



Toxic metal interactions affect the bioaccumulation and dietary intake of macro- and micro-nutrients



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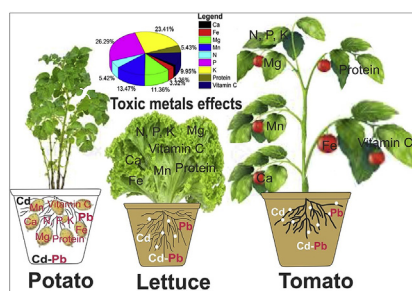
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HIGHLIGHTS

- Cd and Pb have significant negative impacts on macro and micronutrients.
- Toxic metals effects depend upon plants and other metal concentrations.
- Daily dietary intake of nutrients was significantly affected by heavy metals.
- Decrease in essential element concentrations in vegetables can cause malnutrition.

GRAPHICAL ABSTRACT



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ABSTRACT

The present study was conducted to evaluate the effects of heavy metals (cadmium (Cd), lead (Pb) and Cd–Pb mix) on bioaccumulation of different nutrients. Three plant species including potato, tomato and lettuce were grown in pots containing soil contaminated with Cd, Pb and Cd–Pb mix at four different levels. The edible portions of each plant were analysed for Cd, Pb and different macro- and micro-nutrients including protein, vitamin C, nitrogen (N), phosphorous (P), potassium (K), iron (Fe), manganese (Mn), calcium (Ca) and magnesium (Mg). Results indicated significant variations in selected elemental concentrations in all the three plants grown in different treatments. The projected daily dietary intake values of selected metals were significant ($P < 0.001$) for Fe, Mn, Ca and Mg but not significant for protein, vitamin C, N and P. The elemental contribution to Recommended Dietary Allowance (RDA) was significant for Mn. Similarly, Fe and Mg also showed substantial contribution to RDA, while Ca, N, P, K, protein and vitamin C showed the minimal contribution for different age groups. This study suggests that vegetables cultivated on Cd and Pb contaminated soil may significantly affect their quality, and the consumption of such vegetables may result in substantial negative effects on nutritional composition of the consumer body. Long term and continuous use of contaminated vegetables may result in malnutrition.

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1. Introduction

Heavy metals are environmental contaminants and their presence in soil environment is of great concern, as they might be toxic for crop growth, productivity, nutritional status and even for human health (Khan et al., 2015a). Non-essential metals like cadmium (Cd) and lead (Pb) are present and distributed throughout the world. They are released to the surrounding environment through various ways including natural as well as anthropogenic sources (Farahat and Linderholm, 2015; Khan et al., 2014). Different studies revealed that, even at low concentrations the heavy metals are toxic and can cause serious disorders and health problems in plants, animals and human beings because of their non-biodegradability, higher bioaccumulation rate and biotoxicity (Di Salvatore et al., 2008). Toxic metals like Cd can cause serious health hazards on exposure, as they are cytotoxic (Monteiro et al., 2007) and cause cancer and mutation in humans.

In recent years, the interest has been developed towards the heavy metals tolerance mechanism by plants (Caporale et al., 2014) and several studies have been conducted to understand the mechanism of soil-root-shoot transfer of heavy metals (Yin et al., 2011; Khan et al., 2015b). Since, soil is considered as the most important sink for heavy metals and it also act as source of different environmental contaminants (Khan et al., 2015a, 2008).

Heavy metals, released to the environment are extremely relentless, cause serious health hazards and severely affect the ecosystem (Komárek et al., 2013) and ecosystem functions, as they can accumulate in the food crops and contaminate the whole food chain (Bermudez et al., 2012). Previous studies revealed that plants are good bio-indicator to study soil contamination with heavy metals (Zupan et al., 2003). Bini et al. (2012) reported that plants have the capability to uptake heavy metals from metal contaminated soil that remains in their tissues and produce severe morphological changes and health effects. Since, binary mixtures of heavy metals have adverse effects on plants, accredited by their interactions (Cooper et al., 2009). Therefore, metals toxicity to plant can be better assessed by using metal mixture (Frias-Espericueta et al., 2009). Previously, several studies have been conducted to investigate the heavy metal effects on micronutrient concentrations and uptake by plants (Zhang et al., 2002). Furthermore, the interaction and toxicological effects of metals mixture might be due to uptake mechanism and toxicity actions of individual elements (Mendoza et al., 2015).

