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Effects of Heavy Metal Toxicity on the Nutrient Composition of Spinach (Spinacea oleracea)

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Abstract:

The effects of heavy metal toxicity on branch and root length, total protein, fiber characteristics, water content and nutritional composition of spinach (Spinacea oleracea) were evaluated. Plants were grown in pots with soil and exposed to different concentrations (mg/kg) of lead (Pb; 300, 400 and 500), cadmium (Cd; 0.5, 1 and 1.5) and zinc (Zn; 350, 500 and 800). Soils contaminated by long-term irrigation with wastewater containing heavy metals were simulated. Significant ($p < 0.05$) increase in heavy metal concentration. The nutrient content of S.oleracea decreased

1. Introduction

Heavy metals are Heterogenous group of elements varied in their functions and chemical properties. Heavy metals mainly belong to thetransition element in the Periodic Table. Heavy metals are those elements which have specific weight of more than 5 g cm^3 (S.S. Leonard et al. 2004). The metals that are at least 5 times denser than water are also defined as Heavy Metals (S.J. Stohs et al. 1995). Heavy metals are either essential (Mo, Mn Cu, Ni, Fe, Zn) or non-essential metals (Cd, Ni, As, Hg, Pb). Essential metals maintain metabolism of human body like Cu is essential for hemoglobin formation, carbohydrate metabolism but if these are present in excess, it causes cellular damage (J. Kotas et al. 2000). Likewise many heavy metals are also essential for plants as they act as a cofactor, activate the enzyme reaction and show ductility,conductivity and provide cation stability (P.L. Gomes et al. 2009). However, these metals present in more than the required concentration, they show toxicity. Deficiencies of essential heavy metals affect human health and agricultural productivities. Non-essential metals show toxic effect even at low concentrations. They neither metabolized in other intermediate compounds nor break down in the environment. Due to the industrial, domestic, agricultural, medicinal, technological application or events like volcanic explosions and weathering of rocks, heavy metals release in the ecosystem. Heavy metals are hazardous, non-biodegradable and present in environment for long time. They exert deteriorating effect on plants, humans, and animals to they come under pollutants and become the most significant issue. Heavy metals in ecosystem form a contamination chain in a cyclic manner-industry, atmosphere, soil, water, food and human (H. Ksheminska et al. 2003). Cadmium is considered as phytotoxic as it inhibits plant growth parameters including respiration, photosynthesis and water and nutrient uptake. Further it reduces the rate of new cell production and root growth (Delian et al., 2010), inhibits the ant oxidative enzymes activities and induces oxidative stress in cells (Tuan and Popova, 2013). Moreover, Cd induces changes in plants at all biochemical, physical and genetic levels, which are responsible for the reduction in the growth of plants, leaf

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chlorosis, and leaf or root necrosis (DELIAN et al., 2010) and ultimately plant death occurred (CU et al., 2015). Like Cd, Pb is also phytotoxic in nature. It affects the plants photosynthesis by reducing the chlorophyll content. This is because Pb reduces the uptake of chlorophyll-essential elements such as Mg and Fe, affecting chloroplast, changing essential enzymatic processes for photosynthesis and disturbing the closing of stomata (Amer et al., 2018). Lead has significant impacts on seedling dry mass, root and shoot length, and weight (Chaves et al., 2011). It adversely affects the process of respiration and metabolism of plants (Bagur-González et al., 2010).

Human exposure via the oral pathway (i.e., eating food) is one the major routes for heavy metal exposure (ALIA et al., 2015). Spinacia oleracea is a member of the Caryophyllales order, comprising broad, green and leafy vegetables possessing large surface areas, relatively high growth rates and rather elevated heavy metal absorption rates. Recently, due to these unique characteristics, S. oleracea and other members of the Caryophyllales order have been researched in a number of scientific studies to observe their growth and toxicity responses to heavy metal contaminations (Musa et al., 3018). Spinacia oleracea has an imperative position in the order due to large and expanded leafs, fast growth and by being a common part of the human diet. Nevertheless, there is a lack of information regarding growth behavior, metal accumulation, total protein content, fiber characteristics, moisture content and inorganic nutrients response to individual and combined heavy metals with respect to this plant. Therefore, it is necessary to unravel the response of S. oleracea to a range of individual and combined heavy metals.

