Effect of Increased Amounts of Fe, Zn, and Cd on Uptake, Translocation, and Accumulation of Human Health Related Micronutrients in Wheat

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ABSTRACT---- ***Agricultural Scientists has lifted the crop production many folds' overs last 100 years but nutritive quality of crop products has not been addressed accordingly as a result humans in many parts of the world are suffering from malnutrition. The efficient improvement of nutritive quality of important crop species like wheat is dependent on the understanding of the acquisition of micronutrients from soil environment and subsequent translocation and distribution into different tissues. The objectives of this work were to understand the effect of increased concentrations of Fe, Zn, and Cd 1) on overall mineral and metal concentrations, 2) on acquisition, translocation, and distribution of minerals among different tissues, and 3) on the inter-relationship of the minerals and metals as reflected in changing the relationship pattern in wheat. The application of increased concentrations of Fe and Zn resulted in three and 11 folds' increase of these micronutrients in wheat respectively and significantly increased seed Ca, P, and S contents however acquisition and translocation of 20 mineral elements varied from tissue to tissue. The improvement of major crop species for health-related micronutrient is important for combating world-wide malnutrition problem. The higher concentration of one micronutrient element may not always ensure higher concentration of that element in seed but increase concentration of Fe and Zn may ensure higher concentrations of others important minerals in wheat seed. The results from our research unveiled key aspects on interrelation among some minerals and metals due to higher concentration of Fe, Zn, and Cd application in wheat.***

Keywords---- Distribution, micronutrient, tissues, translocation, uptake, wheat

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1. INTRODUCTION

Optimal human health depends on a diverse and well-balanced diet containing complex mixture of both macronutrients and micronutrients. Macronutrients make up the bulk of food and are used primarily as a source of energy. Micronutrients are either organic or inorganic compounds, present in small amounts, not used for energy, but are needed for maintaining good health. In human diet, the essential micronutrients include 17 minerals and 13 vitamins which are required at minimum levels to alleviate nutritional disorders [1]. Among the minerals, B (boron), Ca (calcium) Co (cobalt), Cr (chromium), Cu (copper), Fe (iron), I (iodine), K (potassium), Mg (magnesium), Mn (manganese), Mo (molybdenum), Na (sodium), Ni (nickel), P (phosphorus), S (sulfur), Se (selenium), and Zn (zinc) are essential for normal human growth and reproduction [2,3].

Human derives micronutrients from plant based food products essentially from crop plants. Over the last 100 years, science-driven progress in agriculture increased crop production to meet food demand while nutritive values are largely over looked which resulted in worldwide malnutrition [4]. As a result, food products are deficient in micronutrients and contributing to increased morbidity and mortality rates, diminished livelihoods, and adverse effects on learning ability, development, and growth in infants and children [5,6]. World population is likely to reach over 9.6 billion by 2050 [7]; the malnutrition problem will take a serious turn unless the current status of micronutrient concentrations in crop plants is improved.

Micronutrient contents in plant based food products depend on their availability in the soil environment, acquisition by plant roots, and efficient transportation and storage mechanisms. However, along with essential micronutrients, heavy metals are also present ubiquitously in the soil environments. Heavy metals are toxic at higher concentrations [8] and crops uptake and accumulate these metals along with micronutrients in similar ways and thus cause potential threats to human health [9,10]. Among the heavy metals, cadmium (Cd), arsenic (As), chromium (Cr), nickel (Ni) and lead (Pb) are commonly considered as toxic to both plants and humans [11]. Additionally, Cd and As are considered the two major global environmental pollutants because of their high toxicity and carcinogenic properties.

Developing crop varieties with higher micronutrient through breeding or transgenic approaches require the understanding and manipulation of several processes. These include membrane localized transport proteins and long distance transport systems which ultimately contributes to the final seed composition [12]. The physiological basis of absorption and accumulation of micronutrients and metals in edible portion of seeds and grains are not well understood [13,14]. Plants have evolved complex and well controlled network for uptake, translocation, redistribution, and sequestration of mineral and metal elements. There are many different genes including various metal transporters, reductive agents, specialized storage proteins, metal ligands with different substrate specificities, and regularity proteins such as transcription factors, protein kinase, and receptors are involved in directing and controlling this network [15]. The uptake, translocation, and redistribution of mineral elements also depended on the chemical nature of the elements and their competition between and among each other [16,17].

Biofortification is a recent approach aimed at increasing the health-related micronutrients in the staple crops [18]. It is well established that the traits involved in mineral accumulation and uptake are inherited and could therefore be improved by selective breeding [19]. However, progress utilizing plant breeding approaches is limited [20,18] because in the grain of high yielding cereals, there is lower accumulation of some important micronutrients due to the dilution effect resulting from 1000 kernel weight, negative relationship between irrigation and Fe and Zn uptake, negative relationships among micronutrients such as P and Fe and Zn uptake [21,22]. The effect of one mineral element may influence the concentration of others which may also varied from tissue to tissue. The Cd stress showed various influences on the uptake and translocation of mineral nutrients, such as Fe, Zn, Cu, Mn, etc., in dry land crops like soybean, barley and wheat [23,24,25]. The pattern of Zn and Cd distribution were found similar when *Brassica juncea* plants were simultaneously exposed to both Cd and Zn and when plants were treated with Cd during seed set showed the highest concentrations of Cd in seeds, suggesting that the uptake Cd during seed fill has significant contribution to its seed content [26]. The increased concentrations of one mineral may also influence in ligand binding specificity and stability of other minerals during uptake and translocation of minerals in crop plant [27].

Although, a number of investigations were conducted on the essential minerals and metal contents in horticultural crops, many food crops like wheat were overlooked. Among cereals, wheat is the most important staple food crop for more than one third of the world's population [28] and is a good source of Zn, Fe, Se, Mg, and other micronutrients essential to human health [29,30]. Similarly, wheat is also a major source of minerals for humankind, especially in developing countries where a substantial deficiency of minerals like Fe, Zn, Ca, Mg, P, Mn, Sr, Ni, K, S, Na, and Se are reported [31,32,33,34,35]. In this study, we evaluated minerals and metal content in wheat as affected by the increased application of Fe, Zn, and Cd in three wheat genotypes and the objectives were to understand the effect of increased concentrations of Fe, Zn, and Cd 1) on overall mineral and metal concentrations, 2) on acquisition, translocation, and distribution of minerals among different tissues, and 3) on the inter-relationship of the minerals and metals as reflected in changing the relationship pattern in wheat.

2. MATERIALS AND METHODS

2.1 Plant Materials

Three wheat genotypes (Glenn, Alsen, and Dapps) were used in this experiment. Seeds were planted in 8″x11″ pots filled with "Sunshine Mix" and soaked the sunshine mix with water till seed germination as described [27]. At two-leaf stage, 200 mgL^{-1} Fe (iron), 100 mgL^{-1} Zn (zinc), and 50 mgL^{-1} Cd (cadmium) were applied and Sunshine Mix was kept moisten with mineral/metal solution till harvesting. Reference standard solutions of these minerals and metals were purchased from Fisher Scientific (www.fishersci.com) and diluted to required concentrations in slightly acidic solutions of water.