Vegetables are important part of human diet and contamination of these foodstuffs with heavy metals cannot be ignored, as these diets are rich source of essential macro- and micro-nutrients and their consumption may severely affect the human health (Khan et al., 2008). Leafy vegetables grown on metal contaminated soil have high concentrations of heavy metals because they are potentially hyperaccumulator (Khan et al., 2010). Cd enters the root cells and impound into the root tissues, where it combines with the sulphur because of its high affinity towards heavy metals (Isaure et al., 2006). The phytochelatin-metal complexes could be transported and sequestered in different plant tissues, as reported in *Thlaspi caerulescens* (Küpper et al., 2004).

The aims of this study were to investigate 1) the effects of Cd, Pb and Cd–Pb mix on accumulation of essential macro- and micro-nutrients in selected plants, 2) the influence of toxic metals on daily dietary intake of essential macro- and micro-nutrients, 3) heavy metal effects on plant nutritional values and how they affect the human body nutrient requirements and 4) the effects on daily intakes of light and heavy metals resulting from vegetables consumption grown on metal contaminated soil for the purpose to determine the potential toxic and nutritional impacts of contaminated vegetables.

2. Materials and methods

2.1. Soil sampling, preparation and analyses

Soil samples were collected from a depth of 0–30 cm of the top soil, using stainless steel clean auger, air dried, sieved and then passed through a <2 mm screen. Basic soil physicochemical properties (pH, EC, organic matter, bulk density and soil texture) were investigated. The detail information is given in [Supporting Information \(SI\)](#).

The total metal concentrations in soil samples were determined according to the standard procedure (Nezhad et al., 2014). Briefly, 0.5 g air dried soil samples were digested using concentrated HCl and HNO₃ at 3:1. The extracts were transferred to 50 ml tube and diluted with ultra pure water and the concentrations of elements were determined by Atomic Absorption spectrophotometer (AAS, Perkin–Elmer model 2380). Soil bioavailable metals extracted with 0.05 M EDTA (Merry et al., 1981) and analysed using AAS-MODEL 2380.

2.2. Experimental design

Soil was spiked with selected toxic metals at different concentrations (Cd: 0, 1.0, 2.5 and 5.0 mg kg⁻¹, Pb: 0, 200, 300 and 400 mg kg⁻¹ and Cd–Pb mix: 1.0/200, 2.5/300, and 5.0/400 mg kg⁻¹) using CdSO₄ and Pb(NO₃)₂ salts, respectively. These treatments are represented by the symbols of elements and the figures after them show the level of contamination (e.g. Cd1 refers to level one contaminated with 1.0 mg kg⁻¹), for detail is given in [SI](#). A total mass of 4–8 kg was added in to each pot depending on the type of vegetable. These treatments were performed in triplicates. Before seed sowing pots were irrigated with distilled water to 70% holding capacity of soil. Basal NPK doses of 60, 35 and 40 mg kg⁻¹ were applied to each pot before cultivation. For NPK application, NH₄NO₃ and K₂HPO₄ were added to the soil. Irrigation was performed evenly to all pots when needed. All pots were randomly arranged and changed their position time to time to insure the equal amount of light reach to each pot.

Plant seeds (tomato and lettuce) were purchased from the local market, washed with deionized water and H₂O₂ and germinated in clean petri dishes. The seedlings were kept in control environment for one week with an average room temperature of 23 ± 2 °C and a photoperiod of 12/12 (light/dark). After germination, equal numbers of fresh and healthy seedlings were transferred to the selected pots. Potato seeds were directly introduced to pots. The pots were kept in greenhouse under desired environmental conditions ([Table S1](#)).

2.3. Vegetables preparation and extraction

The vegetable samples were washed with deionized water and oven dried at 70 °C to constant weight (Jordão et al., 2007). After grinding, 0.5 g sample was digested with 5 ml conc. HNO₃ for 1 h at 80 °C and further for 20 h at 120–130 °C using digestion block at the Department of Environmental Sciences, University of Peshawar. The extracts were transferred to 50 ml plastic tubes and the volume was raised to 50 ml by adding deionized water. The metal (Cd, Pb, Fe, Mn, Ca and Mg) concentrations were measured using AAS (Perkin–Elmer 2380). The Phosphorous (P) concentration was measured using UV-spectrophotometer, while potassium (K) concentration was measured using flame photometer.

2.4. Macronutrient analyses

The vitamin C contents were determined by Association of Analytical Chemist (AOAC, 1980) titrimetric procedure using 2,6

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