ZINC AND COPPER UPTAKE BY WHEAT AND BUCKWHEAT UNDER TWO TRANSPIRATION RATES

By

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HEAVY METAL UPTAKE BY WHEAT AND BUCKWHEAT

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ABSTRACT

M. Sc. Fahima Tani Agric. Biosyst. Eng.

Wastewater has become a vital new supply for irrigation; however, concerns are mounting about environmental and health hazards related to heavy metals present in wastewater. Experiments were conducted to evaluate wheat (Triticum æstivum L.) and buckwheat (Fagopyrum esculentum L.) uptake of copper (Cu) and zinc (Zn).

Some 15 plants per pot were allowed to establish themselves in the greenhouse for 4 and 6 weeks for buckwheat and wheat, respectively. Plants were then transferred to one of two growth chambers differing in the vapor pressure deficit (VPD), creating conditions for two different transpiration rates to occur: high (HT) and low (LT). A total 48 pots for each crop were seeded in order to evaluate the effect of 8 treatment combinations of Cu and Zn (0/0, 5/0, 15/0, 30/0, 0/25, 5/25, 15/25, 30/25) levels (mg L^{-1}). Treatments were laid out in a completely randomized design within each growth chamber.

Three plants were harvested from each pot at days 10 and 20 for wheat, and days 6, 12 and 18 days for buckwheat to measure dry mass and Cu and Zn content in different plant parts. Heavy metal treatments had no significant effect on transpiration rate for either crops. The higher transpiration rate increased Cu/Zn uptake. A Zn amendment in the absence of Cu had a beneficial effect on buckwheat growth, whereas with Cu at 15 mg Cu L^{-1} or 30 mg Cu L^{-1} the lowest dry weights were recorded, regardless of the transpiration rate. Roots contained greater concentrations of Cu and Zn, irrespective of the treatment level and transpiration rate, than did stems, leaves or grain. High retention of heavy metals in the roots of cereal crops may be desirable because these parts are not generally utilized as food or feed.

RÉSUMÉ

M. Sc. Fahima Tani Génie Agric. Biosyst. Eng

Les eaux usées sont devenues d'une grande importance dans l'approvisionnement de l'irrigation; cependant, les risques environnementaux et de santé publique pouvant être associés aux métaux lourds dans ces eaux sont une préoccupation croissante. Des études furent entreprises afin d'évaluer l'absorption de cuivre (Cu) et de zinc (Zn) par des plants de blé (Triticum æstivum L.) ou de sarrazin (Fagopyrum esculentum L.).

En serre, il fut permit à quinze plants de sarrazin ou de blé de s'établir par pot, pour 4 ou 6 semaines, respectivement. Les plants furent ensuite placés dans un de deux phytotrons se distinguant par deux régimes de déficit en pression de vapeur (DPV), créant ainsi des conditions où deux taux de transpiration existent: élevé et bas. En tout, 48 pots de chaque espèce furent ensemencés afin d'évaluer l'effet de 8 traitements de combinaisons de Cu et Zn (0/0, 5/0, 15/0, 30/0, 0/25, 5/25, 15/25, 30/25 mg L^{-1}). Les traitements furent disposés dans un plan expérimental complètement aléatoire dans chacun des phytotrons.

Après 10 et 20 jours pour le blé ou après 6, 12 et 18 jours pour le sarrazin, trois plantes furent récoltées dans chaque pot pour mesurer la masse sèche et la teneur en Cu et Zn dans diverses parties des plantes. Pour les deux espèces, les traitments avec métaux lourds n'eurent aucun effet sur leur taux de transpiration. Le taux de transpiration le plus élevé haussa le taux d'absorption de Cu/Zn. Un ajout de Zn en l'absence de Cu améliora la croissance du sarrazin, tandis que les pires masses sèches furent notées en présence de 15 ou 30 mg L^{-1} de Cu, sans égard au taux de transpiration. Les racines retenèrent plus Cu et Zn que les tiges, feuilles ou grains, sans égard ni au niveau de métaux lourds imposé, ni au taux

de transpiration. Une importante rétention des métaux lourds dans les racines de cultures céréalières seraient à desirer puisque cette partie de la plante ne sert généralement pas dans l'alimentation humaine ou du bétail.

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Finally, this study would not have been possible without the sacrifice, support, encouragement and love of my husband, Dr. Abdirashid Elmi and my beloved children Ifrah and Osman.

DEDICATION

I wish to dedicate this work to my daughter and son, Ifrah and Osman, in the hope that it will encourage them in all that they do.

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MANUSCRIPT AND AUTHORSHIP

This thesis has been written in the form of manuscripts submitted or to be submitted to scientific journals. This format has been approved as outlined in the "Guidelines Concerning Thesis Preparation" by the Faculty of Graduate Studies and Research, "must be cited in full in the introductory section of any thesis to which it applies:

"Candidates have the option of including, as part of their thesis copies of the text of paper(s) submitted or to be submitted for publication, or the clearly-duplicated text of a published paper(s). These text must be bound as an integral part of the thesis".

"If this option is chosen, connecting texts that provide logical bridges between the different papers are mandatory. The thesis must be written in such a way that it is more than a mere collection of manuscripts; in other words, results of a series of papers must be integrated.

"The thesis must still conform to all other requirements of the "Guidelines for Thesis Preparation". The thesis must include, as separate chapters or sections: (1) a table of contents, (2) a general abstract, in English and French, an introduction which clearly states the rationale and objectives of the study, a comprehensive review of the literature, and final conclusion and/or summary, and a thorough bibliography or reference list.

"Additional material must be provided where appropriate and in sufficient detail (e. g., in appendices) to allow a clear and precise judgement to be made of the importance and originality of the research reported in the thesis.

"In the case of manuscripts co-authored by the candidate and others, the candidate is required to make an explicit statement in the thesis to who contributed to such work and to what extent. Since the task of the examiners is made more difficult in these cases, it is in the candidate's interest to make perfectly clear the responsibilities of all authors of the co-authored papers. Under no circumstances can a co-author of any component of such a thesis serve as an examiner for that thesis".

This thesis is presented as two papers prepared for publication; all of them co-authored by myself as a lead author and my supervisor; Dr. Suzelle Barrington, Professor within the Department of Agricultural and Biosystems Engineering. The research was planned and conducted under the responsibility of the student, with technical assistance as noted in the acknowledgments. The student wrote the manuscripts. My co-author provided supervisory guidance from the onset of this study, arranged technical assistance during greenhouse and laboratory operations, offered many valuable suggestions and reviewed all of the manuscripts.

1.0 GENERAL INTRODUCTION

1.1 Statement of the problem

The scarcity of fresh water has become one of the most pressing environmental issues of the 21^{st} Century. A growing body of evidence suggests that, in many parts of the world, water scarcity is already limiting agricultural production (Postel, 1996). Most estimates show that the agricultural sector demands about 70% of global fresh water resources (World Resource Institute, 1995). Demand for water is projected to increase sharply as world populations grow and the development of other sectors, such as industry, impose greater demands for water. Water use prioritization may then come to be based upon produce value, such as is currently the case for industrial operations. This could adversely affect food production and food security.

Irrigated agriculture accounts for nearly 40% of world food production, yet covers 17% of cultivated lands, by area. Preventing the growing scarcity of irrigation water from undermining food security is a key challenge facing today's scientific and producer communities alike. Along with improving irrigation efficiency, other alternatives must be explored. Given rising municipal demands for good quality water, the use of poorer quality waters for irrigated agriculture is on the rise. Consequently, wastewaters have become a vital new source of water to meet irrigation requirements. However, urban effluents, even after conventional biological treatment, still contain substantial levels of heavy metals (Tam and Wong, 1996), which may constitute a hazard not only to the crop, but also to those consuming it (Smith and Cook, 1996). With the current emphasis on environmental health and water pollution issues, public awareness of the need to dispose of these wastewaters safely, while deriving the maximum possible benefit from them is rising.

Plant uptake of heavy metals is a major pathway through which heavy metals from wastewater can enter to the food chain. Some heavy metals, considered innocuous plant micronutrients at low doses, can become harmful to human health if their concentrations exceed certain maximum permissible levels. Continuous use of treated wastewater on agricultural land may lead to an accumulation of heavy metals in the soil, which may then result in excessive or toxic levels being taken up and stored by the crop, potentially compromising the health of those consuming the crop (Korcak and Govin, 1979; Latimer *et al.*, 1990). Excessive levels of cadmium (Cd), copper (Cu), lead (Pb), mercury (Hg), or zinc (Zn) in infants' blood have been linked to a range of health problems (Singh and Steinnes, 1994). Since plants may accumulate these and other heavy metals to levels sufficiently high as to be harmful to humans or animals consumers, concerns about heavy metals entering the food chain are justified (Cook and Andrews, 1990; Levine *et al.*, 1989).

When irrigation water containing high levels of heavy metals contaminates land, two or more metals are generally in excess. The question arises as to whether the concentration of a given trace element (heavy metal) may interfere (synergistically or antagonistically) with the bio-availability and transport of other trace elements in the soil-water system. The interaction most thoroughly investigated has been that of Zn and Cd. Moraghan (1993) showed Zn to be antagonistic to the uptake of Cd in a wide range of crop plants. Zinc is relatively mobile in soil solution and is readily adsorbed by plant roots. Hooda *et al.* (1997) reported that among Cd, Cu, Ni, Pb, and Zn, applied to the soil in the form of municipal wastewater, Zn was the element most efficiently accumulated by the wheat grain. However, limited data is available concerning the interaction of Cu and Zn (Luo and Rimmer, 1995).

This study was designed to investigate the uptake of Zn and Cu, separately and in combination, by wheat (*Triticum æstivum* L.) and buckwheat (*Fagopyrum esculentum* Moench.). Wheat is the world's most important largest cereal-grass crop, and the second most important staple food in Asia, the most populous continent in the world. In 2001, in Canada alone, nearly 1.1×10^7 ha were under wheat, representing 30% of cropped lands (Statistics Canada, 2001). Buckwheat is an unusually fast-growing crop. If water is not limiting, the long growing season available in many parts of the world, provides the opportunity to grow buckwheat as a second crop after the wheat has been harvested.

Presently, information on the possible effects of irrigation water laden with heavy metals such Cu and Zn, saparetley or in combination, on the growth of wheat, and their eventual distribution throughout the plant, is limited. My extensive review of the literature could locate no any information regarding heavy metal uptake by buckwheat.

1.2 Objectives

The primary objectives of this study were to:

1) Quantify Zn and Cu uptake by buckwheat and wheat under two potential transpiration rates.

2) Investigate the interaction between Zn and Cu uptake by buckwheat and wheat, using different combinations and single levels of Zn and Cu.

3) Investigate the effect of transpiration rate on heavy metal uptake by plants.

