enzymes of the plant. In the study carried out in the climate cabin, AgNO3 was applied to the plants for 4 weeks at concentrations determined as 1000, 2000, 4000 and 6000 ppm and after 4 weeks analyzes were carried out on the samples taken from the plants. As a result the plant showed resistance against the maximum concentration of 6000 ppm and continued its life activities. the excess accumulation was observed at the root of the plant (207.100 ± 21.49) in the application of 6000 ppm (AgNO3), and in the leaf of the plant (184.20 ± 28.70) (13.20 ± 4.80). Superoxide dismutase (SOD) enzyme activity, responsible for the elimination of superoxide radicals from oxidative stress-induced active oxygen derivatives, has been shown to increase due to increased silver concentrations.

Keywords - Pyhtoremedion, Soil Treatment, Heavy metal Aluminium

I. INTRODUCTION

The use of wastewater as a source of plant nutrients in agricultural production in today's world, where environmental pollution has reached extreme dimensions, has brought about a major problem, soil pollution. Industrial wastes are also considered as one of the important causes of soil pollution. Many heavy metals are the micronutrient required for plants. If they exceed the toxicity limits of the plant, they may be harmful for plant growth. For this reason, the treatment of toxic metals contaminated, especially agricultural areas, is a very important process. In addition to industrialization and rapid urbanization, various agricultural applications, especially fertilization and agricultural drug use, lead to heavy metal pollution in soil and groundwater resources.[1],[2][3]. This situation affects different levels of plant breeding depending on species and variety in agricultural areas. In recent years, environmental and soil clearing has become an extremely important issue[4].

Because many living things on the earth need and are of vital importance. For this reason, the hyperacuma effect of Brassica sp., Which is effectively used in the biological struggle, has been exploited because of the fact that the high doses of heavy metals in the present study are a stratified solution for plants and living things growing in soil and soil. When pollution is eliminated, it will be used as a useful work for the surrounding area and the agricultural lands, helping to develop a mechanism of influence[5].

This study was carried out to investigate the effects of Brassica sp. plants were added silver nitrate at different concentrations to the growing medium to determine the changes in the stress enzymes of the plants and the amounts of silver they could contain in their bodies. Thus, the study is important in terms of improving the soil and environment, in addition to investigating the molecular effects of heavy metal stress in plants in terms of potential results.

II. DETAILS EXPERIMENTAL

2.1. Materials and Procedures

Fundamental materiels used in this study were silver nitrate (AgNO3), and Brassica sp. seeds. During trials, placement of plants were initially conducted.
In first stage of the experiment, Compo Sana soil was placed in seed trays of 85 slots as a starter peat and Brassica sp. seeds were planted. In this trial, Brassica sp. and control group were grown in both clean environments and environments that are contaminated with Aspergillusniger. Planted seeds were grown in our laboratory, within a controlled climate cabin stable at 25 °C temperature, 5 LS and a humidity of %65, with equal irrigation every 2 days. Plants that had become seedlings were placed in seed trays of 45 slots for growing. The strain was incubated for 3 to 4 days, in 30°C, and preserved in + 4°C. Following this stage, again in controlled climate cabin, various amounts of silver nitrate had been administered. Plants were subjected to 1000, 2000, 4000 and 6000 ppm concentrations of silver nitrate, for 4 weeks, at the end of which samples of plants were analyzed according to the following procedures;

1) Detection of Cell Membrane Damage Using Ion Leakage Method

Leaf samples gathered from plants are analyzed according to [6] and [7] modifications, in order to detect cell membrane damage.

2) Total Chlorophyll
Total chlorophyll amount in Brassica sp. leaf samples is determined according to the study of [8]

3) Enzymatic Antioxidants
In order to determine the activities of antioxidant enzymes, 0,5 g of leaf samples are grinded in liquid nitrogen, then, homogenized in 2-3 mL extraction buffer solution. Homogenized samples are kept in 15,000 g centrifuge for 15 minutes. Centrifuged samples are used to determine enzyme activites and protein levels [9]. Evaluation of enzymes and proteins are carried out in spectrophotometre.

Catalase (CAT) activities are determined in 240 nm (E= 39.4mM cm-1) based on fragmentation rates of H202.[10]

Ascorbate Peroxidase (APX) activities are determined by measuring the oxidation of ascorbate in 290nm (E= 2.8mM cm-1).[11]

GlutathionReductase (GR) activities are measured according to [12] and [13], in 340nm (E=6.2mM cm-1) based on NADPH oxidation.

The amount of soluble proteins are evaluated according to [14]in samples processed in centrifuge for enzymes.