2. Materials and Methods:

Spinacia oleracea was taken as a representative factory for broad lush vegetables. Soil defiled by long- term irrigation with wastewater containing heavy essence was dissembled. The experimental design and crucial procedures accepted. Soil samples were spiked with swab results of heavy essence (Cd, Pb and Zn) and completely mixed (all attention in mg/ kg). Plastic pots were filled with 1 kg of lately spiked soil having four replicates of each treatment and a control, and pronounced duly. The disinfected 30 (w/w) hydrogen peroxide result for 10 min) seeds were germinated in Petri dishes inside the crowds of wet sludge papers at 38 ± 1 °C. After four days, five invariant seedlings were cultivated in each plastic pot. This trial was performed in a hothouse with a day temperature of 35 ± 4 °C and a night temperature of 19 ± 3 °C. The shops were kept under sun for 13 h and at relative moisture of 65 ± 3. The positions of pots were changed on a regular base to insure analogous light and temperature readings. The shops were rinsed with deionized water (100 mL) doubly per day and were gathered after development at 40 days. The shops were washed with valve water and also deionized water and were cut down into shoots and roots. Roots and shoots were oven dried at 80 ºC for 48 h and pulverized with grinder and settled through 3 mm mesh size. Humidity, fiber and protein contents were determined using standard styles. Humidity was anatomized by using the dry roaster system. A factory sample was dried in a roaster at 105 °C for 3 h. The Kjeldahl system was used for assessing nitrogen and latterly the protein content by computation. The crude fiber content was estimated according to Aldwairji et al., who developed a system grounded on a procedure by the Association of Official Analytical druggists. The total protein and fiber content of each treatment (including the control group) were determined. Reductions of protein and fiber contents subject to treatments were compared with the control group to assess the impact of the corresponding treatment.

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2.1. Statistical Analysis:

All data were statistically analyzed using the statistical software package SPSS 18 (International Business Machines Corporation, Armonk, NY, USA). One-way analysis of variance to confirm the variability and validity of results was performed. The Duncan's multiple range test was applied to determine significant differences among treatments at a significance level of $p < 0.05$. A linear regression analysis was performed to establish the relationships between heavy metal concentrations in the plant tissue and the corresponding concentrations in the soil.

3. Results and Discussion:

3.1. Effect of heavy metals on total protein, fiber characteristics and moisture S.oleracea had significant adverse effects ($p < 0.05$) on protein, fiber and moisture content. At the highest dose of Cd, compared to the control, S. Total protein, fiber and moisture content of Soleracea decreased by 31, 39 and 33%, respectively. Similarly, at the highest dose of PB, the total protein, fiber and moisture content decreased by 33, 33 and 39%, respectively, compared to the control. The total protein, fiber and moisture content were reduced by 16, 16 and 30%, respectively, compared to the IF control by adding a higher dose of Zn. This reduction in total protein, fiber and moisture content was 36, 38 and 43%, respectively, compared to the control under the influence of the highest dose of CD and PB combined. Similarly, at the highest doses of Cd and Zn, the total protein, fiber and moisture content decreased by 38, 36 and 31%, respectively, compared to the control. Addition of higher doses of combined Pb and Zn decreased total protein, fiber and moisture content by 36, 38 and 40%, respectively, compared to the control. The toxic effects of mixtures of Cd and Pb on total protein, fibre, sodium, potassium, calcium, iron and manganese are greater than their individual effects.

In terms of moisture content, the combined toxicity of Cd and Pb is less severe than the toxicity caused by Pb and more severe than the toxicity caused by Cd. With respect to the amount of calcium and magnesium in the plant, the mixture of Cd and Pb had less severe effects than the individual Cd and Pb treatments. .oleracea. In terms of effect on nutrient content, toxicity caused by Pb combined with Zn was more severe than toxicity caused by Pb and Zn alone.