2. 2. Acid Digestion and Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES)

Digestion and ICP-OES analysis were performed at the Metal Analysis Core Laboratory of ND INBRE at NDSU for minerals/metals analyses. At grain filling stage, samples were collected from root, stem, and head and after harvesting, seeds from each head of individual plant were mixed thoroughly and 100 seeds were randomly taken for chemical analysis. All tissues (root, stem, head, and seed) were ground in liquid nitrogen with mortar and pestle until a relatively homogenous particle size was achieved. Closed acid digestion was performed in a Mars Xpress Microwave system (CEM) and 55 mL PFA (Paraformaldehyde) venting vessels following methods as described [2] with modifications. The digestion mixture consisted of 250 mg of sample, 5 mL of concentrated nitric acid and 5 mL of water. The mixture was digested in microwave for 25 min at 185°C. Analyses of Aluminum (Al), Arsenic (As), Boron (B), Beryllium (Be), Calcium (Ca), Cadmium (Cd), Chromium (Cr), Copper (Cu), Iron (Fe), Potassium (K), Lithium (Li), Magnesium (Mg), Manganese (Mn), Molybdenum (Mo), Sodium (Na), Nickel (Ni), Phosphorus (P), Sulfur (S), Silicon (Si), Strontium (Sr), Titanium (Ti), and Zinc (Zn) concentration were performed on Spectra Genesis ICP-OES using Smart Analyzer Vision software (v. 3.013.0752). Quality control consisted of continuous calibration verification, internal standardization and simultaneous analysis of certified reference plant material. The mineral and metal concentrations were measured and presented in µg.g^{-1}.

2.3 Data Analysis

The statistical analysis was performed using the ANOVA procedure of the Statistical Analysis System (SAS) (Version 8.0, SAS Institute Inc., Cary, NC, USA). Means of mineral/metal concentrations of the whole plant and also individual tissues (root, stem, head, and seeds) in different treatments (control, elevated Fe, Zn, and Cd) were compared using Duncan's multiple range test (DMRT). This way the overall effects of the treatments and their influences in different tissues on uptake and translocation of 22 minerals/metals in wheat could be investigated (Table 1 and 2). All the minerals/metals were also clustered using SAS Cluster procedure considering the correlation matrix (Fig. 1) to understand the interrelationship and changing patterns of interrelation due to increased Fe, Zn, and Cd.

3. RESULTS

3. 1 Effect of increased Fe, Zn, and Cd on other mineral and metal concentrations in wheat

Mean concentrations of all minerals and metals due to increased supply of Fe, Zn, and Cd were analyzed using Duncan Multiple Range Test (DMRT). As evident from Table 1, the increased application Fe resulted over three times of the Fe concentration (311.838 µg.g^{-1} compared to 93.759 µg.g^{-1} in control). Similarly Zn concentration was increased by 11 times (450.788 µg.g^{-1} compared to 39.796 µg.g^{-1} in control) Cd increased about 100 times (21.819 µg.g^{-1} compared to 0.229 µg.g^{-1} in control) in wheat . In addition, increased application of Fe significantly increased the overall concentrations of Al, B, Be, Ca, Fe, K, Li, Mg, Mn, Na, P, S, Si, Sr, Ti, and Zn but decreased the concentrations of Cr, Cu, and Ni. However, no significant changes were observed in As, Cd, and Mo concentrations compared to the control. When the genotypes were treated with increased Zn concentration, the concentrations of Fe and Mn were significantly reduced and the concentrations of Cd, Cu, and Mo were unchanged. In contrast, increased application of Fe significantly increased the concentration of the rest of the 17 minerals and metals. Similarly, Cd application increased the concentrations of Al, Ca, Cd, Cu, Li,Mg, Na, P, S, Si, Sr, Ti, and Zn and decreased the concentrations of As, Fe, Mn, and Ni, while no change were observed in five other minerals and metals.

The concentrations of B, Be, and K were increased significantly due to both Fe and Zn application. Interestingly, the concentration of Cr was increased for Zn application and decreased for Fe application and Cu concentration was increased for Cd application and reduced for Fe application. Mn were increased for Fe application but decreased for both Zn and Cd application.

3.2. Effect of Fe, Zn, and Cd on Uptake and translocation of mineral and metals through different Tissues in wheat

The mineral and metal contents of root, stem, head, and seed of both treated and control (not treated) genotypes were analyzed by DMRT and presented in table 2. In all tissues, Ca, P, and S concentrations were found significantly increased compared to control due to increased `Fe, Zn, and Cd application, however, the concentration of other mineral and metal elements varied from tissue to tissue. For instance, Fe application significantly increased Ca content (4470.15 µg.g^{-1}) followed by Zn (4061.35 µg.g^{-1}) and Cd (2562.67 µg.g^{-1}) in root. Similary in stem, Ca content was increased for Fe and Zn application (6816.90 µg.g^{-1}and 7093.70 µg.g^{-1}) respectively. In head, Ca concentration was 4171.42 µg.g^{-1} for Zn application 3866.46 µg.g^{-1} for Fe, and 2067.63 µg.g^{-1} for Cd applications. In seed, the Ca concentration was significantly higher (1288.62 µg.g^{-1}) for Fe application when compared to Zn (1028.63 µg.g^{-1}) and Cd (728.47 µg.g^{-1}) applications. Increased concentration of P in all tissue (6213.50 µg.g^{-1} in seed, 4554.81 µg.g^{-1} in head, 2140.38 µg.g^{-1} in stem, and 1985.56 µg.g^{-1}in root) was observed due to increased Cd application. Increased Zn application contributed significantly to the P content in both stem and root (2140.38 µg.g^{-1} in stem and 1371.87 µg.g^{-1} in root) compared to Fe application in these tissues (1337.28 µg.g^{-1} and 1259.41 µg.g^{-1} respectively). In all tissues except root, Fe application increased S content (2103.42 µg.g^{-1} in seed, 2384.44 µg.g^{-1} in head and 1786.12 µg.g^{-1} in stem). Although no significant difference between Fe and Cd application on the S content in seed was observed, there was a significant increase in S content in seed due to Cd increase (1786.13 µg.g^{-1}) followed by Fe (1594.74 µg.g^{-1}) and Zn (1264 µg.g^{-1}) application. In stem, the influence of Zn elevation was higher for S increase (1655.42 µg.g^{-1}) than that of (1075.70 µg.g^{-1}) for Cd increase. Among the mineral elements, the K content was higher in stem irrespective of treatments (including control) followed by root, head and seed. In case of P, the concentration increased from root to stem, head and seed including control except the content of P in control stem however in case of S, the concentration reduced from root to stem but increased from stem to head and seed. In seed, Al, Cr, Li, Si, and Ti concentrations were significantly increased (29.60 µg.g^{-1}, 0.272 µg.g^{-1}, 0.172 µg.g^{-1}, 70.45 µg.g^{-1}, and 0.561 µg.g^{-1} respectively) for Cd application and Na concentration (53.62 µg.g^{-1}) was increased for Fe application in this tissue. In seed, the Al concentration did not vary significantly for