Assessing the Amount of Heavy Metals Accumulated in Plant Body; From the beginning of the experiment leaves that fall from plants are collected, labelled and stored until analysis. Moreover, in order to determine their growing rates, throughout the 4 week process plants were measured for their height and recorded in centimetres every week, and temperature and humidity levels were tracked and recorded. Plant roots, stems and leaves, and soil samples were gathered in small amounts, placed in heat-resistent drying oven containers and kept in 60 °C for 5 consecutive days in drying oven, for the samples to reach constant weight. At the end of five days samples were measured for their dry weight. Dried plants and soil samples were then grinded and filtered through a sieve. Samples were then numbered according to their original receptacles and placed in humidity-proof bags. At the end of the process plant and soil samples were prepared for analysis according to the method (AgNO3) in “Standard Methods for the Examination of Water and Wastewater, 20th Edition”.

III. RESULTS AND DISCUSSION

Throughout the research plant growth was observed regularly and each sample’s plant is measured for its height every week. Also, every sample plant’s number of leaves were recorded weekly. As a general appraisal, plants that have not been treated with silver nitrate, the control group, can be said to have grown in height very rapidly, but plants, that were subjected to solutions with 1000 ppm, 2000 ppm, 4000 ppm, 6000 ppm AgNO3, have been slow to grow in proportion with the amount of silver nitrate. Control group of Brassica sp. and plants that were subjected to 1000 ppm, 2000 ppm, 4000 ppm, 6000 ppm of AgNO3,were shown according to their weekly height measurements. Growing rate of plants that were in the control group was 8 cm, whereas the growing rate of plants that were subjected to 1000 ppm, 2000 ppm, 4000 ppm AgNO3 is 3 cm, and plants that were subjected to 6000 ppm of AgNO3 had a growing rate of 2 cm. shows the number of leaves of Brassica sp. plants in the control group and in the groups that were subjected to solutions with 1000 ppm, 2000 ppm, 4000 ppm, 6000 ppm of AgNO3. In the first 10 days all of the plants’ leaves grown in number. But following the 10th day, until the 14th day, all of the plants that had been subjected solutions started losing leaves. After the 14th day, until 16th week none of the plants’ leaves have grown in number. At the end of the 16th day, both control group and plants that have been subjected to solutions were seen to be lively.

Brassica sp.’s capacity to absorb the AgNO3 within its root, stem and leaf have been examined according to different levels of AgNO3. The plant was able to withstand the 6000 ppm solution, and also the highest amount of absorption was observed in plants that have been subjected to 6000 ppm of AgNO3, in their root (207.100±21.49), stem (184.20±28.70), and leaf (13.20±4.80).

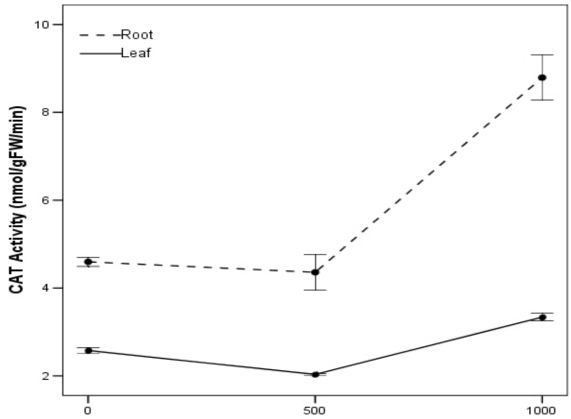

Table. I Brassica sp. CAT enzyme activity level

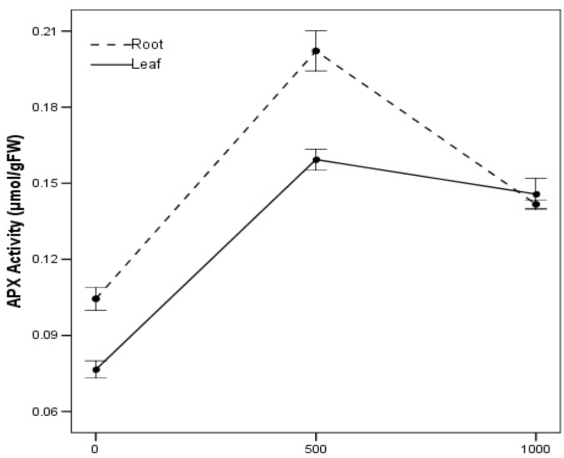

Table II. Brassica sp. AP enzyme activity levels

Superoxide dismutase enzyme activity, which is active oxygen, that occur as a result of oxidative stress, superoxide radical, is observed to rise according to the level of silver nitrate. GlutathionReductase (GR) and Ascorbate Peroxidase (APX) enzymes are a part of antioxidant defense mechanism, which play a crucial role in clearing out the Hidrogen Peroxide from chloroplasts and mitochondria. Catalase (CAT) enzyme, plays a role in eliminating reactive oxigen derivatives, like hidrogen peroxide, that occur as a result of oxidative stress, through conversion to water and molecular oxigen.