1.3 Scope of this study

Controlled environment growth chambers were used to study Zn and Cu uptake by wheat and buckwheat plants. Copper and zinc were the only heavy metals investigated, in combination with two vapour pressure deficit (VPD) levels, that resulted in two transpiration rates: high (HT), and low (LT). Plants were grown in 2 L pots containing 1.5 kg of moist sand. Buckwheat was harvested at the flowering stage.

1.4 Method of Thesis Presentation

Chapters 1 and 2 present the general introduction and literature review. The results of experiments are reported in Chapters 3 and 4 in the form of two papers with connecting text, followed by general conclusions in Chapter 5. Literature cited within a chapter is cited at the end of that chapter. The format has been changed to be consistent within this thesis. Tables and figures are presented in sequence at the end of each chapter. Pages with figures were left unnumbered. This chosen procedure was used consistently throughout the thesis.

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CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Population growth and food production

The world's capacity to sustain a favourable food-population balance has become a serious global concern in view of continued population growth and a drastic slowdown in the growth of cereal production since the 1990s (Brown, 1996). Estimates based on the latest UN population projections indicate that the world's population will grow from 6.1 billion in 2000 to 8 billion in 2025 (UN, 2001). Feeding a growing world population at a time when the amount of freshwater available for irrigation is dropping demands the development of strategies to meet the challenge. Chief among the various strategies for sustaining an increase in food production is the improvement of water resource management. Historically, the development of irrigation technology was an important contributing factor to the success of the green revolution.

Water is presently recognized as the most critical resource for future agricultural development. A growing scarcity of freshwater has already become a major impediment to food production in many regions of the world (Postel, 1996). The world population withdraws enormous quantities of freshwater from lakes, streams, and rivers each year. The relative distribution of agricultural, municipal and industrial water withdrawals in selected countries is presented in Table 2.1. Arid regions, where irrigation plays a crucial role in agriculture, have the highest level of water withdrawal for agriculture. In Africa, approximately, 85% of water withdrawals are directed towards agriculture, but this figure varies considerably from one region to another. Globally, about 70% of water is used for crop and livestock production (World Watch Institute, 1995). When demand exceeds supply, Water shortages may occur even in areas with abundant supplies. While Canada houses 9% of the world's freshwater supplies, ranking third among nations, water shortages are common in many parts of Canada. The majority (60%) of Canada's wheat production occurs in the Province of Saskatchewan, where irrigation may become necessary to supplement precipitation. In 2002, for example, wheat production in Western Canada fell to two-thirds of its five-year average because of drought (AAFC, 2002).

Treated sewage water is a potential source of water that could play a critical role in meeting the requirements of the agriculture sector. Thus, there is an urgent need to more thoroughly investigate the suitability of treated wastewater irrigation for cereal crops such as wheat and buckwheat.

As population increases, water shortages will become more pronounced. The demands of agriculture, industry and domestic use will rise significantly, causing even greater shortages than presently exist. Therefore, to help alleviate this problem, municipal wastewater will have to be used for irrigation, leaving water of higher quality for domestic consumption. Moreover, it is worth noting that there are agronomic and economic benefits associated with a careful use of treated wastewater in agriculture. Treated wastewater can contain significant amount of plant nutrients, reducing fertilizer requirements in crop production systems, and minimizing the release of residual contaminants into waterways. FAO (1991) estimated that typical wastewater effluent from domestic sources could supply all the nitrogen and much of the phosphorus and potassium normally required for agricultural crop production. In addition, wastewater use results conserves fresh water for purposes other than irrigation. This study focuses on the impact on plant uptake of the irrigation of wheat and buckwheat with copper- and/or zinc-laden water designed to mimic wastewater.

2.2 The role of wheat and buckwheat in global food security

Wheat is the world's most important food crop cultivated in nearly all regions of the world, its consumption exceeding that of any other cereal crop (Khush, 1999). As a leading global food source, wheat will no doubt become even more important in view of growing food security concerns. Water limitations are generally cited as a major constraint to crop productivity. Consequently irrigation is being expanded in many parts of the world as a way to enhance food production.

The expected increased in the world population from 6 billion in 2000 to more than 8 billion in 2025 (UN, 2001) will place enormous pressure on the world's limited water supplies.. As demand on existing water supplies continues to increase throughout the world, options are being explored to re-use irrigation drainage water and municipal wastewater for irrigation purposes. Continuous use of wastewater, especially untreated or poorly treated wastewater, leads to a constant input of heavy metals into the soil environment. Accumulation of heavy metals in soils, which can then be taken up by plants, poses many health risks, both to humans and to the environment.

Although not a crop of major economic interest, buckwheat is an important crop in a number of countries where it serves as an insurance or important secondary crop, mainly after wheat. Buckwheat is a broadleaf plant native to northern Asia. Seeds are brown in colour, roughly the size of a soybean (*Glycine max* Merr.), but irregularly shaped, with four triangular surfaces. The seeds germinate and emerge rapidly when planted in warm soil, typically in three to four days.

Buckwheat is mostly consumed in the form of flour, which is used in bread, pancakes, noodles, and other food items. Buckwheat consumption has nutritional and health implications. Although its digestibility is relatively low, buckwheat protein has been reported to have positive health effects (Ikeda and Kishida, 1993). Kashayita *et al.* (1995) reported that a buckwheat protein extract reduced, to a greater degree than either soy protein or casein, blood hepatic cholesterol concentrations in rats fed a cholesterol-enriched diet. Some claims of health benefits and its use as a functional food source, in noodles, have been made in China (Li *et al*, 1997).

2.3 Health and environmental risks associated with wastewater

The increasing demand for domestic water due to rapid increases in urban population results in large quantities of municipal wastewater being produced. Consequently, it is prudent to use these wastewaters safely and beneficially according to the guideline of environmental pollution regulations. A number of uses can be found for properly treated wastewater: irrigation, industrial cooling, etc. However, there are mounting concerns about the potential public health hazards related to pathogens, heavy metals, and organic contaminants present in wastewater.

The principal health risks associated with wastewater used for irrigation arises from contamination of crops and groundwater (Pescode, 1992). The use of untreated or poorly treated wastewater to irrigate vegetables and certain fruit crops has been one of the main factors in the outbreak of gastrointestinal diseases, Cholera (Shuval, 1993). Other constituents, in particular nitrogenous compounds, present in wastewater can lead to water quality problems which contribute to health and environmental problems. Elevated nitrate (NO_3^-) levels in drinking water can cause methæmoglobinæmia, or blue baby syndrome, in infants. The current regulatory threshold for nitrate in drinking water in Canada is 10 NO_3^--N mg L^{-1} or 45 NO_3^- mg L^{-1} (Health and Welfare Canada, 1996).

Similarly, pollution caused by municipal wastes and urban storm runoff has become a global environmental issue leading to the degradation of aquatic ecosystems. Phosphorous and nitrogen are often the limiting nutrients in aquatic ecosystems. Their introduction into such ecosystems may contribute to significant eutrophication of surface waters by stimulating algal growth (Spalding and Exner, 1993). Dissolved oxygen levels drop drastically when algae die and begin to decompose, which can result in fish kills and loss of biodiversity in aquatic ecosystems (Carpenter *et al.*, 1998). Certain algal species produce toxins making waters unfit for human consumption (Carpenter *et al.*, 1998). Navigation and recreational uses may also be impaired.

This review highlights the fact that wastewater usage can create an unacceptable health and environmental risk if its use is not strictly controlled. This has increased the urgency to more thoroughly investigate and identify possible detrimental effects of wastewater irrigation on plant growth and develop safe production methods to meet production needs.

Wastewater effluents generally contain high concentrations of suspended and dissolved solids, both organic and inorganic. Traditionally, wastewater treatment plants were designed to reduce the organic pollution of rivers and lakes, but rarely were they designed to remove all pathogenic organisms. The primary objective of any modern wastewater treatment must however be to reduce or eliminate all potential risks. To safely use wastewater for irrigation, safety criteria must be developed. A central point in the question of the risks associated with a given substance/organism is whether or not there is a threshold level below which no adverse effects occurs.

2.3.1 Specific health risks associated with heavy metals

Health risks associated with the reuse of wastewater are not solely restricted to pathogenic organisms. Considerable concern also relates to possible presence of heavy metals in the wastewater. Table 2.2 summarizes the levels of heavy metals typically recmmanded in wastewater, for irrigation water, and in crop tissues. Heavy metals exhibit densities greater than 5 g cm^{-3} and account for 53 of the 90 naturally occurring elements (Niel, 1999). Heavy metals are a special group of trace elements, which have been shown to create definite health hazards when taken up by plants which are later consumed by animals or humans. This group includes, arsenic, (As), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), mercury (Hg), and zinc (Zn). In conventional irrigation waters heavy metals are usually present at relatively low concentrations, usually less than a few mg L^{-1}, but such is not the case with sewage effluents, particularly if they are contaminated with industrial discharges.

Urban wastewater may contain heavy metals at concentrations that will give rise to elevated levels in the soil and cause undesirable accumulations in plant tissue, suppressing crop growth. Tam and Wong (1996) presented an extensive discussion of levels at which accumulation of heavy metals in the soil is likely to have deleterious effects on crops, and secondarily on human and animal health. They suggested that more than 85% of heavy metals were likely to accumulate predominantly at the soil surface, where they are readily available for plant uptake. Furthermore, Alloway and Jackson (1991) indicated that anomalously high concentrations of heavy metals accumulate in the soil and remain available for several years after the application of sludge.

Plant uptake is one the of major pathways by which contaminants from wastewater-amended soil enter the food chain. Plants may absorb heavy metals to levels elevated enough to become a potential health hazard to consumers. Chaney (1990) showed that Cd, Ni, and Zn pose the greatest threat among the metals studied, whereas levels of Cu and Pb tended to remain relatively low or did not show an appreciable increase compared to their background concentrations in the food crops. Moreover, heavy metals borne in wastewater have been shown to move with percolation water (Kirkham, 1986; Alloway and Jackson, 1991), suggesting that with a constant input of metal-bearing wastewater over time, substantial amounts might reach below the root zone to subsoils. In humans, excess Cu and Zn accumulate chiefly in the blood, liver, and kidneys (Gupta and Gupta, 1998), causing severe health problems such as renal damage, hepatic necrosis, anaemia, etc (Sanstead, 1977; Smith *et al.*, 1976)

2.3.2 Effects of heavy metals on soil quality

The extent of the microbial biomass has been widely used to evaluate the detrimental effects of heavy metals on soil health (Frostegard *et al.*, 1996). Several investigations have shown that soil microorganisms may be negatively affected by elevated levels of heavy metals (Baah, 1989; Chander and Brookes, 1993). For example, the *Rhizobium* populations have been found to decrease significantly in soils as a result of heavy metal contamination associated with wastewater irrigation (Chander and Brookes, 1993; Dahlin *et al.*, 1997). These findings clearly indicate that microbial populations are sensitive to metal pollution, and hence, can be used as bioindicators. What is perhaps more surprising is that soil microorganisms subject to long -term metal stress, even at modest levels of exposure, are not able to maintain the same overall biomass as in unpolluted soils (Frostegard *et al.*,1993). Development of tolerance and shifts in community structure could be expected to compensate for loss of more sensitive populations. Instead, results from ecotoxicological studies suggest that microbial diversity decreases along with the soil microbial biomass (Frostegard *et al.*, 1996).