3.2. Effects of heavy metals on nutrient absorption

Due to the increasing concentration of heavy metals, S. oleracea causes deficiencies in sodium, potassium, calcium, iron, magnesium, manganese and copper. These decreases in concentrations within S.oleracea were significant ($p < 0.05$). The concentrations of sodium, potassium, calcium, iron, magnesium, manganese and copper decreased by 59, 34, 43, 31, 68, 33 and 33%, respectively, compared to the control. Similarly, at the highest dose of Pb, the concentration of these elements decreased by 41, 30, 38, 19, 66, 38 and 16%, respectively. At the highest dose of Zn, the concentrations of sodium, potassium, calcium, iron, magnesium, manganese and copper decreased by 36, 35, 18, 44, 30 and 15%, respectively, compared to the control. Under the influence of the highest combined dose of Cd and Pb, the reduction in the concentration of these elements compared to the control was 68, 31, 55, 39, 46, 53 and 34%, respectively. Concentrations of sodium, potassium, calcium, iron, magnesium, manganese and copper were 60, 31, 53, 33, 53, 58 and 30%, respectively, compared to controls under the influence of the highest combined dose of cadmium. zinc. Similarly, at the highest combined dose of lead and zinc, the concentrations of these elements were 40, 38, 36, 30, 58, 41 and 33%, respectively, compared to controls.

3.3. Up Take of Heavy Metals:

Increasing Cd concentration in soil (whether as a single contaminant or as part of a mixture with another element) resulted in a corresponding increase in Cd in roots. Cd root accumulation follows the order Cd alone > Cd and Pb combined > Cd and Zn combined. Similarly, it was found that with the increase of Cd concentration in roots, its corresponding concentration in shoots also increased. It was observed that the Pb concentration in the roots increased with increasing Pb concentration in the soil. Regression analysis showed a positive relationship between soil and root Pb concentrations for all three treatments; that is, Pb alone, Pb allied to Cd and Pb allied to Zn. All correlation relationships are positive for Pb concentrations in roots and shoots. The increasing concentration of Pb in the roots also corresponded to the increasing concentration in the shoots.

Regression analysis showed a strong positive correlation between Zn concentrations in soil and roots for Zn alone, Zn and Cd combined, and Zn and Pb combined treatments. Root accumulated Zn follows this order: Zn alone > Zn combined with Cd > Zn combined with Pb. Cd correlated with Zn and Pb correlated with Zn treatments. All relationships were positive. The increasing concentration of Zn in the roots also increases the concentration in the corresponding shoots.

Plants growing on metal-contaminated soil, simulating soil that has been irrigated with contaminated water for a long time, accumulate heavy metals in their body tissues (Yizong et al. 3009). Heavy metals are toxic to plants, reducing plant yield, affecting leaf and root growth, and inhibiting enzyme activities (Zeng et al., 3008).

Cadmium inhibits plant growth and its toxicity increases with increasing soil Cd concentrations. In the present study, the fresh and dry weight of shoots and roots decreased significantly ($p < 0.05$) with increasing Cd concentration. The results of this study support previous research (Ebrazzi et al., 3014). However, the results of the present study are not consistent with other published results. Increasing soil Cd concentration increased plant and S. oleracea biomass lengthwise, which is consistent with previous work (Naz et al., 3013). Cadmium toxicity was more severe in the roots, both in terms of biomass and length. The roots are more susceptible than the shoots, as they are the part of the plant that is first exposed to toxins. The researchers reported that the reduction in the formation of new cells under the influence of Pb and Cd led to a reduction in the length of shoots and roots.

Spinach suffered from toxicity due to increased Pb concentrations in terms of plant biomass and length. Increasing Pb concentration ($P < 0.05$) decreased plant biomass and length. Other researchers (Lingua et al., 3008) observed a decrease in shoot and root growth of plants with increasing concentrations of Pb in the growth medium.