Fe and Zn increase compared to the control. In both root and stem, Al concentration was increased for Fe, Zn, and Cd applications (1397.15 µg.g^{-1}, 724.49 µg.g^{-1}, and 620.17 µg.g^{-1} respectively in root compared to 329.98 µg.g^{-1} in control) and (1280.44 µg.g^{-1}, 681.89 µg.g^{-1} , and 997.17 µg.g^{-1} respectively in stem compared to 288.14 µg.g^{-1} in control). On the contrary Fe, Zn, and Cd applications showed significantly lower influence on AL concentration in head compared to control (81.95 µg.g^{-1}, 90.08 µg.g^{-1}, and 68.31 µg.g^{-1} respectively compared to 120.02 µg.g^{-1} in control). In both treated and non-treated genotypes, AL concentrations were found lower in head and seed compared to those in root and stem. In seed, no significant influence was observed for Fe and Zn increase on Cr concentration compared to control. In head, the influence of Fe and Zn elevation did not vary but their influence significantly reduced Cr concentrations in this tissue (0.656 µg.g^{-1} and 0.636 µg.g^{-1} for Fe and Zn elevation respectively and 0.748 µg.g^{-1} in control). In stem, the Cr concentration reduced significantly for Fe, Zn, and Cd applications but it was higher in roots when treated with Fe, Zn, and Cd (1.67 µg.g^{-1}, 3.03 µg.g^{-1}, and 1.71 µg.g^{-1} respectively compared to 1.18 µg.g^{-1} in control). Significantly higher influence of Cd elevation was observed for Li content in seed and head but no influence of Fe and Zn elevation compared to control. In both stem and root, the effect Cd application was not significant on Li concentration but Fe and Zn elevation increased Li contents (in stem 1.489 µg.g^{-1} and 1.39 µg.g^{-1} for Fe and Zn elevation respectively compared to 0.398 µg.g^{-1} in control; in root 3.61 µg.g^{-1} and 4.05 µg.g^{-1} for Fe and Zn elevation compared to 0.555 µg.g^{-1} in control). The increase Zn application caused significantly higher concentration of Si in both root (3926.40 µg.g^{-1} compared to control 944.20 µg.g^{-1}) and head (1505.22 µg.g^{-1} compared to control 1456.67 µg.g^{-1}) but insignificant influence of this treatment was observed in seed. Both Fe and Cd application increased Si content in root and stem but its concentration was reduced in head. The reduced concentration of Ti in head was observed due to Fe application followed by Zn and Cd applications (2.26 µg.g^{-1} for Fe, 2.16 µg.g^{-1} for Zn and 1.68 µg.g^{-1} for Cd compared to 2.29 µg.g^{-1} in control). However, Ti concentration was higher for increased Fe, Zn, and Cd application in both root and stem when compared to respective controls (Table 2). A reduced trend of Ti concentrations was observed from root to seed in both treated and non-treated genotypes (seed<head<stem<root). In both root and stem, lower concentrations of As was observed for Cd elevation while Zn elevation showed higher concentration of As in root (24.93 µg.g^{-1} compared to 12.15 µg.g^{-1} in control) but lower in stem (8.37 µg.g^{-1}). Fe application increased As content (10.14 µg.g^{-1}) in stem compared to control (9.78 µg.g^{-1}). In root and head, Fe and Zn application increased B concentration (7.98 µg.g^{-1} and 11.60 µg.g^{-1} respectively) when compared to control (4.03 µg.g^{-1}) in root and in head (5.43 µg.g^{-1} and 4.46 µg.g^{-1} respectively) compared to control (2.08 µg.g^{-1}). The application of Cd reduced B concentrations in root (3.07µg.g^{-1}) and stem (1.14 µg.g^{-1}) but increased in head (4.49 µg.g^{-1}). In root, Zn application showed higher concentration of K while Fe and Cd applications showed lower concentration compared to control. In stem, K concentration significantly increased for both Fe and Zn applications while Cd elevation reduced K concentration in this tissue (23656.40 µg.g^{-1}, 23728.90 µg.g^{-1}, and 12360.60 µg.g^{-1} respectively for Fe, Zn, and Cd applications compared to 15886.90 µg.g^{-1} in control). In head, Zn and Cd applications increased K in head but Fe application reduced K content compared to control (8160.43 µg.g^{-1}, 9004.36 µg.g^{-1} and 11560.82 µg.g^{-1} for Fe, Zn and Cd applications respectively, compared to 7819.40 µg.g^{-1} in control). In root, stem, and head, the Fe, Zn, and Cd applications significantly increased Mg contents compared to control. In both stem and head, Fe, Zn, and Cd applications decreased Mo concentration compared to control but higher concentration of Mo was observed for Zn application in root but lower concentration was observed for Cd application in this tissue. In root, Ni concentration was found higher for Zn application (9.09 µg.g^{-1}) but lower for Fe and Cd applications (4.29 µg.g^{-1} and 3.07 µg.g^{-1} respectively) compared to control (7.05 µg.g^{-1}). However, Sr concentration was increased in root, stem and head for the application of Fe, Zn, and Cd. In seed, Cu concentration was significantly decreased for Zn application (1.91 µg.g^{-1} compared to 3.33 µg.g^{-1} in control). On the other hand Fe application increased Cu concentration (2.52 µg.g^{-1}) in head and Cd elevation decreased it (2.26 µg.g^{-1}). In stem, Zn application (2.78 µg.g^{-1}) significantly increased the Cu concentration while Fe application decreased its concentration (1.82 µg.g^{-1}). The Cd uptake and translocation in different tissues of wheat were only significantly influenced by Cd application but the influence of the of Fe and Zn application did vary significantly compared to respective controls. The applications of Fe and Cd significantly increased the Fe content in seed (99.33 µg.g^{-1} and 69.17 µg.g^{-1} to 31.17 in control) but in both head and stem, Fe, Zn, and Cd applications significantly increased Fe concentration in these tissues (in head 95.03 µg.g^{-1}, 49.08 µg.g^{-1}, and 57.38 µg.g^{-1} for Fe, Zn, and Cd elevations respectively compared to 36.78 µg.g^{-1} in control and in stem 104.00 µg.g^{-1}, 60.95 µg.g^{-1}, and 58.37 µg.g^{-1} for Fe, Zn, and Cd elevation respectively compared to 24.55 µg.g^{-1} in control). However, in root significantly higher concentration of Fe was observed for Fe elevation but lower concentrations were observed for both Zn and Cd elevation compared to control. In seed, Zn and Cd application increased Zn concentration (218.85 µg.g^{-1} and 102.46 µg.g^{-1} compared to 41.59 µg.g^{-1} in control). In head, Fe, Zn, and Cd application significantly increased Zn concentration, but no significant difference was observed for Fe and Cd applications. In both stem and root, only Zn elevation significantly increased the Zn concentration in these tissues.

