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Research Article

EFFECTS OF SOME HEAVY METALS ON TOTAL PROTEIN AND PEROXIDASE ACTIVITY IN CLOVER AND VETCH PLANTS

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ABSTRACT

Forage crop farming is shown as the most important way of continuous and safe forage production. Clover and vetch are the most used forage plants. In this research clover (Medicago sativa) and vetch (Vicia sativa) plants were planted in large pots (48x18x18cm) containing peat: perlite mixture (3:1). The seeds were kept in distilled water for 3 hours before planting and swelling was ensured. After the peat perlite mixture prepared in pots was watered sufficiently with distilled water and then seeds were planted. 1000 ppm heavy metal stock solutions have been prepared for lead nitrate Pb(NO3)2, copper nitrate Cu(NO3)2.3H2O, and cadmium nitrate (CdNO3)2.4H2O. Prepared heavy metal solutions were given to the plants at once, with irrigation water, in 300 mL for each pot. Total protein amount and peroxidase activity were examined by spectrophotometrically 72 hours after the heavy metal application. Three different heavy metals applications to the forage plants, have been showed different physiological reactions in these plants. These reactions have been measured by comparing control group of plants. According to research results, it was determined that the maximum increase in the total protein amount in M. sativa compared with the control group was 100 ppm applied lead. The maximum increase in the total protein amount in V. sativa compared with the control group was 30 ppm applied cadmium, respectively. The increase in peroxidase activity has been determined in M. sativa and V. sativa plants compared with the control groups were 30 ppm applied cadmium. As a result of our research, it was determined that cadmium has been found to be more effective than lead and copper for stimulation of plant defense system.

Key words: *Clover, Vetch, total protein, peroxidase, forage plants.*

1. INTRODUCTION

The accumulation of heavy metals in the soil has important effects not only on soil fertility and ecosystem functions, but also on animal and human health through the food chain (Jiwan and Ajay, 2011). Some heavy metals in high concentrations affect animals, plants and who feed on plants adversely (Hejna et al., 2018). Heavy metals, which are poisonous in all respects, are released from various sources to the environment. It is one of the important causes of environmental pollution (Jaishankar et al., 2014). Plants being ion in soil solution that usually take the heavy metals in the form by their roots (Pinto et al., 2014).

Lead (Pb) is the first metal which causes the greatest damage to the ecological system with human activities. The passage of lead into the soil and atmosphere being in various ways. Among these ways, fumes from the chimneys of industrial establishments and vehicle exhaust, solder, battery, paint, waste from the electricity, petroleum industry and pesticides (Okcu et al., 2009). Cadmium (Cd), one of the heavy metals, is a highly toxic metal that has come to the fore with its important role in pollution today.

Important sources of cadmium which affecting human life; cigaret burning smoke, refined foodstuffs, water pipes, coffee, tea, coal, shellfish, fertilizers used in the seed stage and industrial production flue gases formed in stages (Seven et al., 2018). Copper (Cu) is an important element due to its involvement in plant enzyme activation, nitrogen fixation, protein metabolism, antioxidant activity, cell wall metabolism, carbohydrate and lipid metabolism (Emiroğlu et al., 2018). Copper toxicity is often found in plant root systems and protein synthesis, photosynthesis, respiration, ion causes disruption of some physiological events such as uptake and cell membrane stability (Sosse et al., 2004).

Forage crop farming is shown as the most important way of continuous and safe forage production (Ozkan, 2020). Forage crops agriculture, which has a very important place in agricultural activities, is the insurance of crop and animal production. Considering the situation of the agricultural feed plant in Turkey available forage crops are grown most clover plant within our acreage (36.6%) vetch with it as well (31.9%), corn (21.4%) and sainfoin (9.7%) is followed by plants (Yolcu and Tan, 2008).