Since conventional sewage treatment (secondary and tertiary) removes a very limited portion of the salts present in domestic and industrial wastewater, these salts are applied to the soil when wastewater is used for irrigation. The presence of these salts in the soil results in clay dispersion and plugging (or size reduction) of soil pores, as evidenced by reduced hydraulic conductivity (HC) in wastewater-treated soils (Vandevivere and Baveye, 1992). Chemical phenomena affecting soil hydraulic properties include changes in the swelling properties of soils and in the dispersion of colloids, affecting natural physical, chemical, and biological processes within the plant-soil-water matrix (Borreli and Fedler, 1988).

2.4 Heavy metals interaction and plant uptake

The major sources of heavy metals, influencing both soil solution concentrations and their uptake by plants, are fertilizers and irrigation water. Soil heavy metal availability impacts plants by affecting their growth. Some soil micronutrients, even in minute quantities, are essential for plant growth. However, the presence of any one of these metals at abnormally elevated concentrations in the root zone may affect the absorption, uptake and utilization of other nutrient elements (Robson and Pitman, 1983). In many cases, metal pollution in the soil is a multi- element problem. Heavy metals may exert antagonistic, additive, and/or synergetic effect on each other's uptake by plants (Luo and Rimmer, 1995). Supplemented in culture solutions to levels exceeding 28 mg L^{-1}, Cd was found to decrease concentrations of K, Zn and Mn in wheat biomass, but did not affect those of Fe and Cu (Jalil *et al.*, 1994). Dudka *et al.* (1994) reported that Zn enhanced Cd uptake by spring wheat grown in sand at Zn levels of 1.042 and 1.542 mg kg^{-1}.

The amount of a given metal taken up by plants from contaminated soil is an important indicator in assessing the risks of toxicity. Heavy metals can be toxic to plants by directly or in directly affecting metabolic processes such as respiration, photosynthesis, CO_2 -fixation, and gas exchange (Ouzounidou *et al.*, 1998). In the cases of multi-element contamination, interaction between metals may occur both at the root surface, affecting uptake to the plant, and in the shoot, affecting translocation and toxicity (Pahlsson, 1989) The interaction most thoroughly investigated is that of Zn and Cd. Moraghan (1993) found mainly antagonistic effects, in which applied Zn reduced the plant uptake of Cd in a range of soil-grown crop plants. Similar effects for lettuce (*Lactuca sativa* L.) and spring wheat grown in nutrient solutions were reported by MacKenna *et al.* (1993). In a nutrient solution experiment, Beckett and Davis (1978) found that Cu had little antagonistic effect on Zn uptake by barley (*Hordeum vulgare* L.) or *vice versa*. In a greenhouse experiment wherein ten soil/metal-contaminated sewage sludge combinations were tested, Sanders *et al.* (1987) found a decrease in the yield of red beet (*Beta vulgaris* L.) in only one of the four soils tested. By comparing treatments with equivalent amounts of applied Zn, either as Zn alone or in combination with Cu or Ni, they found little effect of the presence of Cu and Ni. For the heavy metal levels used in their study, they concluded that yield effects could be attributed to the Zn alone. However, with maize (*Zea mays* L.), a synergistic interaction between Cu and Zn has been reported (Agarwala *et al.*, 1995). In higher plants, Zn competes with Cu at the site of absorption and uptake and, thus, may induce severe nutrient deficiencies in the plant. Interaction between Cu and Zn in wheat crop has rarely been reported.(Agarwala et al., 1995; Pahlsson, 1989) with no study reported with respect to buckwheat crop.

2.5 Heavy metal phytotoxicity

Heavy metals such as Cu and Zn are essential for normal plant growth and development since they are constituents of many enzymes and other proteins (Taiz and Zeiger, 1998). However, elevated concentrations of both essential and non-essential heavy metals in the soil can lead to toxicity symptoms and inhibition of plant growth (Das *et al.*, 1997). Toxicity results occur when the plant is incapable of sequestering or excluding excess concentrations of heavy metals (see Table 2.2). Most trace elements show signs of toxicity at soil solution levels ranging from 1 to 2 mg kg^{-1} tissue (Truhaut, 1979). The amount of heavy metal taken up by a given plant from contaminated soil is of central importance in assessing the risk of toxicity (Luo and Rimmer, 1995).

The major heavy metal environmental contaminants are considered to be Cd, Cu, and Zn, with Cd exerting the greatest effect on plants, followed by Cu and Zn (Kastori *et al.*, 1992). Cadmium has no function in the plant (Das *et al.*, 1997). Cadmium is toxic to plant, causing membrane damage and inducing the production of activated oxygen radicals (Prasad, 1995). Baszynski *et al* (1980) found that Cd affected plant photosynthetis, specifically leading to lower plastid pigment content and lower net photosynthesis.

Copper is an essential plant micronutrients that is required for a number of enzymatic activities, particularly in nitrogen metabolism (Taiz and Zeiger, 1998). High concentrations of Cu inhibit photosynthetic electron transport. Baron *et al.* (1995) found that Cu has a negative impact on photosynthesis, at high concentrations inhibiting photosynthetic electron transport. Similarly, Ouzomidous *et al.* (1998) indicated that plants treated with water containing 160 m Cu showed symptoms of toxicity. According to Moustakas *et al.* (1997), wheat plants showed a lower leaf photosynthetic rate and grain production under Cu stress, suggesting a decrease in CO_2 assimilation and plant chlorophyll content. In contrast, Rousos *et al.* (1989) found no effect on transpiration rate or net photosynthesis for different cabbage (*Brassica oleracea* var. *capitata* L.) cultivars exposed to a Cu concentration of 2.5mg L^{-1}. No data is currently available for buckwheat. More research is needed to establish its toxicity threshold levels for heavy metals, particularly Cu and Zn.

The concentrations of metals in different crops grown on wastewater-irrigated soils show that metal uptake is metal species dependent. Hooda and Alloway (1993) demonstrated that wheat grains accumulated greater amounts of Cd and Ni than Pb or Zn. Thus, municipal wastewater effluents should be checked for trace element toxicity hazards, particularly when heavy metal contamination is suspected. Table 2.3 presents threshold levels of selected heavy metals.

A large body of research on factors controlling the uptake of heavy metals by crop plants has shown that soil pH is an important factor affecting trace metals mobility (Williams *et al.*, 1987; Alloway and Jackson, 1991; Smith, 1994). Alloway and Jackson (1991) suggested that a pH above 6.0 immobilizes heavy metals and thus minimizes the risk of food chain contamination. Increasing the pH involves an increase in the binding of metals to soil constituents, and thereby a decrease in the mobility of soil metals. For example, it is well known that Cu bio-availability and hence Cu toxicity is greater in acidic *vs.* calcareous soils. This is attributable to the greater proportion of Cu^{2+}/Cu^{+} ions under acidic conditions and the fact that the divalent form is that most easily taken up by plants (Tyler and Olsson, 2001).

Concentrations of metals and their distribution in different plant parts grown on wastewater-irrigated soils is also plant-species dependent. Tyler and Olsson (2001) found that in wheat grains Cd and Ni concentrations were much higher than those of Pb or Zn, whereas in carrots (*Daucus carota* L.) Zn levels were highest. McBride *et al.* (2000) showed that total soil Mo concentrations as low as 2 to 3 mg kg^{-1} can lead to serious diseases in cattle grazing on pastures. Kirkham (1986), however, suggested that even if moderately high concentrations of heavy metals were to occur in wastewater, they may not cause harmful effects as the food may normally be deficient in Cu and Zn. Furthermore, heavy metals taken up by vegetables grown with wastewater tend to remain in the roots, with only a fraction being transported to the tops (Kirkham, 1986).

2.5.1 Metal ions speciation in soil

Heavy metals in soils are present in many different physicochemic al forms. The bioavailability and, hence, potential toxicity of metals in the surrounding environment, either added as pollutants or naturally occurring, depends on their total concentrations in the soil, soil solution, and the metal ion speciation in the soil (Katbata-Pendias, 1993; Alloway, 1995). These parameters are largely determined by free metal ion concentration in the soil-water system. Both concentration and speciation of metals in soil solutes are central to the behaviour of the contaminated soils in terms of their potential long term impact on groundwater and on their impact on metal uptake by the biota (Canceset al, 2003). High concentrations ofbioavailable heavy metals in soils may cause long-term risks to ecosystems and humans. Heavy metals are well known to be toxic to most organisms when present in excessive concentration.

The chemical behaviour of metals are primarily governed by retention and release reactions of solute with the soil matrix. The solubility behaviour of Zn, Cu, and Cd in soils varies from soil to soil and is influenced by soil properties, such as pH, organic matter content, clay content and iron oxide content (Babich and stoizky, 1980). Of these, soil pH is often found to have the largest influence, due to its strong effect on solubility and speciation of metals both in the soil as a whole and particularly in the soil solution. Sanders et al. (1986), for example, found that each unit decrease in pH results in approximately 2-fold increases in the concentrations of metals such as Zn ,Ni and Cd in the soil solution. The speciation process thus affects metal availability to plants and leach ability to ground and surface waters.

2.6 Summary

Increased food production will depend to a large extent on the availability of water. The crisis of water scarcity may to some degree be a management problem, and not simply a supply problem. Wastewater has increasingly been recognized as a promising alternative for irrigation. However, there are mounting concerns about the potential public health and environmental hazards related to pathogens, trace elements, and organic contaminants, contained in wastewater. As treated urban effluents always contain some heavy metals, and these may constitute a hazard not only to plants but also to those consuming the plants, measures must be undertaken to maintain the crop quality. A primary objective of this study was to investigate the heavy metals Cu and Zn, their distribution throughout the crop plant and to attempt to understand the effects of their interactions on potential toxicity.

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Table: 2.1: Water use in selected countries (World Resource Institute, 1995)

Countries	Water use (m^3 yr^{-1} $person^{-1}$)	Domestic (%)	Agriculture (%)	Industry (%)
India	612	3	63	4
United States	1868	13	42	45
Nigeria	37	31	54	15
Egypt	1028	7	88	5
Afghanistan	1775	0	99	1
Tanzania	35	21	74	5
Indonesia	95	13	76	11
Bangladesh	212	3	96	1
Mexico	921	6	86	8
United Kingdom	253	20	3	77
Canada	1688	18	12	70
Japan	732	17	50	33

Table 2.2: Threshold levels of some heavy metals for crops (FAO, 1992).