3.3. Clustering of minerals with the application of increased Fe, Zn, and Cd in wheat

To deduce the inter-relationship of the minerals and metals and relative influence of increased Fe, Zn, and Cd concentration in changing the relationship pattern in wheat, a cluster analysis was performed based on correlation matrix and presented in Fig. 1. In controlled condition, four major cluster of the minerals and were observed (Fig. 1A): cluster 1 grouped S, P, Mn, and Mg, cluster 2 grouped Fe, Ni, Na, Cu, and Zn, cluster 3 grouped Zn, Si, and K, and cluster 4 grouped Cr, Mo, B, Ca, Be, Sr, Li, Cd, As, Ti, and Al. When the genotypes were treated with Fe, three major clusters were formed (Fig. 1B): Mo, Ni, and B in cluster 1, Mg, K, Sr, in cluster 2, and in cluster 3 comprised with three sub-clusters. As evident, the sub-cluster 1 consist of Cu, Cr, Na, Fe, and Cd, sub-cluster 2 consists of Zn and S, and sub-cluster 3 consists of Li, Be, As, P, Mn, Si, Ti, and Al. The treatment of genotypes with Zn formed 3 clusters (Fig. 1C); cluster 1 consist of Mg, Sr, K, Zn, Ca, Be, cluster 2 consist of S, Si, Cu, and cluster 3 contains Fe stand alone in one arm and in another arm, contains two sub-clusters consist of Na, Cr, Cd, Li, B, Ni, Mo, As and P, Mn, Ti, and Al respectively. The Cd treatment formed two major clusters (Fig 1D): cluster 1 consists of Zn, S, P, Mn, Mg, and B and cluster 2 consists five sub-clusters. Sub-cluster 1 consists of Ni, Na, Cu, and Cd, in sub-cluster 2 Fe stand alone, sub-cluster 3 consists of Cr, Sr, and Cd, and sub-cluster 4 consists of K, Be, Mo, Si, As, Li, Ti, and Al.

The cluster analysis shows the effect of different treatment in changing the pattern of grouping the minerals/metals due to changes in their correlations. Some minerals like Ti and Al tend to cluster tightly together regardless of the treatment. However, minerals like Mn and Mg clustered differently based on the treatments the are subjected to (Fig. 1). The Mg and Mn are highly correlated in normal condition while they get separated in different groups by Fe, Zn or Cd treatments. The dendrograms (Fig1.) also is useful to follow the influence of each treatment in increasing the minerals/metals in each group closely related to each other. For example, the Zn, K, Ca group together in control condition but separated with Mn. However, they grouped with Zn in the Zn treatment and they all increased for higher concentration of Zn application.

4. DISCUSSION

The uptake of mineral elements from soil environment and their subsequent distribution within plant have been the subject of studies for many decades[8,36-40] however there are limited studies on the influence of one or more important minerals or metals on the concentration of others at whole plant level. In our study, we treated wheat genotypes with higher concentration of Fe, Zn, and Cd and for the first time, revealed the influence of these minerals and metals on other minerals. The elements of groups 3–12 in periodic table (www.chemicool.com) generally represent transitional states between metallic characters of alkali group (s block) and non-metallic characters of p-block. These elements can form paramagnetic compounds and compounds with many oxidation states[41]. Transitional elements also can be bound with various ligands within and among the elements of same group as well as across groups with varying degrees of specificity[42]. In our study, the mineral elements Fe, Zn, Cd, Cr, Mn, Ni, Cu, and Mo are transitional, Li, Na, and K are alkali metals, Be, Ca, Mg and Sr are alkali earth metals, B, Si, and As are metalloids, Al and Ti are other metals and P and S are other non-metals (www.chemicool.com). We observed that the increased concentrations of Ti and Zn for Fe, Zn, and Cd increase; concentration of Cr increased for Zn increased and reduced for Fe increase; Cu concentration increased for Cd increase and reduced for Fe increase; Fe concentration reduced for both Zn and Cd increase whereas Mn concentration increased for Fe increase and reduced for both Zn and Cd increase. All of these are transitional elements which can form compounds in two or more oxidation states involving a single atom of the element and one or more unpaired elements and can compete to each other for enhancing or inhibiting uptake and translocation of other elements in this group. The uptake and translocation of mineral nutrients in the same group, such as Fe, Zn, Cu, Mn, etc., under Cd stress have been reported in dry land crops, such as soybean, barley and wheat[23-25]. While Cu in human diets is not a limiting on a widespread basis like Fe and Zn, but Cu interacts with Fe and Zn, and shares common transport proteins and mechanisms[26] and Fe, Zn, and Cu are in transitional group. Cadmium and Zn have similar chemical properties and similar pathways are involved in uptake and translocation of these elements within plants[43,44]. In plants, several studies reported that Zn competitively inhibits Cd uptake in roots, also suggesting a common transport mechanism[45]. Several studies reported that that many ZIP family proteins can transport several micronutrients, including Mn, Zn, Cu and Cd[46-51]. Both antagonistic and synergistic interactions between Cd and micronutrient uptake have been reported in different plants in greenhouse and field experiments[52]. In our study, we observed that concentration of Zn increased due to Cd increase which is synergistic in nature and has been reported in wheat and corn[53]. We also observed that the Fe and Mn concentration decreased due to Cd increase which is antagonistic and similar interaction between Cd with Fe and Mn was reported in soybean[23]. The addition of Cd also decreased the concentration of Fe and Mn in cabbage, ryegrass, maize and white clover[54]. In our study, we observed that although the elements like Al, Ca, Li, Na, P, S, and Sr are not transitional elements but their concentrations increased due to Fe, Zn, and Cd increase. It is suggested that in cellular membrane, some ligand can bind with more than one element either with similar specificity and stability or with different specificity or stability. Such as ligand like cysteine and histidine bind to Zn with higher specificity and stability than Fe. Ligand binding with variable specificity and stability has also been reported among metals of different groups[42,55] and the increase of P concentration due to Cd increase in cabbage, ryegrass, maize and white clover[54] could explain the ligand binding among elements of different groups. In our study, the analyses of the mineral element concentrations encompassing root, stem, head, and seed due to Fe, Zn, and Cd application provided a better understanding of these elements on the mineral and metal content in wheat.