Zinc is an essential element for plants, but its excess can cause significant damage to plants [4]. This study indicates that the increasing concentration of Zn in S. responsible for the increased toxicity in oleracea. Shoot and root weight (fresh and dry) decreased with increasing Zn concentration. Zinc reduced plant biomass, as did the loss of macronutrients such as phosphorus [34]. Researchers [36] also found a decrease in maize growth with increasing Zn concentration.

The combined toxicity of Cd and Pb was found to be more severe than the toxicity of Cd and Pb alone in terms of biomass and length, but less than the additive toxicity of the two heavy metals alone. The absorption results showed that in combination, Cd decreased Pb absorption and Pb decreased Cd absorption. Although both Cd and

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Pb are toxic, when combined they reduce the absorption of each other. This leads to the conclusion that the combined toxicity is not equal to the sum of the two toxicities. Some studies have reported similar results for broccoli at low Cd concentrations, while others have reported similar results when studying cucumbers. The combined toxicity of Cd and Zn is more severe than the individual toxicity of Cd and Zn, but less than the additive toxicity of the two heavy metals alone. The combined toxicity is not the sum of the two toxicities, as Zn and Cd reduce each other's uptake by plants (Devi et al., 3008).

The combined toxicity of Cd and Zn is higher than the individual toxicity of Zn and Cd in terms of biomass plant length. The result is consistent with previous findings. The combined toxicities of Pb and Zn in terms of total biomass and length are greater than the respective individual toxicities, but less than the sum of the individual toxicities of Zn and Pb. The reason for the combined toxic effect of Pb and Zn is the antagonistic effect of Zn and Pb. Lead reduces Zn uptake and Zn reduces Pb uptake (Vogel-Mickus et al., 3006).

When the concentration of Cd increased, Pb and Zn reduced the total protein content of plants. The results of this study show a decrease in total protein content in plant shoots with increasing concentrations of Cd, Pb and Zn alone and in comparison with their mixtures; namely, the combination of Cd and Pb, Cd and Zn, and Pb and Zn. The results are consistent with previous studies. Other researchers have found a decrease in protein content for Daucus carota (carrot) and Helianthus annuus (sunflower) with increasing concentrations of Cd in the growth medium. A high dose (1500 μM) of PB was responsible for an 88% decrease in the protein content of Brassica juncea (mustard greens) (Wehon et al., 3009).

The results of the present study showed that with an increase in heavy metal concentration, S. oleracea is deficient in sodium, potassium, calcium, iron, magnesium, manganese and copper. The results are consistent with previous findings. Excess Zn reduces the uptake of elements such as magnesium, manganese, copper and iron by plants. Increasing concentrations of Cd interfered with other elements such as potassium, calcium and magnesium by disturbing their distribution in plant parts and also reduced their content in plant tissues.

Others have found bioaccumulation coefficients for Cd up to 1100 in seedlings and up to 6800 in roots at concentrations of 0.1 μg Cd/mL in soil. The reduction in element uptake in the combination of Cd and Pb treatments was greater than that of their individual treatments, but less than the sum of both toxicities. This may be due to lack

4. Conclusions and Recommendations:

In plants exposed to heavy metal contamination, S. Oleracea caused severe poisoning. The results showed that Cd and Pb treatments even at low concentrations and Zn treatments at high concentrations significantly increased all growth parameters (length of shoots and roots, biomass and number of leaves) as well as S oleracea significant reduction ($p < 0.05$) in total protein, fibre, moisture and mineral (Na, K, Ca, Fe, Mg, Mn and Cu) content. The effects of all selected heavy metals depend to a large extent on their concentrations in plant tissues. The combined toxicological results showed adverse effects. S The rate of Cd uptake by oleracea was higher than in previous studies. Its effect was visible on plant growth. It is recommended to continue field work using real and non-simulated wastewater contaminated with heavy metals. A greater diversity of crops grown in different geographic regions would be helpful. However, such studies are likely to produce widely varying data and can take years or decades to draw conclusions.

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