Element	Level recommended for irrigation water (mg L^{-1})	Remarks
Cd	0.01	Toxic to beans (*Phaseolus vulgaris* L.) and beets at concentrations as low as 0.1 mg L^{-1} in nutrition solutions. Conservative limits recommended due its potential accumulation in plants and soils to concentrations that may be harmful to humans.
Co	0.05	Toxic to tomato (*Lycopersicon esculentum* Mill.) plants at 0.1 mg L^{-1}. Tends to be inactivated by neutral and alkaline soil.
Cu	0.2	Toxic to a number of plants at 0.1-1.0 mg L^{-1} in nutrient solution.
Mo	0.01	Not toxic to plants at normal concentration in soil and water. Forage grown in soil with high concentrations of available Mo, can be toxic to livestock.
Zn	2	Toxic to many plants at widely varying concentrations. Reduced toxicity at pH.6.0 and in fine textured or organic soil.
Pb	5	Can inhibit plant cell growth at high concentrations.

Table 2.3: Levels of heavy metals typically found in wastewater, irrigation water, and crop tissues (AAFC, 1985; Reed *et al.*, 1995)

Heavy metal	Level of heavy metal in			
	raw wastewater (mg L^{-1})	irrigation water (mg L^{-1})*	Crops (mg kg^{-1})**	Livestock (mg kg^{-1})***
Cd	0.005	0.01	5-700	0.5
Pb	0.008	5	2-5	30
Zn	0.04	2	15-150	500-1000
Cu	0.18	0.2	25-40	25-300
Ni	0.04	0.02	50-100	50-300

*Safe limit

**Toxic level

***Maximum level tolerated for continuous consumption.

PREFACE TO CHAPTER 3

Ensuring food security for a growing global population poses a challenge for scientists, farmers and policy-makers alike. A primary physical constraint to wheat production is shortage of water. Use of treated wastewater can play an important role in alleviating water shortage problems. Unfortunately, wastewater may contain heavy metals at concentrations that will give rise to elevated levels in the soil and cause undesirable accumulations in plant tissue, suppressing the crop growth. At this project's inception, the need for more research was evident.

The uptake by wheat of Cu and Zn applied in irrigation water, singly or in combination, and the effects of these heavy metals on wheat growth were studied. This chapter which reports the results of these investigations is drawn from a manuscript prepared for publication by myself and co-authored by my supervisor, Dr. Suzelle Barrington, as outlined in the manuscript and authorship section. The format has been changed to be consistent within this thesis.

CHAPTER 3

TRANSPIRATION RATE EFFECT ON COPPER AND ZINC UPTAKE. PART I. WHEAT RESPONSE

ABSTRACT

Freshwater scarcity has increasingly been recognized as a major constraint to agricultural production. As population continues to increase, water shortages will become even more pronounced. Reuse of wastewater for irrigation can provide a vital new source of water for agriculture. This study, undertaken in growth chambers, was designed to evaluate the effects of various concentrations of copper (Cu) and zinc (Zn), singly and in combination on their uptake and accumulation in different portions of the wheat (*Triticum æstivum* L.) plant. Heavy metal treatments were imposed independently under one of two relative humidity (70% or 90%), maintained in growth chambers. For each relative humidity (RH) level, two levels of Zn (0 mg L^{-1} and 25 mg L^{-1}) were factorially combined with four levels of Cu (0, 5, 15, and 30 mg L^{-1}), in a thrice replicated completely randomized design. The Zn and Cu contents of shoots and grain were measured 10 and 20 days after the RH treatments were imposed, and the contents in roots was measured at 20 days.

Transpiration rate was not affected by either heavy metal treatment. The Cu and Zn contents of plants increased with increasing application rates of the individual heavy metals. Concentration of Cu and Zn were highest in the roots. Generally speaking, the presence of Zn in the irrigation water reduced Cu content in the various portions of the plant, but higher Cu levels did not affect the Zn content of plants. In the terms of percentage dry weight, the

quantity of Zn taken up by wheat was roughly two-fold that of Cu. This may be ascribed to the relatively greater mobility of Zn in the soil solution.

3.1 Introduction

Wheat (*Triticum æstivum* L.) is an important food crop worldwide. Growing food demands have led to the extensive introduction of wheat in many parts of the world. Estimates based on the latest UN population projections (UN, 2001) indicate that world's population will exceed 8 billion in 2025, with most of the increase taking place in developing countries such as Ethiopia, Nigeria, Pakistan, etc. This sharp increase in population growth will continue to increase food grain demand. Some of the greatest growth in wheat consumption has occurred in Sub-Sahara Africa, mainly in the form of food aid. Similarly, wheat is the second most important staple food in Asia, the most populous continent in the world, and its consumption has been growing much faster than that of rice (FAO, 1996). Most of the wheat in Asia is now grown on irrigated lands, resulting in dramatic yield increases (Khush, 1999).

The technological advances to which the dramatic rise in world food production over the past 40 years can be most tightly linked were the development of high-yielding cereal varieties, particularly for wheat and rice, and improvements in irrigation technology. Now that the expansion of cultivated lands has ended in many parts of the world, water shortages have emerged as the chief physical constraint to wheat production (Brown, 1996). Lack of water also limits the area potentially available for crop production.

Use of treated wastewater can play an important role in reducing water shortage problems. Unfortunately, wastewater may contain heavy metals at concentrations that can give rise to elevated soil levels and undesirable accumulation in plant tissue, sometimes to the extent of suppressing crop growth (FAO, 1985). Soil solution concentrations and plant uptake of heavy metals are mainly influenced by fertilizer and irrigation water inputs, and the level of binding to soil particles. There are growing concerns that the consumption of foodstuffs containing unacceptably high levels of heavy metals may lead to chronic toxicity (Wagner, 1993). Copper (Cu) and Zinc (Zn) are easily taken up by plants and translocated to different plant organs, posing, through the food chain, a potential health hazard for livestock and humans. Heavy metals may exert antagonistic, additive, synergistic or no effects on their mutual rates of uptake by plants (Luo and Rimmer, 1995). Jalil et al. (1994) showed that Zn accumulation in shoots and roots were decreased by the addition of cadmium (Cd) to the growth medium. However, little information is available regarding plant uptake of Cu and Zn, singly or in combination.

This experiment was designed to evaluate, under two transpiration rates, plant uptake of Cu and Zn, singly or in combination, and their eventual distribution in different plant organs. Specific objectives included:

(i) quantify Zn and Cu uptake by young wheat plants grown under two transpiration rates,

(ii) investigate the interaction between Zn and Cu in their uptake by wheat, using different combinations and single levels Zn and Cu, and

(iii) investigate the effect of heavy metals in the irrigation solution on plant growth

3.2 Materials and methods

3.2.1 Pretreatment growth conditions

Forty-eight 160 mm-high, 155 mm I.D. polyethylene pots were lined with an impermeable plastic bag, to facilitate water balance measurements, and each filled with 1.5

kg of dry sandy soil. Soil was obtained from the B-horizon of an Upland series soil profile consisting of a 1.2 m deep sandy soil layer, overlying a marine clay. The sand's particle size ranged mainly between 0.25 and 0.5 mm, with 10% (w/w) larger than 0.5 mm and 35% (w/w) smaller than 0.25 mm. Initial soil pH was neutral (7.0), P and K levels fairly high at 173 and 222 mg kg^{-1}, respectively, and the cation exchange capacity (CEC) low at 2.02 cmol kg^{-1}. Other selected properties of the soil are reported in Table 3.1. Analyses of available nutrients followed the Mehlich3 extraction procedure (Canadian Society of Soil Science,1993).

Each pot received 300 ml of water, bringing the dry soil to field capacity, and was then seeded with 20 wheat seeds. Seeds were treated with Captan (*N*-trichloromethyl-4-cyclohexene-1,2-dicarboximide), a fungicide. Pots were placed in a greenhouse on the Macdonald Campus of McGill University, Montreal, Canada. Day and night temperatures ranged from 20-22°C and from 16-18 C, respectively. After emergence, the number of plants per pot was reduced to 15, and these were allowed to develop for 6 weeks. Every two days soil water content was estimated by weighing the pots, and soil moisture returned to field capacity with tap water. Initially, and 2, 4, and 6 weeks after emergence the pots were irrigated with a fertilizer (20-20-20) solution, which amounted to a total input per pot of 135 mg each of N, P and K. Any contribution of trace levels of Cu and Zn in the fertilizer were likely negligible and would be accounted for by the measurement of Cu and Zn levels in plants just prior to the imposition of Cu/Zn treatments. Flowering began roughly 4 weeks post-emergence and was largely finished by 5 weeks. At the end of the six weeks, plants had reached a height of 0.60 to 0.65 m and grain filling had begun.

3.2.2 Experimental conditions

At 6 weeks post-emergence, 24 treatment pots and 3 unseeded soil-filled and normally irrigated pots, were transferred to each of two identical growth chambers [Model E15, Conviron Ltd., Winnipeg, Manitoba, Canada) one set to maintain a RH of 70% the other to maintain a RH of 90%. A 12:12 day:night cycle was imposed, with a daytime lighting intensity of 250μ lux at the top of the canopy. Day- and night-time temperatures were maintained at 22 C and 16 C, respectively. The conditions in the 70% and 90% RH chambers led to day-time vapour pressure deficits (VPD) of 0.80 and 0.26 kPa, respectively, leading, in turn, to higher and lower transpiration rates. The chamber had an available floor area of 1.5 m^2 (roughly 1 m × 1.5 m). Pots were arranged on a rectangular grid 50 mm apart, rim to rim, in both directions.

Once in the growth chambers, irrigation to replace water lost by evapotranspiration (i.e. back to field capacity) on a two day interval continued, but, except for the untreated control, the irrigation water contained Cu or Zn singly or in combination. The rate of water loss never exceeded 50 ml per 2-days, representing roughly 20% of soil water content at field capacity. Fertilization was insured by irrigating with the fertilizer solution, rather than a heavy metal solution, on day 14. Temporal differences in weight of the three unseeded pots in each growth chamber served to estimate the evaporation rate. The transpiration rate of the plants was calculated by subtracting the evaporation rate from the daily water loss of plant-bearing pots (the evapotranspiration rate). Transpiration was expressed on the basis of the number of plants in each pot and their dry weight on the day of measurement. Plant dry weights were measured at 10-day intervals and daily values interpolated.

Upon the transfer to the growth chambers the Cu and Zn treatments were imposed. For each RH level, two levels of Zn (0 mg L^{-1} and 25 mg L^{-1}) were factorially combined with four levels of Cu (0, 5, 15, and 30 mg L^{-1}), in a thrice replicated completely randomized design. Irrigation with the assigned Cu/Zn solutions was carried out for 20 days to simulate the application of treated wastewater during the grain filling period of the plant. Zinc and Cu solutions were prepared from $ZnCl_2$ and $CuCl_2$ salts (Mandel Scientific) dissolved in distilled water. The levels of Cu and Zn we chosen to be large enough to have an impact on the Cu and Zn content of the plants, without causing phytotoxicity. The natural pH of these solutions was relatively low, at 5.5, and thus, all soil pH values were measured at the end of the experiment.