The studies on the physiology and regulation of minerals uptake from rhizosphere have been increasing; however, knowledge on minerals moving in and out from vascular tissues, translocating to vegetative and storage tissues is generally lacking[56]. In this study, the higher accumulation of some minerals in wheat tissues from root to head did not ensure higher concentrations of those into the storage tissue (seed). A similar observation was also reported in rice[57]. We also did not find any particular pattern of uptaking minerals or metals by root and translocating them to stem, head, and seeds. This differential mobility of minerals was observed in wheat particularly during uptaking and translocating of P and Mn[58,59] and upaking and translocating of Mn, Fe, and Zn in olive[60].

In this study, we observed significantly higher concentrations of Cd contents in different tissues due to Cd application with highest being in root followed by shoot and seed. We also observed significant variation among mineral and metal elements in different tissues of wheat due to Cd application such as the increase of Al, Ca, Cr, Fe, Li, P, S, Si, Ti, and Zn concentrations in seed when it increased the P and S concentration in all tissues and increased the concentrations Ca and Cr in head and root, Li and Zn in head, and Fe in stem and head. Similar to our study, differential accumulation of several minerals and metals was in wheat and rice due to Cd application[25,61]. Differential influence of Cd application among tissues of wheat was also observed when the Cd levels were increased among different genotypes of wheat[25]. In rice, when Cd level was increased decrease of Zn, Fe and Mn concentrations in shoots and Zn, Cu and Mn concentrations in roots were observed[61]. It was also found that Mn concentration was much higher in shoots, Zn concentrations were almost similar in both root and shoot in rice and a higher Cd level led to a decrease in the Zn concentration in shoots. It was concluded that the Cd interacts with Mn, Zn, Fe, and Cu thus changes in Cd concentration may influence the uptake and translocation of these minerals and consequently may influence reduction or balance in sink tissues[25].

In all tissues, significant influence of Fe application was not only observed in increasing Fe concentration but also observed in increasing in Ca, Na, P, and S concentrations however no significant influence on seed Zn was observed. Except for Fe, similar influence of Zn increase was observed on Ca, Na, P, and S while it significantly increased seed Zn concentration. Among these four minerals, Ca and Na concentrations were found much lower in head and seed compared to those in root and stem. Except for reducing seed Mn concentration for Zn application, no significant influence of Fe and Zn applications were observed for seed Al, As, B, Cr, Cu, K, Li, Mg, Mo, Ni, and Sr. The influence of Fe and Zn application varied from tissue to tissue such as Fe increase significantly increased the concentrations of As, B, Be, Cu, K, Mg, Mn, Ni, Sr, and Zn in head but reduced the concentrations of Al, Cr, Li, Mo, Sr, and Ti in this tissue whereas Zn increase increased the concentration of B, Be, K, Mg, Ni, Si, and Sr, and reduced the concentration of Al, Cr, Li, Mn, Mo, and Ti. We have analyzed mineral concentrations of different tissues at leaf senescence and observed a general tendency of deceasing of Al, As, Ca, Cr, Cu, Fe, Na, Si, Sr, and Ti concentrations from root to seed irrespective of treatments however Mg, Mn, P, and S were found increasing from root to seed. Plants uptake mineral and metals from soil environment through roots and xylem directed those into the transpiration stream to translocate to shoots. The redistribution of the minerals and metals from shoots largely depend on the phloem mobility which varied among minerals and metals[64]. Among the minerals, K, S, Mg, P, and Ni, considered highly mobile, Na, Fe, Zn, cu, B and Mo considered intermediate or conditionally mobile and Ca, Si, and Mn are low mobile[8,61]. The potentiality of translocation of mineral and metal also affected by ligand binding[64,65] , also it was reported that the binding sites should be saturated with a specific mineral or metal to translocate into the tissue. In our study, we analyzed 22 minerals, and the higher mobility patterns were observed for Mg, P, and S which could be due to either intermediate or lower mobility associated with these elements.

5. CONCLUSION

The development of high yielding varieties of major crop species have been ensuring required energy supply worldwide but have not ensured optimum human health. The word population is likely to be 9.5 billion by 2050 thus father improvement of crop yield must be accompanied with the nutritional improvement of food grains otherwise there will be tremendous malnutrition problem worldwide. In this work, we have increased the concentration of important health related micronutrients, Fe and Zn, and a toxic metal Cd in wheat to understand the acquisition, translocation, distribution, and interactions of these minerals and metal among the tissues of wheat. We observed that the acquisition, translocation, and distribution of micronutrients and metal varied from tissue to tissue and the higher accumulation of some minerals in wheat tissues from root to head do not ensure higher concentrations of those into the seed. We also found that the acquisition, translocation, and distribution do not always follow the chemical nature (periodic table) of the mineral elements, non-specific ligand binding among mineral elements of different group may lead to increase or decrease of minerals belong to different group. We observed significant increase of Fe and Zn in wheat grain while these two micronutrients were increased separately however increase of either of these micronutrients can be accompanied by increasing other important minerals like Ca, Na, P, and S. Therefore, tracing the uptake and movement of one mineral or metal could be helpful to understand the transport of the others and could be an attribute in increasing multiple health related mineral in a single breeding effort.

Table 1. Effect of increased Fe, Zn, and Cd on 22 mineral and metal concentrations at whole plant level of wheat derived from the comparison of mean concentrations of minerals and metals with control by Duncan Multiple Range Test (DMRT). Means with same letter are not significantly different.

Treatment	Al	As	B	Be	Ca	Cd	Cr	Cu	Fe	K	Li	Mg	Mn	Mo	Na	Ni	P	S	Si	Sr	Ti	Zn
	$\mu g.g^{-1}$	$\mu g.g^{-1}$	$\mu g.g^{-1}$	$\mu g.g^{-1}$	$\mu g.g^{-1}$	$\mu g.g^{-1}$	$\mu g.g^{-1}$	$\mu g.g^{-1}$	$\mu g.g^{-1}$	$\mu g.g^{-1}$	$\mu g.g^{-1}$	$\mu g.g^{-1}$	$\mu g.g^{-1}$	$\mu g.g^{-1}$	$\mu g.g^{-1}$	$\mu g.g^{-1}$	$\mu g.g^{-1}$	$\mu g.g^{-1}$	$\mu g.g^{-1}$	$\mu g.g^{-1}$	$\mu g.g^{-1}$	$\mu g.g^{-1}$
Co	188.63^{D}	6.3549^{B}	2.4658^{B}	2.937^{C}	1287.41^{C}	0.2292^{B}	0.95436^{B}	4.5769^{B}	93.759^{B}	9679.2^{C}	0.29322^{C}	944.6^{B}	34.164^{B}	2.3505A	404.27^{D}	2.54008^{B}	2576.66D	1105.43^{D}	923.92^{D}	2.8679^{D}	3.7861^{D}	39.796^{C}
Fe	692.32^{A}	6.6155^{B}	4.9312^{A}	9.182^{A}	4110.54^{A}	0.3867^{B}	0.79528^{C}	4.1461^{C}	311.838^{A}	11985.6^{B}	1.31297^{A}	1909.8^{AB}	40.498^{A}	1.635A	1767.27^{A}	2.09281^{C}	3034.31C	1967.18^{A}	1277.03^{B}	7.4984^{A}	10.6219^{A}	62.584^{B}
Zn	378.41^{C}	9.1009^{A}	5.3541^{A}	4.5293^{B}	4088.77^{A}	0.5945^{B}	1.21869^{A}	4.7656^{B}	67.594^{C}	12561.2^{A}	1.39111^{A}	1699.2^{AB}	25.755^{C}	2.1446^{A}	973.38^{B}	3.59167^{A}	3288.69^{B}	1654.68^{C}	1720.48^{A}	6.8625^{B}	6.6815^{C}	450.788^{A}
Cd	428.81^{B}	4.4837^{C}	2.9865^{B}	2.8792^{C}	1874.56^{B}	21.819^{A}	0.95653^{B}	7.9744^{A}	68.018^{C}	9947.1^{C}	0.47253^{B}	2178.9^{A}	24.887^{C}	1.3513A	855.84^{C}	2.15433^{C}	3712.39A	1784.28^{B}	1016.22^{C}	4.174^{C}	7.5029^{B}	74.935^{B}