When animal feeds contaminated with heavy metals are consumed by livestock, they may pass into products such as meat, milk and eggs and reach levels that threaten human health. There are not many scientific studies on the heavy metal levels of the feed consumed by the animal. Therefore, this research is important in terms of filling the gap in this field.

2. MATERIAL AND METHODS

2.1. Plant Materials

M. sativa and *V. sativa* seeds were planted in large pots containing soil perlite mixture (3:1) at the temperature of 24±2˚C, 28.000 lux light, under the conditions of 16 hours light and 8 hours dark. Plantlets were grown in controlled plant chamber.

The seeds are swollen before planting by soaking in pure water for 3 hours. The soil: perlite mixture prepared in pots was watered sufficiently with distilled water. After that seeds belonging to two plant species has been planted. Seeds has been planted forms the following groups;

- 1 = *M. sativa* control
- 2 = *M. sativa* application lead (100 ppm)
- 3 = *M. sativa* application cadmium (30 ppm)
- 4 = *M. sativa* application copper (50 ppm)

- 5 = *V. sativa* control
- 6 = *V. sativa* application lead (100 ppm)
- 7 = *V. sativa* application cadmium (30 ppm)
- 8 = *V. sativa* application copper (50 ppm)

2.2. Preparation of Heavy Metal Stock Solutions and Application to Plants

The following procedure has been followed in the preparation of stock solutions.

1000 ppm (1000 mg/L) stock solution was prepared for lead nitrate $Pb(NO_3)_2$, solution. The solution of 100 ppm was diluted with distilled water in 1000 mL each. 1000 ppm (1000 mg/L) stock solution was prepared for copper nitrate $Cu(NO_3)_2.3H_2O$ solution. The 50 ppm solution was diluted with pure water in 1000 mL each.1000 ppm (1000 mg/mL) stock solution was prepared for cadmium nitrate $(CdNO_3)_2.4H_2O$ solution. The 30 ppm solution was diluted with distilled water as 1000 mL each. Prepared heavy metal solutions were given to the plants at once 300 mL for each pot with irrigation water.

2.3. Harvesting Plants for Homogenization and Analysis

After 72 hours of heavy metal applications on *M. sativa* and *V. sativa* seed for four weeks, the above-ground parts of the plants were harvested and the standard homogenization method was used. After the homogenates belonging to all groups were centrifuged, their upper parts were taken and used for total protein and peroxidase analysis of the preliminary trials. All trials were carried out in triplicate.

2.4. Determination of Total Protein Amount

The protein standards used in this method were prepared from Bovine Serum Albumin (BSA) stock solution. For this purpose, 0.02 mg/mL from 2 mg/mL stock ampoule BSA; 0.04 mg/mL; 0.08 mg/mL; 0.12 mg/mL; 0.16 mg/mL and 0.20 mg/mL concentrations were taken and transferred to test tubes and the final volume was completed to 1000 µl. For the protein amount measuring, homogenates were transferred to the glass test tubes as 100 µl from the eppendorf and 5 mL of Protein Reagent Blue G-250 was added to each test tube. The amount of total protein was measured by according to Bradford (1976) method. All trials were carried out in triplicate.