3.2.3 Sampling and parameters measured

Prior to the imposition of Cu/Zn treatments, and on days 10 and 20 after their imposition, three plants from each pot were randomly selected and harvested by removing their aerial portion. Plant material was separated into grain and shoots, and each component was analyzed separately for dry matter, Cu and Zn content. On day 20, which represented the end of the experiment, roots of the sampled plants were removed from the soil, washed with distilled water to remove all traces of soil and similarly analyzed. Dry matter content was determined by weighing the freshly harvested material, drying it for 48h at 70 C then re-weighing and expressing the dry weight as a percentage of the fresh weight. Dried plant parts were then stored in individual plastic bags, until their analysis.

Plant materials for Cu and Zn analysis were ground in a stainless steel mill, and subsamples of 2 g were digested with concentrated sulphuric acid and hydrogen peroxide (50%) at 500 C. The Cu and Zn levels were then determined by Atomic Absorbtion Spectrometry (Model 903 Single beam atomic absorption spectrophotometer, GBC Scientific Equipment, Dandelong, Australia) and expressed in mg kg^{-1} dry weight of plant material. The percentage uptake of Zn or Cu was calculated as one hundred times the total quantity per plant (mg) divided by the total amount applied to the pot in irrigation water (concentration in mg L^{-1} × volume of irrigation water applied in L).

Analyses of variance (ANOVA) was performed using general linear models (GLM) available in Statistical Analysis System (SAS) package for windows, (SAS Institute, Cary, NC). The data from each RH regime was analyzed independently, as the layout of the CRD in each chamber was different. Significant differences within the 8 Cu/Zn treatment combinations were assessed using Sheffe's multiple comparison test. Unless otherwise noted, a 5% probability level (P 0.05) was used to declare treatment differences to be significant.

3.3 Results and discussion

3.3.1 Transpiration rate

Transpiration rate was not significantly affected by heavy metal treatments ($P > 0.05$; data not presented), but changed with VDP regime. Plants grown under a high VDP showed consistently greater transpiration rates than plants grown under a low VDP (Fig. 3.1). Regression analysis showed that transpiration per plant increased linearly with time under both VDP regimes (Fig. 3.1), suggesting that while grain filling is occurring there is some leaf area expansion continuing.

3.3.2 Dry matter yield

For both VDP regimes and both sampling days, dry matter yields of shoot and grain were largely unaffected by the presence of heavy metals in the irrigation solution (Tables 3.2 and 3.3). Under both transpiration rates, there was a progressive slight decline in shoot and grain yields with the additions of Cu at Zn 25 mg L^{-1} Zn. The opposite was true with additions of Cu at Zn 0 mg L^{-1} Zn. Overall, the greatest shoot and grain dry weights were obtained with no Cu amendment and Zn at a level of 25 mg L^{-1}, while the lowest weights (24% reduction) occurred with no Zn amendment and Cu at a level of 5 mg L^{-1}. Decreases in dry weight ranged from 4% to 24%, but the differences were largely statistically not significant. Although no symptoms of toxicity were visible, the negative effects of the high levels of Cu with Zn amendment may be due the large concentrations absorbed and translocated to the plant parts, especially towards the end of the growing period. In contrast, averaged across Cu treatments dry matter yield increased with the addition of Zn. We, therefore, speculate that the low yields measured in pots receiving no Zn amendments may be explained by Zn deficiency.

3.3.3 Distribution of Cu and Zn in plant parts

The Cu and Zn contents of whole wheat plants prior to the imposition of the treatments were 1.34±0.48 mg kg^{-1} and 2.05±0.44 mg kg^{-1} (mean±Std. Dev., n = 12), respectively. These levels are clearly below those of plants receiving Cu or Zn in their irrigation water (Figure 3.2). To examine the significance of the effects of Cu and Zn it is particularly important to evaluate the distribution of these metals between tops and roots. The Cu content of shoots increased with increasing concentrations of Cu in the irrigation solution (Fig. 3.2). However, throughout the experimental period, Cu uptake was affected primarily by its interaction with Zn. Mean values and their statistical significance are reported in Appendix A (Table A.1). Strikingly, under both transpiration rates and at both sampling dates, the Cu/Zn interaction appeared to be consistently antagonistic. For example, at 10 days, with no Zn in the irrigation solution, shoot Cu contents was slightly greater than when Zn was present (25 mg L^{-1}) in the irrigation solution (Fig. 3.2a). Similar trends were also apparent for the Day 20 harvest (Fig. 3.3a). Unlike in young barley, where a weak antagonistic interaction between Cu and Zn, Cu slightly reducing Zn uptake, was reported (Beckett and Davis, 1978), we, in wheat, found the presence of Zn to reduce Cu uptake. With respect to the effect of Cu on Zn uptake, the dominant effect in our study was a synergism by which increasing Cu additions proportionately increased Zn uptake (Figs. 3.2b and 3.3b). This is in broad agreement with the results of Luo and Rimmer (1995) who carried out plant growth experiments on soils irrigated at with water bearing, singly or in combination, Zn at 0, 10, and 100 mg L^{-1} and Cu at 0 and 50 mg L^{-1}. On the other hand, Sanders et al. (1987) found no evidence of Cu/Zn interaction.

Uptake of Cu and Zn into the grain followed a pattern similar to that of the shoot material, but with lower Cu and Zn contents for each corresponding sampling day and VPT regime (Figs. 3.4 and 3.5). Mean values and a summary of statistical significance are presented in Appendix A (Table A.2).

For both sampling days and both VPT regimes, Cu and Zn contents of roots were greater than in the shoot or grain (Fig. 3.6). Mean values and statistical significance among treatments is shown in Table A.3 (Appendix A). This suggests that roots served as a barrier to further Cu and Zn movement into the shoot of wheat plants. Chainon et al. (2002) showed higher Cu contents in tomato (*Lycopersicon esculentum* Mill.) and oilseed rape (*Brassica napus* L.) roots than in their respective aerial portions. Similar to our results, Zhang et al. (2002) recently reported the highest cadmium (Cd) contents in wheat roots and the lowest contents in the grain. The retention of heavy metals in roots of cereal crops may be desirable as these parts are not generally used as food or feed; however the consumption of root crops grown on soils heavily contaminated with Cu/Zn could constitute a health hazard. While Cu/Zn concentrations in our study were not excessively high to lead toxicity, the possibility of serious grain contamination does exist with repeated loadings of water containing these metals. Wallace and Wallace (1994) who studied Pb transfer from plant roots to fruit seeds suggested that the transfer process is generally low, but sometimes can be significant. Although the mechanism by which Cu and Zn are bound within the root is not well known, Brun et al. (2001) proposed that root concentrations of Cu/Zn can be used as an indicator for the presence of these metals in the soil. However, under certain circumstances, Cu and Zn may reach sufficiently high (phytotoxic level) contents in the root system to limit root development and this may actually lead to reduced uptake in the above-ground tissues (Lexmond, 1980).

3.4 Conclusions

Plant growth was not significantly affected by the levels of Cu and Zn in the irrigation water. With increasing levels of Cu in the irrigation water, higher Cu contents were measured in different parts of the plant. The Cu and Zn contents were markedly higher in roots than shoots. Additions of Cu enhanced Zn uptake (synergism), while the dominant effect of Zn on Cu uptake appeared to be antagonist. More Zn was taken up by plants than of the Cu. This may be due, in part, to the relatively higher bioavailability of Zn.

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Table 3.1: Selected chemical and physical properties of the soil.

Property	Value
Particle size distribution [% (w/w)]	
>1.0 mm	0
1.0-0.50 mm	5
0.50-0.25 mm	55
0.25-0.10 mm	38
< 0.10 mm	2
Available nutrient elements (mg kg^{-1})	
P	173
K	190
Mg	4
Ca	90
Al	120
Zn	0.73
Cu	0.92
B	3
Mn	1.4
Fe	104
Other characteristics	
Total Cd [mg kg^{-1}]	0.5
pH	7.00
Organic matter [% (w/w)]	0.4
CEC [cmol kg^{-1}]	2.02

Table 3.2: Dry weight of wheat shoot (g plant^{-1}) in response to irrigation with water bearing different concentrations of Cu and Zn, singly or in combination, by transpiration rate and by sampling date.

Treatments (mg L^{-1})	Transpiration rate			
	Low		High	
	Day 10	Day 20	Day 10	Day 20
Cu_0/Zn_0	0.341	0.544	0.348	0.549
Cu_0/Zn_{25}	0.385H	0.591H	0.391H	0.601H
Cu_5/Zn_{25}	0.375H	0.576H	0.380H	0.598H
Cu_{15}/Zn_{25}	0.371H	0.566H	0.376H	0.587H
Cu_{30}/Zn_{25}	0.365H	0.567H	0.372H	0.585H
Cu_5/Zn_0	0.310L	0.508L	0.312L	0.510L
Cu_{15}/Zn_0	0.321L	0.512L	0.325L	0.516L
Cu_{30}/Zn_0	0.335L	0.535L	0.339L	0.542L

Note: L = significantly lower than control

H = significantly greater than control

Table 3.3: Dry weight of wheat grain (g plant^{-1}) in response to irrigation with water bearing different concentrations of Cu and Zn, singly or in combination, by transpiration rate and by sampling date.

Treatment (mg L^{-1})	Transpiration rate			
	Low		High	
	Day 10	Day 20	Day 10	Day 20
Cu_0/Zn_0	0.182	0.225	0.188	0.23
Cu_0/Zn_{25}	0.205H	0.254H	0.209H	0.265H
Cu_5/Zn_{25}	0.190H	0.230H	0.198H	0.240H
Cu_{15}/Zn_{25}	0.185H	0.228H	0.195H	0.237H
Cu_{30}/Zn_{25}	0.184H	0.226H	0.192H	0.234H
Cu_5/Zn_0	0.146L	0.202L	0.150L	0.205L
Cu_{15}/Zn_0	0.148L	0.208L	0.155L	0.215L
Cu_{30}/Zn_0	0.155L	0.212L	0.161L	0.219L

Note: L = significantly lower than control

H = significantly greater than control

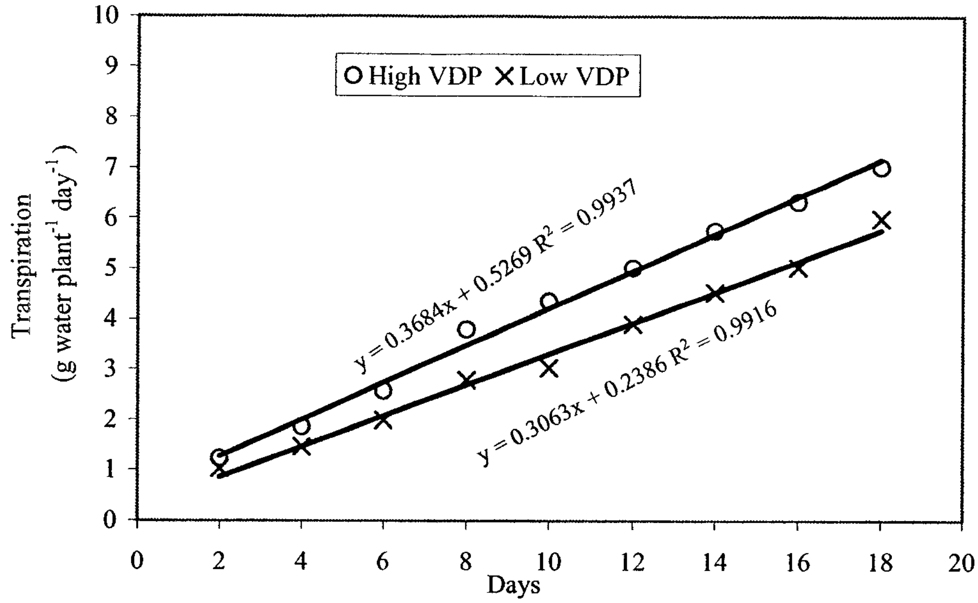

Fig. 3.1: Transpiration rate of wheat plants in growth chambers with imposed low (70%) and high (90%) relative humidity regimes (I.e. high and low vapour pressure deficits, respectively).