Table 2. Comparison of means of 22 mineral concentrations in different tissues of wheat influenced by the increased concentrations of Fe, Zn, and Cd derived from Duncan Multiple Range Test (DMRT). Means with same letter are not significantly different.

Treatment	Al	As	B	Be	Ca	Cd	Cr	Cu	Fe	K	Li	Mg	Mn	Mo	Na	Ni	P	S	Si	Sr	Ti	Zn
	$\mu g.g^{-1}$	$\mu g.g^{-1}$	$\mu g.g^{-1}$	$\mu g.g^{-1}$	$\mu g.g^{-1}$	$\mu g.g^{-1}$	$\mu g.g^{-1}$	$\mu g.g^{-1}$	$\mu g.g^{-1}$	$\mu g.g^{-1}$	$\mu g.g^{-1}$	$\mu g.g^{-1}$	$\mu g.g^{-1}$	$\mu g.g^{-1}$	$\mu g.g^{-1}$	$\mu g.g^{-1}$	$\mu g.g^{-1}$	$\mu g.g^{-1}$	$\mu g.g^{-1}$	$\mu g.g^{-1}$	$\mu g.g^{-1}$	$\mu g.g^{-1}$
										Root												
Co	329.98^{D}	12.15^{B}	4.03^{C}	4.73^{B}	2102.53^{D}	0.485^{B}	1.18^{C}	10.71^{C}	282.54^{B}	8812.45^{B}	0.555^{C}	595.67^{D}	21.92^{A}	3.18^{B}	1456.40^{D}	7.05^{B}	945.10^{D}	1137.15^{D}	944.20^{D}	5.22^{D}	7.47^{D}	39.62^{B}
Fe	1397.15^{A}	12.36^{B}	7.98^{B}	19.45^{A}	4470.15^{A}	1.22^{B}	1.67^{B}	9.84^{C}	949.00^{A}	8126.14^{C}	3.61^{B}	1604.15^{A}	15.98^{B}	3.14^{B}	6522.90^{A}	4.29^{C}	1259.41^{C}	1594.74^{B}	2127.40^{B}	10.32^{A}	20.33^{A}	49.69^{B}
Zn	724.49^{B}	24.93^{A}	11.60^{A}	4.37^{B}	4061.35^{B}	1.97^{B}	3.03^{A}	12.06^{B}	117.24^{C}	10037.85^{A}	4.05^{A}	1531.06^{B}	10.45^{D}	6.57^{A}	3533.10^{B}	9.09^{A}	1371.87^{B}	1264.46^{C}	3926.40^{A}	8.05^{B}	13.65^{B}	408.54^{A}
Cd	620.17^{B}	7.12^{C}	3.07^{D}	3.25^{C}	2562.67^{C}	46.63^{A}	1.71^{B}	24.16^{A}	86.95^{C}	8003.94^{C}	0.591^{C}	1282.61^{C}	11.94^{C}	1.85^{C}	2998.90^{C}	3.07^{D}	1985.56^{A}	1789.13^{A}	1496.78^{C}	6.77^{C}	11.31^{C}	64.35^{B}
										Stem												
Co	288.14^{D}	9.784^{B}	2.51^{C}	4.94^{C}	1941.10^{B}	0.321^{B}	1.70^{A}	1.95^{B}	24.55^{C}	15886.90^{B}	0.398^{D}	480.83^{D}	16.28^{C}	2.37^{A}	119.39^{D}	2.26^{C}	822.58^{C}	325.00^{D}	1244.85^{C}	3.07^{D}	5.07^{D}	45.54^{B}
Fe	1280.44^{A}	10.14^{A}	2.98^{B}	13.33^{A}	6816.90^{A}	0.264^{B}	0.66^{D}	1.82^{C}	104.00^{A}	23656.40^{A}	1.489^{A}	2249.56^{A}	26.47^{A}	0.649^{D}	427.48^{A}	1.30^{D}	1337.28^{B}	1786.12^{A}	1704.56^{A}	12.77^{A}	19.77^{A}	47.21^{B}
Zn	681.89^{C}	8.37^{C}	3.29^{C}	10.76^{B}	7093.70^{A}	0.413^{B}	1.01^{B}	2.78^{A}	60.95^{B}	23728.90^{A}	1.39^{B}	1750.96^{B}	19.67^{C}	1.39^{C}	295.00^{C}	3.07^{B}	2140.38^{A}	1655.42^{B}	1403.19^{B}	12.05^{B}	10.68^{C}	854.12^{A}
Cd	997.17^{D}	5.32^{D}	1.14^{D}	4.62^{C}	2139.50^{B}	12.97^{A}	0.840^{C}	2.01^{B}	58.57^{B}	12360.60^{C}	0.879^{D}	1249.84^{C}	14.79^{D}	1.51^{B}	347.23^{B}	3.43^{A}	2095.63^{A}	1075.70^{C}	1399.10^{B}	4.58^{C}	16.46^{B}	52.28^{B}
										Head												
Co	120.02^{A}	2.19^{C}	2.08^{C}	2.07^{D}	715.19^{D}	0.111^{B}	0.748^{B}	2.32^{B}	36.78^{D}	7819.4^{D}	0.104^{B}	1059.48^{C}	42.4^{B}	2.12^{A}	20.7^{D}	0.746^{C}	3992.29^{C}	1461.24^{D}	1456.67^{B}	1.72^{D}	2.29^{A}	32.43^{C}
Fe	81.95^{C}	3.19^{B}	5.43^{A}	3.95^{A}	3866.46^{B}	0.059^{B}	0.656^{C}	2.52^{A}	95.03^{A}	8160.43^{C}	0.069^{C}	1877.47^{A}	62.51^{A}	0.45^{C}	65.11^{A}	2.52^{A}	4481.52^{B}	2384.44^{A}	1236.93^{C}	5.06^{B}	2.26^{B}	81.93^{B}
Zn	94.08^{B}	1.56^{C}	4.46^{B}	3.00^{C}	4171.42^{A}	0^{C}	0.636^{C}	2.32^{B}	49.08^{C}	9004.36^{B}	0^{D}	1773.09^{B}	38.35^{C}	0.35^{C}	27.31^{C}	1.92^{B}	4455.44^{B}	1826.67^{C}	1505.22^{A}	5.70^{A}	2.16^{D}	321.64^{A}
Cd	68.31^{D}	4.00^{A}	4.49^{B}	3.64^{B}	2067.63^{C}	13.5^{A}	1.00^{A}	2.26^{C}	57.38^{B}	11560.82^{A}	0.248^{A}	1782.94^{B}	32.46^{D}	1.03^{C}	44.1^{B}	1.88^{B}	4554.81^{A}	2022.35^{B}	1098.56^{D}	3.73^{C}	1.68^{D}	80.65^{B}
										Seed												
Co	16.40^{B}	1.29^{A}	1.25^{A}	0	390.84^{D}	0^{B}	0.191^{B}	3.33^{A}	31.17^{C}	6198.00^{A}	0.116^{B}	1642^{A}	56.06^{A}	1.73^{A}	20.58^{B}	0.108^{A}	4546.70^{C}	1498.32^{C}	49.94^{B}	1.45^{A}	0.311^{B}	41.59^{C}
Fe	9.73^{B}	0.773^{A}	3.34^{A}	0	1288.62^{A}	0^{B}	0.200^{B}	2.41^{AB}	99.33^{A}	7999.40^{A}	0.086^{B}	1908^{A}	57.04^{A}	2.31^{A}	53.62^{A}	0.264^{A}	5059^{B}	2103.42^{A}	39.24^{C}	1.84^{A}	0.126^{C}	71.5^{BC}
Zn	13.17^{B}	1.55^{A}	2.07^{A}	0	1028.63^{B}	0^{B}	0.200^{B}	1.91^{B}	43.12^{BC}	7473.80^{A}	0.125^{B}	1742^{A}	34.55^{B}	0.277^{A}	38.17^{AB}	0.291^{A}	5187.1^{B}	1872.14^{B}	47.09^{B}	1.64^{A}	0.227^{BC}	218.85^{A}
Cd	29.60^{A}	1.49^{A}	3.24^{A}	0	728.47^{C}	14.18^{A}	0.272^{A}	3.47^{A}	69.17^{AB}	7863.10^{A}	0.172^{A}	4400^{A}	40.35^{AB}	1.02^{A}	33.20^{AB}	0.238^{A}	6213.5^{A}	2249.95^{A}	70.45^{A}	1.61^{A}	0.561^{A}	102.46^{B}

