Evaluation of heavy metal tolerance at different clover plant growth stages

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Abstract

In order to study heavy metal tolerance at different clover (Trifolium resupinatum L.) plant growth stages, a pot experiment contaminated by cadmium, copper, nickel, or zinc at 500 mg/l was conducted in three replications. Applied seven days after germination, all heavy metals except zinc had lethal effects on the clover seedlings. Furthermore, when they were applied 14 days after germination, nickel still had a lethal effect on the seedling, and cadmium and copper killed more than half of the plant population. While approximately 90% of the plants exposed to cadmium, copper and zinc were able to grow without apparent negative effects, 21 days after germination nickel still showed lethal effects. In this study, the concentration of heavy metals in shoot dry tissues were 940 mg/kg for cadmium, 781 mg/kg for copper and 670 mg/kg for zinc. These results suggest that the tolerance of clover to cadmium, copper and zinc positively correlates with the age of the plant.

Keywords: Clover plant; growth stages; tolerance; heavy metals


Introduction

Modern civilization introduces a wide range of pollutants to the atmosphere through various anthropogenic activities (Houshmandfar and Tehrani 2008). Although it is almost impossible to visualize a soil without trace levels of heavy metals, most of the heavy metals such as zinc and copper are essential elements to living organisms. Despite this, the excess amounts of heavy metals are generally harmful to plants and animals (Mielke and Reagan, 1988).

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Elevated levels of heavy metals in urban and industrialized areas atmosphere are reported in many parts of the world (Momani et al., 2000). The discovery that certain plant species are capable of tolerating high concentrations of heavy metals opened new possibilities to use these plants to remediate contaminated soils. Nowadays, Phytoextraction or the plant-mediated removal of contaminants from polluted waters and soils is evolving rapidly. Many studies have been conducted to determine the toxic levels of heavy metals for certain plants, especially those metals considered as public health threats (Fernandes and Henriques, 1991). The aim of many of these studies has been to identify plant species capable of accumulating undesirable toxic compounds such as heavy
metals. For example, Reeves and Baker (2000) compiled an exhaustive list of plant species that hyperaccumulate cadmium, chromium, nickel, lead, selenium and zinc. Other researchers have also identified some plant species that accumulate one or more of these heavy metals. Monni et al. (2000) found that Empetrum nigrum absorbed high concentrations of copper and nickel, while Rout et al. (2000) tested the ability of Echinochloa colona to accumulate chromium and nickel. Furthermore, Ouzounidou et al. (1995) studied the interaction of copper with calcium and iron assimilation and Bates (1971) studied the interaction and differential uptake of macro and microelements by several plant species. But few studies have been conducted to elucidate the relationship among various effects of heavy metals on plants at various growth stages. Peralta et al. (2004) demonstrated that in alfalfa (Medicago sativa L.) seedlings, there is a correlation among the heavy metal mixture and the age of the plant. Another study conducted with runner bean plant (Phaseolus coccineus L.) has proved the association among the copper and cadmium susceptibility and the growth stages of the plant (Tukendorf et al. 1997). To our knowledge, no study has been reported with clover plant. Hence, the objective of this study was to evaluate the relationship among various effects of cadmium, copper, nickel, and zinc on clover plant at various growth stages.

Materials and methods

Experimental setup

Approximately 40 seeds of Persian clover (Trifolium resupinatum L.) cultivar Dehpir were placed in 400 g plastic pots, which were filled with 250 g of soil. The soil was classified as a clay loam with 27.9% Sand, 26.2% Clay, 45.9% Silt, and a pH of 7.4 (saturated paste). The plants were grown in a green house at 25 °C temperature. At the planting time, 25 ml of a modified Hoagland’s nutrient solution containing macro and micro elements were applied to each pot (Peralta et al. 2001). The heavy metal salts used in this study included Cd(NO$_3$)$_2$, 4H$_2$O; Cu(NO$_3$)$_2$, 2.5H$_2$O; Ni(NO$_3$)$_2$, 6H$_2$O and Zn(NO$_3$)$_2$, 6H$_2$O. The heavy metal treatments were applied every seven days after seed germination up to three weeks and all of the pots received 25 ml of distilled water every three days. A 50 ml solution including a concentration of 500 mg/l for each pot was used in cadmium, copper, nickel, or zinc for each treatment. The control treatment plants received 50 ml of distilled water instead of the heavy metals and each treatment was repeated three times.

Evaluation of plant growth and metal uptake

A sample of 20 plants from each pot was randomly selected to evaluate the biomass accumulation including root dry biomass, shoot dry biomass, root length, and shoot length. The root and shoot length were measured from the main apex to the crown and from the crown to the main apex respectively. All treated plants in were collected after two weeks of exposure to heavy metals.
the heavy metal stress and washed with distilled water, oven dried at 70 °C for 72 h, and weighed. Finally, metal concentration of the plants and approximately a sample of 1 g of pot soils were measured using an Atomic Absorption apparatus (Perkin–Elmer 3110). The data were statistically analysed through one-way analysis of variance (ANOVA).