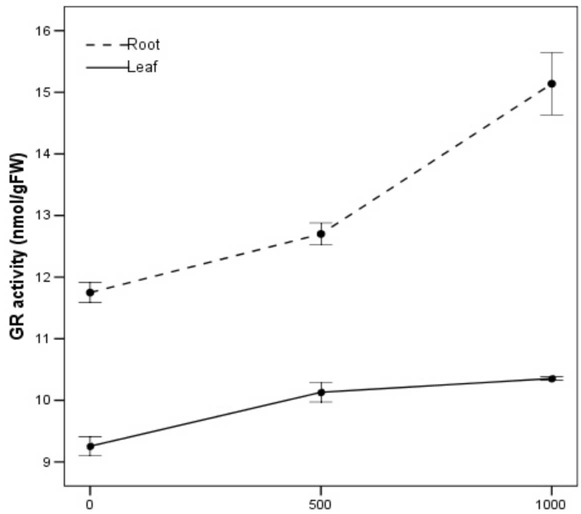

Table III. Brassica sp. GR enzyme activity level

This study has been conducted as an alternative to methods that aim to improve and augment the soil using plants, by subjecting different levels of $AgNO^3$ heavy metal to the soil. Both natural and man-made activities polute the soil, and cleaning the heavy metals that are in the soil through physiochemical processes is very expensive and limited in usage. Thus, to use hyperaccumulator plants in order to clean the soil from heavy metals, in accordance with the type of metal and its concentration levels, which are easy to apply and cheap, gains critical importance. Interaction of silver and the organism starts through the process of absorbation. Studies conducted have shown a correlation between organic acids within the soil and absorbation of Ag by plants[15]. [16]in their studies, have found that increased levels of organic acids in the soil lead to increased levels of Ag absorbed in plant body without any effect on distribution of Ag within the plant.

Plants differ in Ag absorbation. Some take in Ag through active transportation[17]. (Some others, similar to microorganisms, use sulfate transportation system to absorb AgNO3. Translocation of silver within the plant, and its accumulation, differs according to the type of plant and the level of concentration of Ag ion within the environment [18][19].

Ag compounds within the environment are very toxic for the plants, they directly have a negative effect on growth and development. In the studies conducted, it has been observed that plants subjected to low levels of silver (3,8.10-4 µM) are not affected, whereas more than 100 µM Cr/kg dry weight solutions are seen to be very toxic for the plants [20].

Phytoremediation is a system that uses plants to lower the level of polution in the soil, to acceptable levels. Thus, it is important to select plants that have a higher capacity to accumulate toxic material, and improve soil conditions. In this study, Brassica Oleracea, a durable plant that which is used in soil improvement and found in tropical and moderate climates, has been observed. Brassica genus plants, especially BrassicJuncea, which has been used in this study, are one of the most studied plants in the literature. There are studies which indicate that this plant can absorb 2000mg/kg of Zn, 75 mg/kg of Cu, 55 mg/kg of Pb, 100 mg/kg of Cr (Clemente et al., 2005). Similarly, as a result of their ability to absorb silver from the soil, Betula and Salix trees and Brassica Juncea plant are useful for improving underground water reserves contaminated with Ag, likewise Coconut shells and pulps are used to absorb Ag from river inflows. Studies conducted in recent years prove that Brassica Juncea and Brassica Oleracea plants accumulate silver and phytoremediate the soil. At the end of the study, all climatic data, phenological observations and laboratory practices are recorded. All the plants that have been subjected to various levels of AgNO3 are observed to retain their vitality, even the ones subjected to highest level of 6000 ppm, and all have been observed to accumulate Ag ion in their roots.

As a result of the oxidative enzyme activity analyses, it has been observed that in order to eliminate superoxide radical, which occur as a result of oxidative stress, levels of superoxide dismutase (SOD) enzyme activity increase, in proportion to the amount of silver in the environment. GlutathionReductase (GR) and Ascorbate Peroxidase (APX) enzymes are a part of the antioxidant defense mechanism, and play a crucial role in clearing out the hyrdogen peroxide (H202) from chloroplasts and mitochondria. Catalase (CAT) enzyme also is responsible for eliminating reactive oxygen derivatives, like hyrogen peroxide, by transforming into water and molecular oxygen.

It can be advised, for attaining a deeper understanding of the subject, in future studies electrical conductivity, soil pH level, available P and N levels within the soil, amount of heavy metals within the soil (e.g. Al, Fe, Zn, Cd, Cr), organic acids in the soil, plant biomass parametres to be measured. This way, it will be easier for the data collected to be correlated and lead to a conclusive result. Also, it is possible for the heavy metals used to be regained from the ashes of plants.

ACKNOWLEDGMENTS

This project was supported by the BAP Coordination Unit of Çukurova University as MMF2013BAP11 project. Thanks to the BAP unit providing financial support for the work so that can be realized.

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