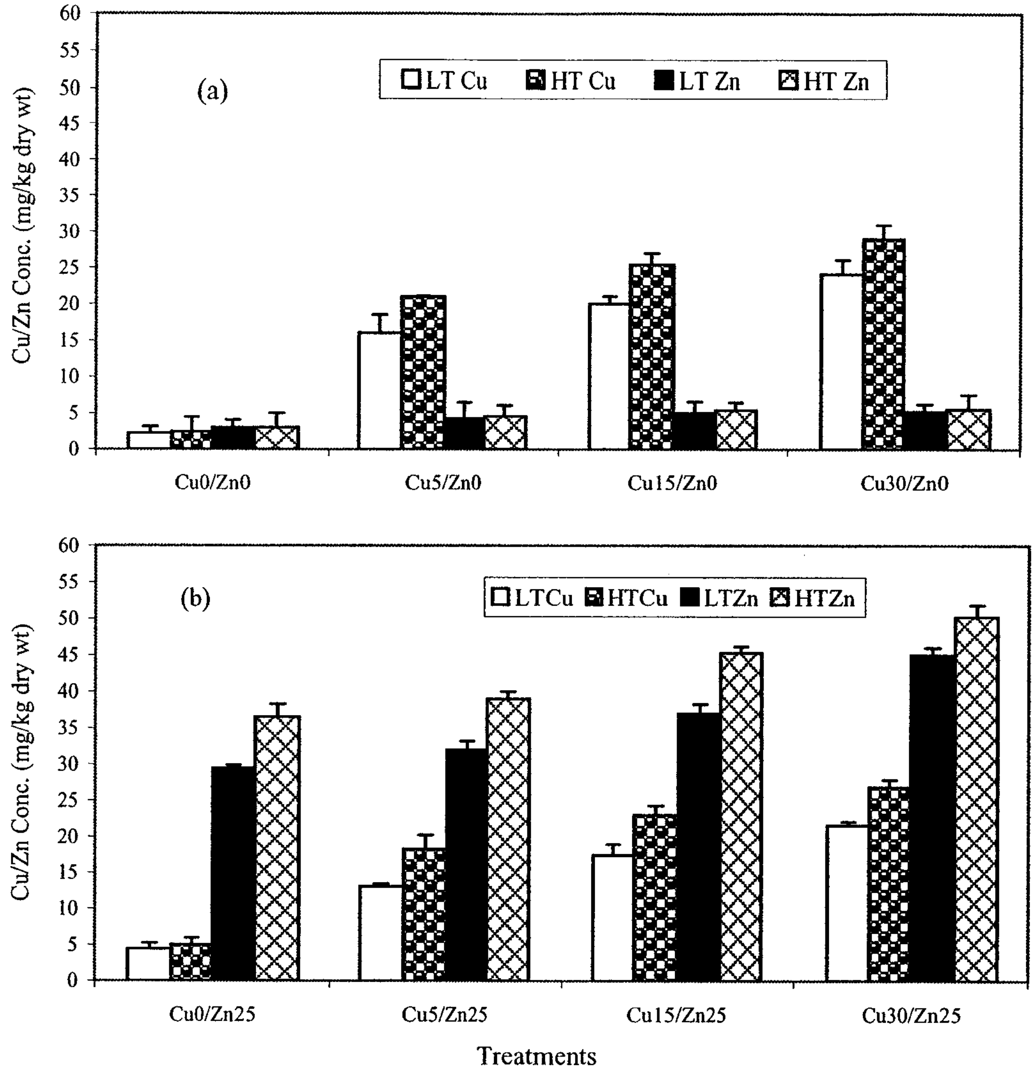

Fig. 3.2. Day 10 Concentration of Cu/Zn in wheat shoots,for both relative humidity regimes, in respose to irrigation with water bearing different concentrations of Cu, (a) no Zn, (b) 25 mg L^{-1} Zn in irrigation water. Bars are standard error (n=3)

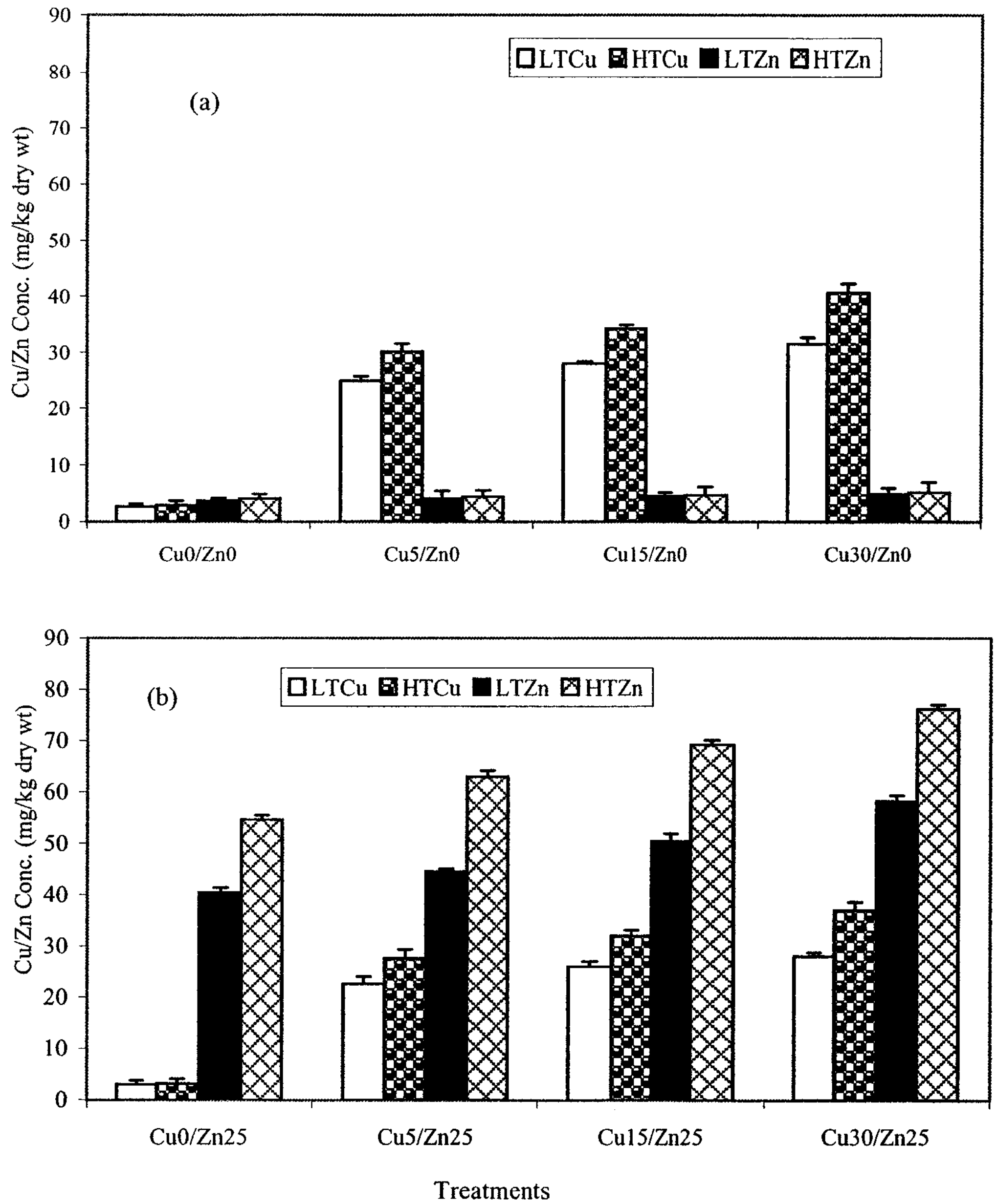

Fig. 3.3. Day 20 Concentration of Cu/Zn in wheat shoots, for both relative humidity regimes, in response to irrigation with water bearing different concentrations of Cu, (a) no Zn, (b) 25 mg L^{-1} Zn in irrigation water. Bars are standard error (n=3).