2.5. Determination of Peroxidase (POD) Activity

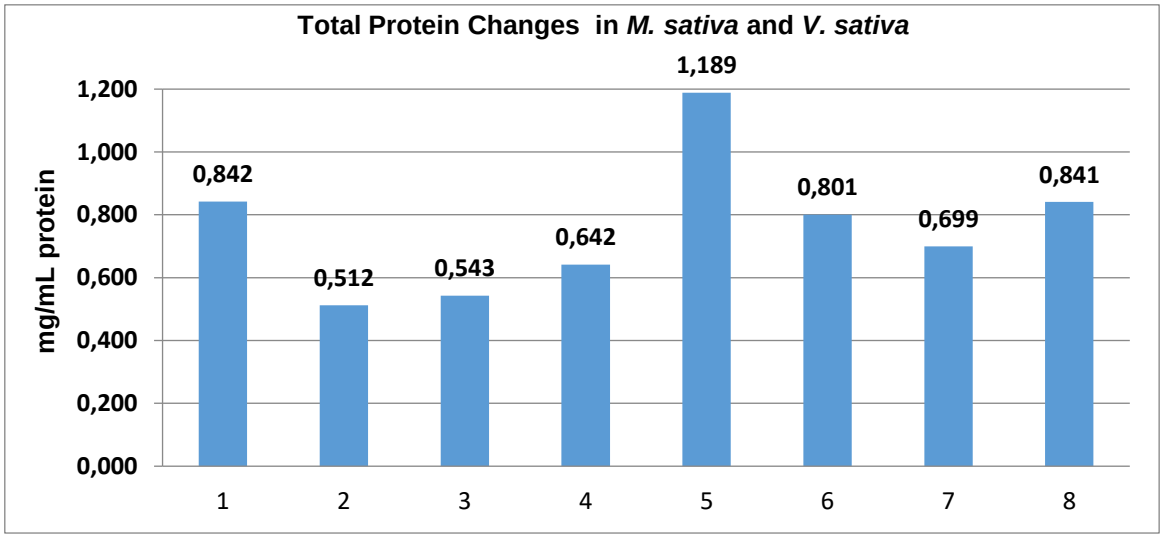
POD activity changes were performed by spectrophotometrically according to Kanner and Kinsella (1983). During the determination of the POD kinetic reaction, enzyme activity has been measured by spectrophotometer at 300nm for 120 seconds. The biggest differences between the absorbance values taken in every 10 seconds periods. The differences have been converted to mg protein level and given as mg/mL/min POD enzyme activity. All POD activity measurements were performed in three replicates.

3. RESULTS AND DISCUSSION

3.1. Total Protein Results

Changes in total protein amount in *M. sativa* (clover) and *V. sativa* (vetch) plants compared to the control groups are as follows (Fig. 1).

Fig. 1: Changes in Total Protein Amount of *M. sativa* (1-4) and *V. sativa* (5-8) Plants.