Results

Evaluations of clover shoot and root growth

The plants treated with zinc did not show any symptom of toxicity even if zinc was applied at seventh day after germination. Except zinc, all of the heavy metals tested had lethal effect on clover plant seedlings when applied at 7th day after germination. On the other hand, nickel had a lethal effect on clover seedlings of 14 days of plant growth whereas cadmium and copper killed approximately 50% of the plants after the same time period. After 21 days of plant growth, the clover seedlings did not show any tolerance to cadmium, copper and zinc. This happened without any apparent symptoms of toxicity. However, even after 21 days, clover plant was not able to tolerate nickel at 500 mg l⁻¹. Figure 1 shows the size of root and shoot length of 35-day-old clover plant treated with cadmium, copper and zinc at 500 mg l⁻¹ at the growth stage of 21 days. The roots of the plants treated with cadmium grew statistically the same as the control treatment plants and significantly more than the roots of plants treated with copper and the control treatment plants (Figure I). Furthermore, as shown in Figure II, the root mass of plants treated with zinc was significantly higher (P≤0.01) than the root mass of plants treated with copper and the root mass of the plants treated with cadmium and the root mass of the plants treated with copper. In addition, the shoot mass of zinc treated plants was significantly higher than the shoot mass of cadmium treated and control treatment plants.

Heavy metal uptake by clover plant

Figure III shows the concentration of cadmium, copper and zinc found in clover plant tissues. The cadmium treated plants accumulated up to 3460 mg of Cd kg⁻¹ of root dry tissue, whereas the roots of copper and zinc treated plants accumulated 2060 and 2150 mg of metal kg⁻¹ of dry tissue, respectively. The ANOVA showed that the averages of cadmium, copper and zinc found in root tissues were statistically different at P≤0.01. The average concentration of cadmium, copper and zinc found in the shoot tissues of clover plants did not show statistical differences. However, the shoot tissue of cadmium treated plants accumulated up to 940 mg of Cd kg⁻¹ of dry tissue. The concentration of copper and zinc in shoot dry tissues were 781 mg kg⁻¹ for copper treated plants and 670 mg kg⁻¹ for zinc treated plants, respectively. Figure IV indicates the ratio of plant to soil-water soluble fraction concentration of heavy metal in clover plant. Clover plants exposed to heavy metals at the growth stage of 21 days accumulated in the shoots up to 80 times more cadmium and copper than concentrations of these heavy metals in the water-soluble fraction of the soil utilized in this study.

Discussion

According to the results about evaluations of clover shoot and root growth, other researchers have proven that heavy metals such as cadmium reduce shoot growth by reducing the chlorophyll content and the activity of photosystem I. (Waldemar and Baszynski 1996; Skorzynska-Polit and Baszynski 1997). Skorzynska-Polit and Baszynski (1997) reported that cadmium affected young plants more than old plants of P. coccineus. These researchers found that cadmium applied to the younger plants caused a stronger reduction in growth parameters such as leaf area and fresh weight accumulation. However, they also found that cadmium caused only slight changes in growth parameters in plants exposed to this metal at the final growth stage of the primary leaves. Furthermore, according to the observations about shoot and root mass of zinc treated plants, it is hypothesized that even at such doses tested in this research; zinc can act as a nutrient, which could explain the higher mass content of the zinc treated plants. According to the results about heavy metal uptake, reports state that the accumulation of more than 100 mg of cadmium in the aerial plant tissues is extraordinary (Reeves and Baker 2000). In this regard,
Tukendorf et al. (1997) mentioned that if plants of *P. coccineus* were exposed to cadmium, they would produce homophytochelatins, which are organic compounds that complex cadmium and convert it into non-toxic complexes, allowing the plants to accumulate higher amounts of cadmium in their tissues. In conclusion, this study showed that the susceptibility of living clover plant to cadmium, copper and zinc was correlated to the age of the plants. Clover seedling tolerated zinc at 500 mg l\(^{-1}\) at the growth stage of seven days after germination. The addition of cadmium and copper at 500 mg l\(^{-1}\) was tolerated by clover plant solely at the growth stage of 21 days after germination. At this growth stage, the root of clover plant that was treated with zinc at 500 mg l\(^{-1}\) grew significantly more than the cadmium treated plant and the control. Furthermore, the cadmium stressed plant accumulated up to 3460 mg of cadmium kg l\(^{-1}\) of root dry weight biomass and translocated up to 27\% of the metal to the shoot tissue. However, plants of 21 days old did not survive the addition of nickel at 500 mg l\(^{-1}\).

References