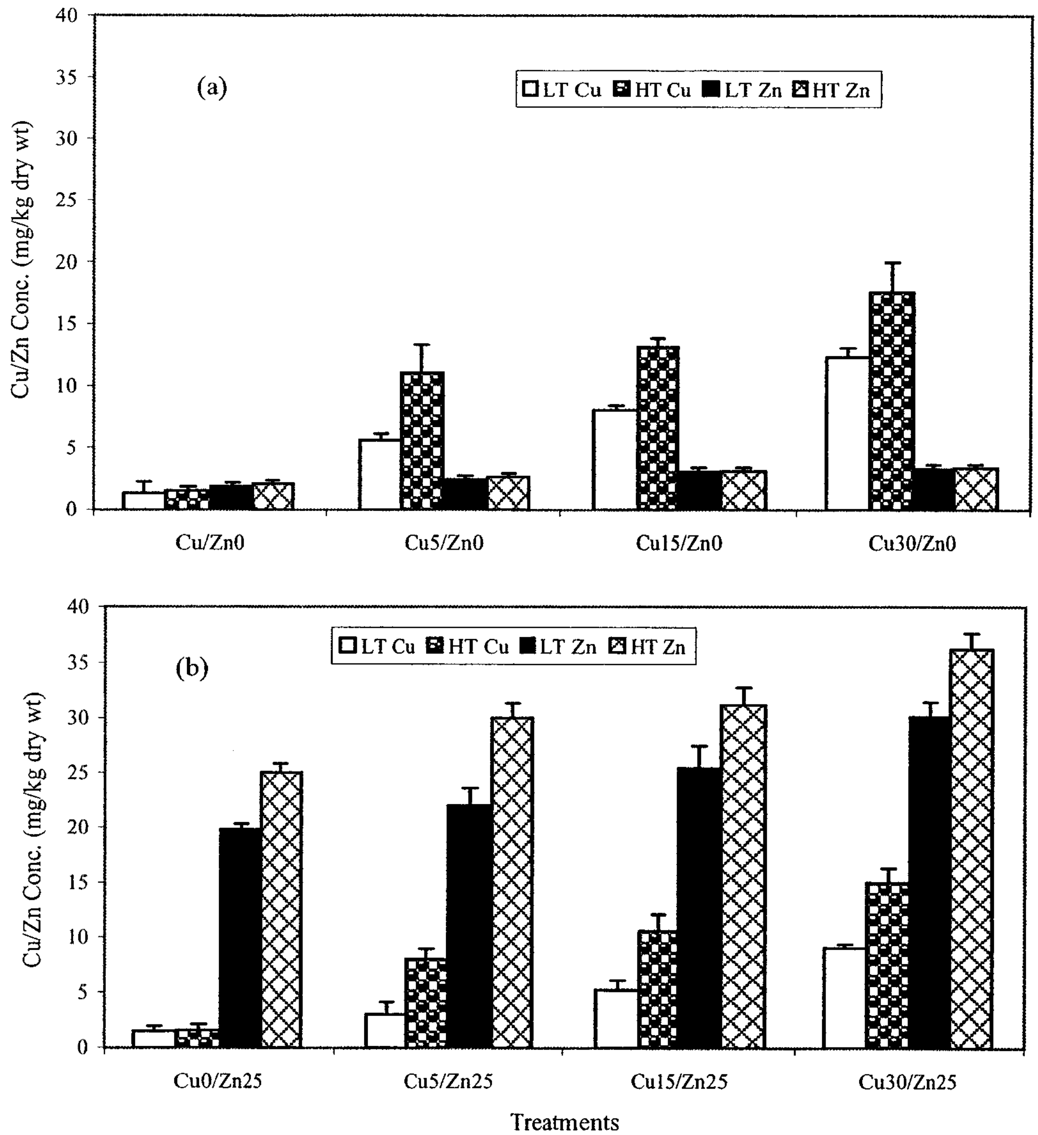

Fig. 3.4 : Day 10 Concentration of Cu/Zn in wheat grain, for both relative humidity regimes, in response to irrigation with water bearing different concentrations of Cu, (a) no Zn, (b) 25 mg L^{-1} Zn in irrigation water. Bars are standard error (n=3).

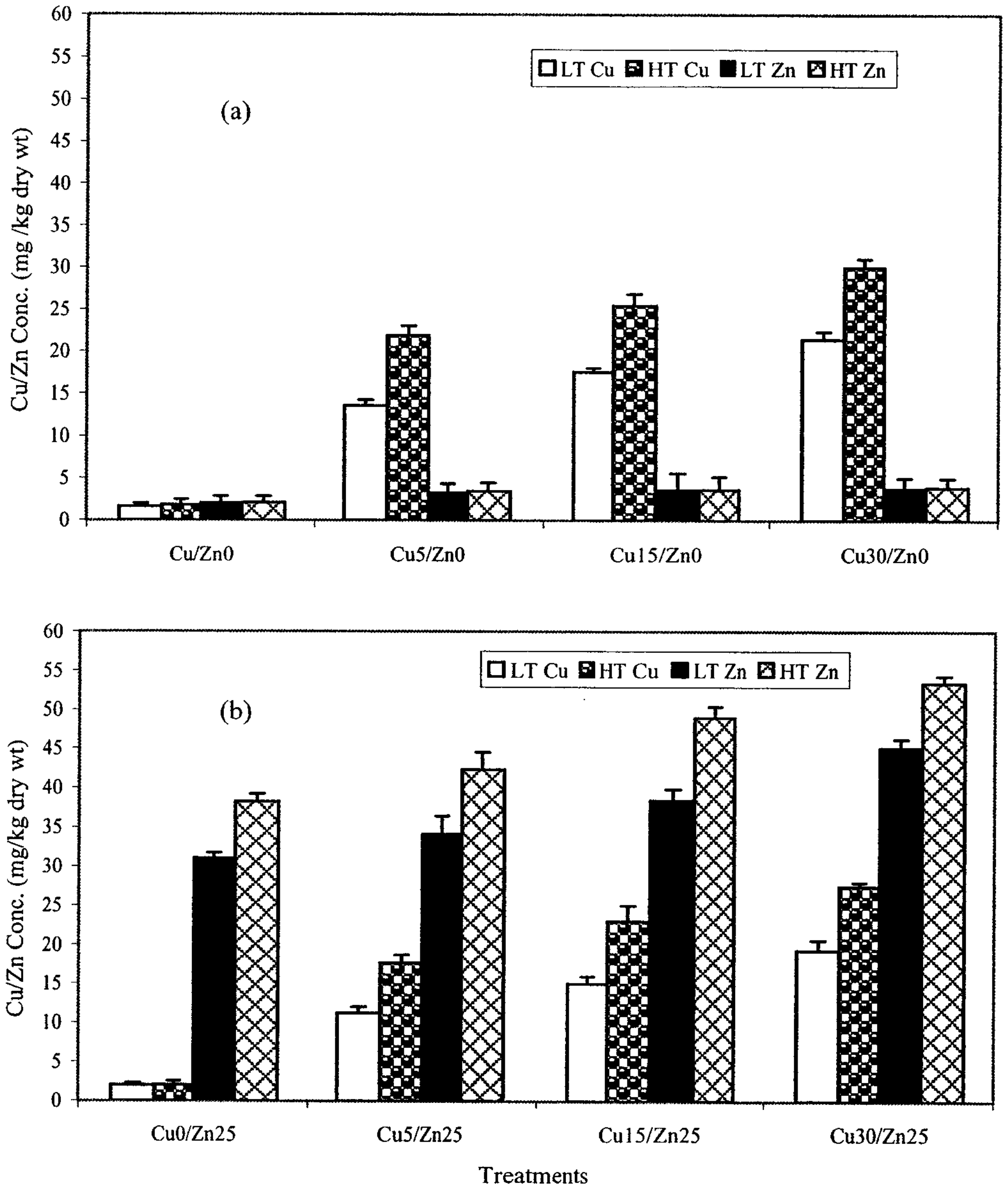

Fig. 3.5: Day 20 Concentration of Cu/Zn in wheat grain, for both relative humidity regimes, in response to irrigation with water bearing different concentrations of Cu, (a) no Zn, (b) 25 mg L^{-1} Zn in irrigation water. Bars are standard error (n=3).

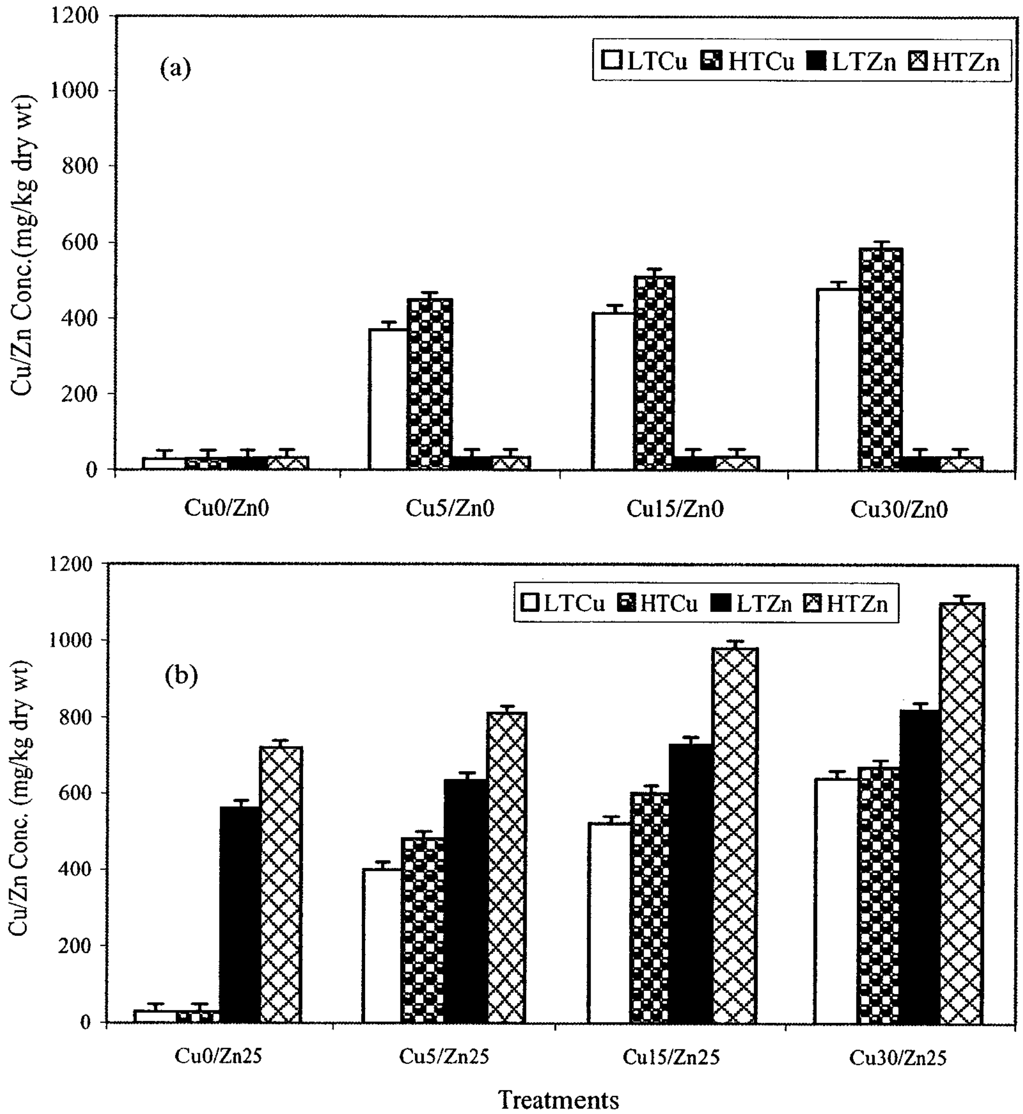

Fig.3.6: Day 20 Concentration of Cu/Zn in wheat roots, for both relative humidity regimes, in response to irrigtion with water bearing different concentrations of Cu, (a) no Zn , (b) 25 mg L^{-1} Zn in irrigation water. Bars are standard error (n=3).

PREFACE TO CHAPTER 4

In many regions of the world food security is contingent on the availability of irrigation water for agriculture, the primary freshwater-consuming production sector worldwide. Freshwater availability for irrigation may, in many regions, be further threatened by its rapidly increasing non-agricultural uses. As a result, the role of wastewater for irrigated agriculture has received substantial attention in recent years. Chapter 4 presents the results of a study investigating Cu and Zn uptake by a buckwheat crop irrigated with simulated wastewater bearing various levels, individually or in combination, of Cu and Zn. Although detailed studies on heavy metal uptake have been reported for wheat, no such data is available for buckwheat.