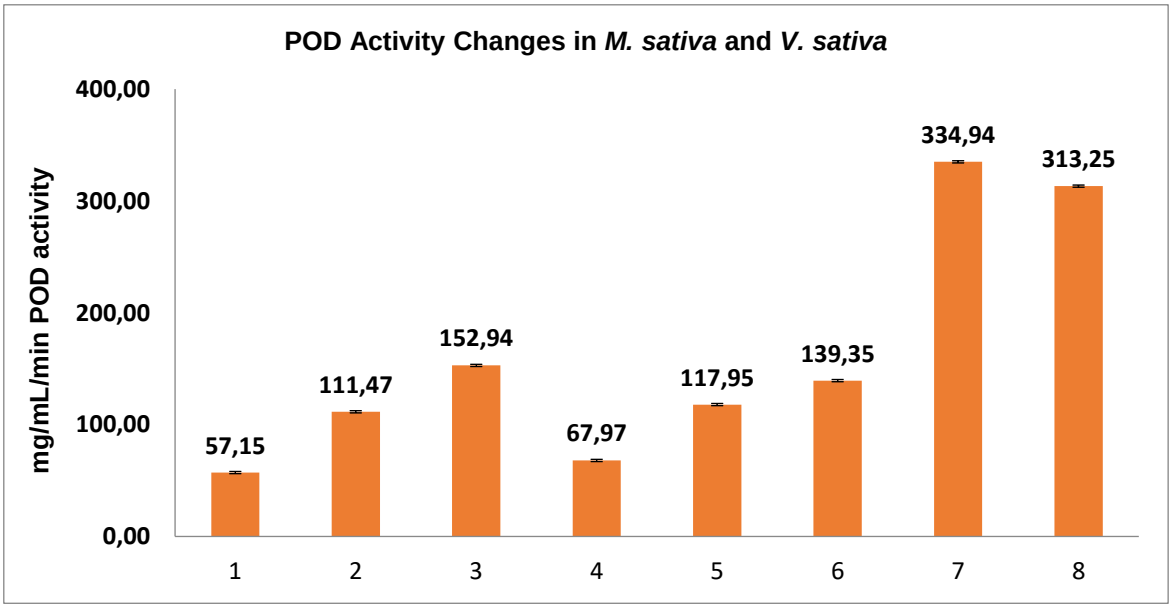


When compared to the control group in *M. sativa* plant, it was determined that the maximum decrease in terms of total protein amount occurred as a result of application of 100 ppm lead (Group 2).When compared to the control group in *V. sativa* plant, it was determined that the highest decrease in terms of total protein amount occurred after the application of 30 ppm cadmium (Group 7). According to these results, it is thought that the moderate application of lead in *M. sativa* has been inhibited protein mechanism at a level of 39%. In the *V. sativa* plant, it has been determined that the application of cadmium has been inhibited protein mechanism at a level of 41 (Fig. 1).

3.2. Peroxidase Results

Changes in peroxidase enzyme activity in *M. sativa* and *V. sativa* plants when compared with the control groups are as follows; (Fig. 2).

Fig. 2: Changes in Peroxidase (POD) Activity in *M. sativa* (1-4) and *V. sativa* (5-8) Plants



When compared with the control group of *M. sativa*, it was determined that the highest increase in POD activity occurred after the application of 30 ppm cadmium (Group 3). When compared with the control group of *V. sativa*, it was determined that the highest increase in

POD activity occurred after the application of 30 ppm cadmium (Group 7). According to our research results, it is thought that cadmium can stimulate the plant defense system in both plants and increase POD activities as plants perceive this heavy metal as a threat signal. Increases in POD activity against to the control group have been measured as 168% in *M. sativa* and 184% in *V. sativa* respectively (Fig. 2).

3.3. Discussion

According to our results regarding the total amount of protein and peroxidase enzyme activity in *M. sativa* and *V. sativa* plants 72 hours after heavy metal applications have been found to produce different responses in the two plants depending on the type of heavy metals. In our research, literature reviews were effective for selecting the heavy metal concentrations which applied to plants.

In the research of Kısa (2018), ascorbate peroxidase (APX), peroxidase (POD), and superoxide dismutase (SOD) activities are measured in the leaf and root of *Lycopersicon esculentum* which grown under the heavy metal conditions. All three enzyme activities showed induction after the treatment of Cd, Cu and Pb in the leaves of *L. esculentum* compared with control groups. Reduced peroxidase activity measured in all treatments of heavy metals in the roots. Cd treatment increased the SOD activity on the contrary, copper showed opposite effect in the increasing doses of copper in roots.

In another research, Cd application showed reduction in plant growth. Different doses of $CdCl_2$, added to the growth media reduced the area of the leaf, chlorophyll and carotenoid contents in the radish plant. In addition that, increasing in catalase (CAT), guaiacol peroxidase (GST) and POD enzymes have been measured (El-Beltagi et al., 2010).

Similar to our research, there are studies in which the same heavy metals are used in different plant systems. It is normal to see physiological differences in different plants as a response. In our research, analyzes were carried out from the regions belonging to the aboveground parts of vetch and clover plants. Heavy metals used in our research are currently and widely used by other researchers.

4. CONCLUSION

As a result of the widespread use of heavy metals, the load on the ecosystem is increasing day by day. As seen in our research, it was determined that Pb, Cu and Cd heavy metals applications caused different physiological responses in clover and vetch plants. The presence of heavy metals that may come from irrigation water or other sources in areas where such forage crops are grown poses a great threat. In terms of the quality of soil and source of irrigation water are very important for plant development. If we notice to this point, forage plants which economically importance can growth well in this healthy ecosystems and by this way we can prevent the damages which such plants can cause to other living things through the food chain.

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