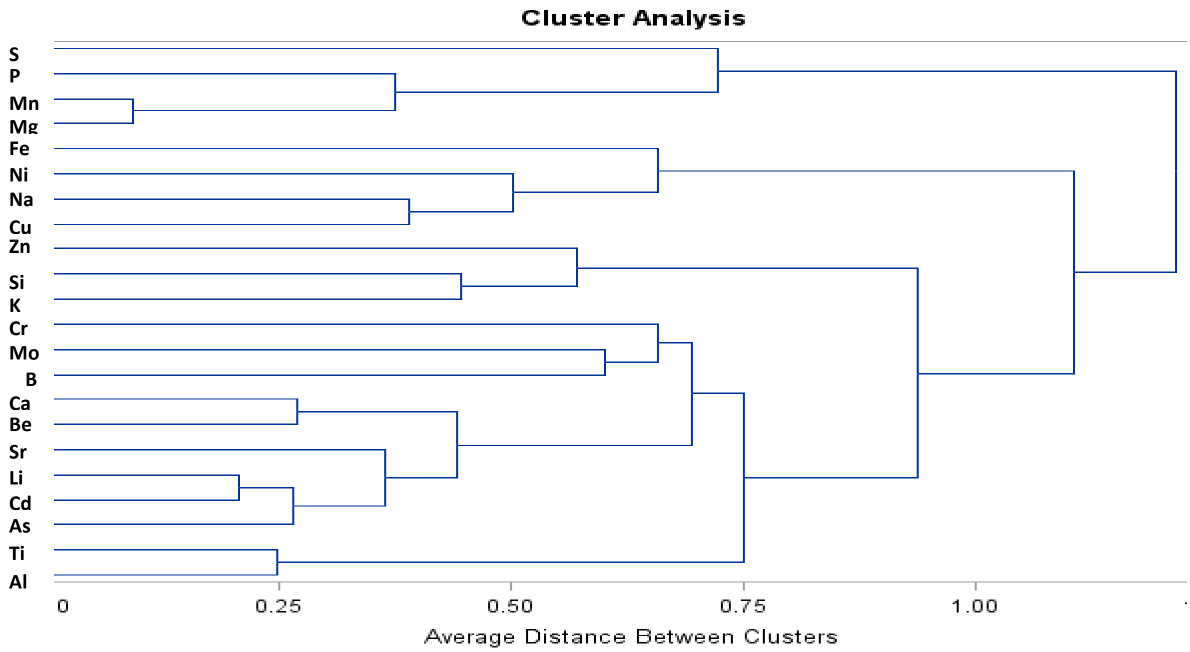

Fig 1A. Grouping of minerals and metals (control condition)

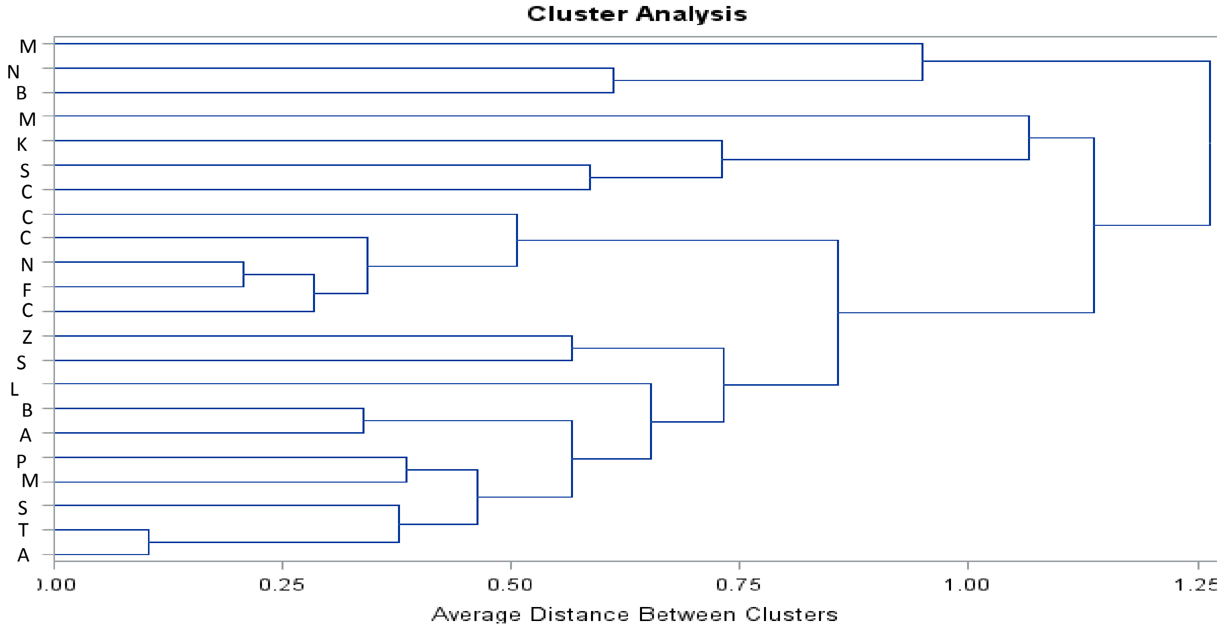

Fig 1B. Effect of increased Fe in grouping of minerals and metals

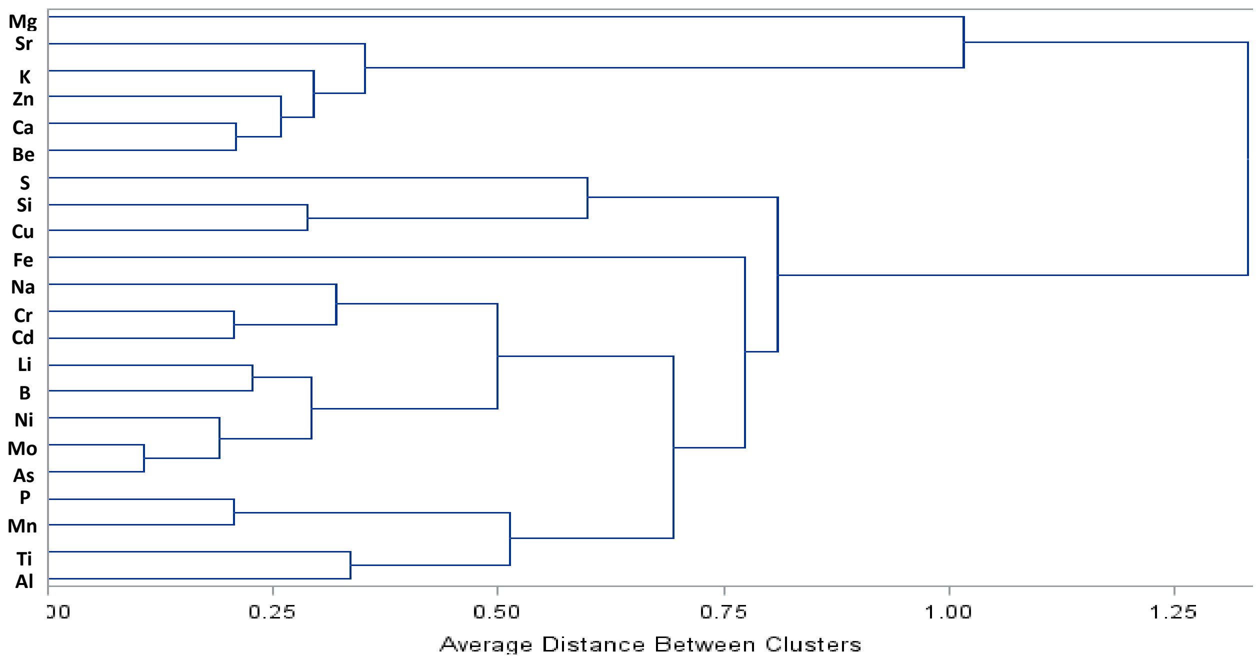

Fig 1C. Effect of Increased Zn on minerals and metals grouping

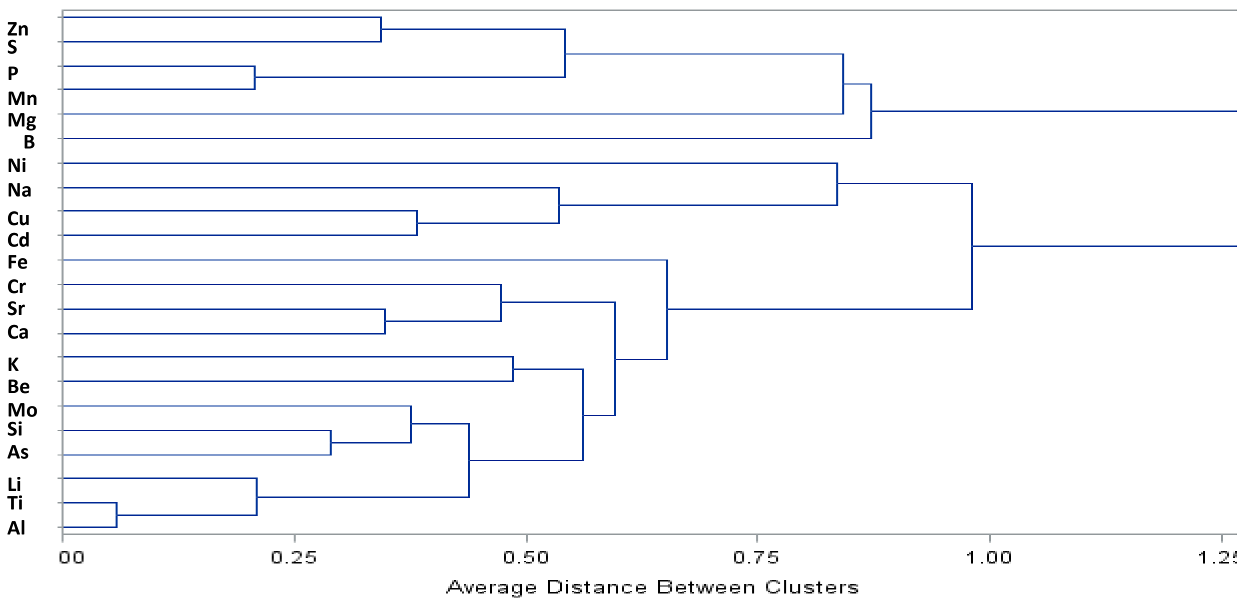

Fig 1D. Effect of increased Cd in grouping of minerals and metals

Fig 1. Cluster analysis of wheat derived from correlation matrix showing the grouping of minerals and metals in control condition and changes in grouping when treated the genotypes with Fe, Zn, and Cd

6. ACKNOWLEDGEMENT

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