This chapter is drawn from a manuscript prepared for publication by the author of the thesis and co-authored by her supervisor, Dr. Suzelle Barrington. The co-author collaborated solely in reviewing the experimental procedure, assisting with the experimental design and analyzing and reviewing the publication. The format has been changed to be consistent within this thesis.

CHAPTER 4

TRANSPIRATION RATE EFFECT ON COPPER AND ZINC UPTAKE. PART II. BUCKWHEAT RESPONSE

ABSTRACT

To evaluate the environmental risks of irrigating crops with treated wastewater, a study was undertaken to quantify Cu and Zn uptake by a buckwheat *(Fagopyrum esculentum* L.*)* crop as a function of transpiration rate. Buckwheat plants were grown during four weeks in a greenhouse before being transferred into one of two growth chambers, each operating under either a high or low vapour deficit pressure (VDP) and resultant high or low transpiration rate, respectively. Pots, each bearing 15 plants, were exposed to a thrice replicated factorial combination of two Zn and three Cu irrigation solution concentrations. The resulting Cu and Zn levels, in mg L^{-1}, were : Cu_0/Zn_0, Cu_0/Zn_{25}, Cu_5/Zn_0, Cu_{15}/Zn_0, Cu_{30}/Zn_0, Cu_5/Zn_{25}, Cu_{15}/Zn_{25}, Cu_{30}/Zn_{25}. Evaporation and evapo-transpiration rates were monitored every other day by weighing plant-free and plant-bearing pots and calculating the water lost from them. Three randomly selected plants were harvested on each of days 6, 12, and 18 after the initiation of artificial wastewater irrigation. Shoots and leaves were analyzed for Cu/Zn content. On day 18, Cu and Zn contents were also determined for the roots. The treatments had no significant effect on transpiration rate (mm kg^{-1} dry weight), indicating that treatments produced no toxic effects. The addition of Zn in the absence of Cu increased dry biomass production, whereas the lowest biomass occurred at Cu concentrations of 15 and 30 mg L^{-1}. The higher VPD/transpiration rate enhanced plant Cu/Zn uptake. The roots contained the greatest concentrations of Cu and Zn, indicating their role in moderating heavy metal uptake. Higher rates of Cu led to acidification of the soil by the end of the study.

Keywords: Contamination, Copper, Heavy metal, Transpiration, Zinc

4.1 Introduction

Buckwheat (*Fagopyrum esculentum* L.) is gaining an increasing popularity as a cash crop because of its rapid growth and potential for use when other crops fail or when weather or soil conditions are unfavourable. Consequently, this crop can play a vital role in alleviating food security problems. In Missouri, Emily and Myers (1998) concluded that despite its lower yield, increased flexibility in planting date makes buckwheat an acceptable alternative double-cropping with soybeans. Furthermore, buckwheat possesses a high level of protein, short vegetative period and is not susceptible to most cereal diseases (Marshal and Pomeranz, 1984).

Historically, buckwheat was once common on farms in the north-central United States and Canada. Buckwheat has been grown in Canada for many years, with Manitoba recording the highest production in Canada (Pomeranz, 1984). The decline in importance of this crop results from the lack of research effort to improve its breeding and diversify its varieties. Also, unlike other crops, buckwheat yields show little response. Buckwheat grows well in a variety of soil types, and in moist and cool climates is more likely than other crops to produce a satisfactory grain yield on poor soil.

Given these factors, buckwheat remains an interesting crop for developing countries. Because of water shortage in such countries, irrigation using treated wastewater provide a rational management of the water resource. Nevertheless, no information is currently available regarding the performance of buckwheat and its heavy metal uptake when irrigated with wastewater containing high levels of heavy metals.

4.2 Objective

The objective of the project was to evaluate Cu and Zn uptake by 4-week old buckwheat plants, under two transpiration rates. Plants were exposed to Cu and Zn by irrigating them with a solution containing one of two levels of Zn (0 or 25 mg L^{-1}) combined with one of four levels of Cu. This combination was designed to measure the interactive and individual effects of Zn and Cu on plant uptake.

4.3 Materials and methods

4.3.1 Experimental material

The experiment was conducted using 4-week old buckwheat plants, grown in a glass greenhouse. Some 20 seeds of buckwheat were planted in each of 48 pots (I.D. 155 mm) and each containing 1.5 kg of dry sand wetted with 300 ml of distilled water. The sand was held in a plastic bag to prevent any leakage and to be able to conduct a water mass balance analysis later on in the growth chambers. A total of 48 pots were seeded to evaluate in triplicates, 8 treatments or combinations of Cu and Zn levels in the irrigation water, exposed to two transpiration rates. At emergence, all pots were thinned down to 15 plants, for a uniform plant population among treatments. The plants were grown in a standard glass greenhouse for 4 weeks after emergence. Until this time, no heavy metal solution was used in the irrigation water. The pots were irrigated with a fertilized solution once every two weeks, from plant emergence onwards, at a rate of 135 mg each of N, P and K per pot or roughly 76 kg of N, P and K ha^{-1}. The fertilizer also contained traces of heavy metals, but these were not significant as compared to the amounts of Cu and Zn given to the plants through the experimental irrigation water. At the end of the fourth week, the plants had reached a height of 0.65-0.68 m and were well developed (still vegetative stage). They were then transferred to the growth chambers.

The experimental soil was a sand obtained from the B-horizon of a soil profile of the Upland series which consists of sandy soil layer of over 1.2 m in depth, overlying a marine clay. The sand's particle size distribution ranged mainly between 0.25 and 0.5 mm, with 10% of its mass larger than 0.5 mm and 35% smaller than 0.25 mm. The experimental soil had a pH of 7.0 and contained a relatively high amount of P and K (173 and 222 mg kg^{-1} of dry soil). It's cation exchange capacity was low at 2.02 $cmol^{(+)}$ kg^{-1}. Some selected soil characteristics are shown in Table 4.1.

Two growth chambers were used to grow plants , one for each transpiration rate. The temperature of these growth chambers was controlled at 22 C while the relative humidity was controlled at either 70% or 90%, to induce high and low transpiration rates. A daytime light intensity of 250μ lux was used for 12 hours. A night time temperature of 16 C was used in both chambers, for the other 12 h period.

Copper and Zn solution were prepared from $ZnCl_2$ and $CuCl_2$ salts dissolved in distilled water. The natural pH of these solutions was relatively low (5.5), and thus, soil pH was measured at the end of the experiment (Table 4.2).

4.3.2 Experimental procedure

The 4 week old plants were exposed to one of the 8 heavy metal solutions under one of two transpiration rate induced by different vapour pressure deficits (VPD). Thus, half of the 48 seeded pots were randomly selected and placed in the growth chamber with a high VPD (70% relative humidity) to invoke a high transpiration rate (HT), while the other half was placed under low VPD (90% relative humidity) for a low transpiration rate (LT). The pots were arranged in a completely randomized fashion within each growth chamber. Once in the growth chamber, 8 sets of three pots were randomly selected and each three randomized pots were assigned an irrigation treatment consisting of one of two levels of Zn (0 or 25 mg L^{-1}) in factorial combination with one of four levels of Cu (0, 5, 25 and 30 mg L^{-1}), resulting in treatment combinations of: Cu_0/Zn_0, Cu_0/Zn_{25}, Cu_5/Zn_0, Cu_{15}/Zn_0, Cu_{30}/Zn_0, Cu_5/Zn_{25}, Cu_{15}/Zn_{25}, Cu_{30}/Zn_{25}. Three plant-free pots were also placed in each growth chamber to monitor the evaporation rate. The plant transpiration rate was calculated by measuring the water loss from each seeded pot (the evapo-transpiration rate) and subtracting the evaporation rate measured from the plant-free pots in every two days. All transpiration rates were calculated based on the number of plants in each pot.

Every two days, the water content of each pot was returned to field capacity (300 ml pot^{-1}) with the assigned Cu/Zn solution. Once every two weeks the irrigation solution was a fertilizer solution with a fraction of the amount of fertilizer specified previously. The Cu/Zn solution was applied for 18 days to simulate the application of treated wastewater to plants under active growth. The quantities of metal added in the irrigated waters were intended to be large enough to have an impact on the Cu and Zn content of the plants, without causing phytotoxicity. The evapo-transpiration rate never exceeded 60 ml per 2-days, thus representing less than 20% of the field capacity water content of the soil, and thus having a limited effect on the transpiration rate among treatments.

For each treatment three plants per pots were harvested at each of 6, 12, and 18 days of treatment. Samples were divided into stems and leaves, carefully rinsed with distilled water, and dried at 70°C for 48 h. All plant parts were then stored in individual plastic bags, until ground in a stainless steel mill for Cu and Zn analysis. On day 18, the end of the experimental period, roots were also removed from the soil, washed with distilled water and analyzed for dry matter, and Cu and Zn content. All Cu and Zn levels of dried plant material was determined by atomic absorption spectrometry, after their digestion with concentrated sulphuric acid and hydrogen peroxide at 500 C.

4.3.3 Statistical analysis

Analyses of variance (ANOVA) was performed on each day separately using the general linear model (GLM) of the SAS package (Statistical Analyses System) for windows, (SAS Institute, Cary, NC). Sheffe's multiple comparison was used to assess the significance of treatment differences. Unless otherwise stated, a 5% probability level (P 0.05) was used to declare difference significance among treatments.

4.4 Results and discussion

4.4.1 Transpiration rate and plant dry yield

Heavy metal treatments did not have a significant effect on transpiration rate for either VPD treatment. This is in accordance with the results of Grifferty and Barrington (2000), who did not observe any significant effect of a 50 mg Zn L^{-1} irrigation solution on the transpiration rate of wheat plants. Similarly, Sharif (2001) observed no toxic effect of heavy metals on wheat exposed to Cd/Zn combinations as high as 5/50 mg L^{-1}.

Plants grown under the higher VPD showed a higher transpiration rate (33.3 g water $plant^{-1}$ day^{-1}) than under the low VPD (19.5 g water $plant^{-1}$ day^{-1}). Given continued plant growth, transpiration rate under both VDP regimes increased linearly over time (Fig. 4.1)..

Dry matter yield of leaves and stems increased with time under both high and low transpiration rates (Tables 4.3, 4.4). The Cu/Zn treatments had a significant effect on stem and leaf dry mass. The Cu_0/Zn_{25} and Cu_5/Zn_{25} treatments consistently yielded greater plant masses, whereas the Cu_{15}/Zn_{25}, Cu_{30}/Zn_0 and Cu_{30}/Zn_{25} treatment combinations consistently yielded poorly compared to the control, Cu_0/Zn_0 (Tables 4.3 and 4.4). Thus, Zn at low Cu levels (less than 5mg L^{-1}) had a beneficial effect on plant growth while Cu levels above 15 mg L^{-1} produced poorer dry weight yields, regardless of the level of transpiration rate. Similar results were obtained by Luo and Rimmer (1994) who reported that Cu addition decreased barley (*Hordeum vulgare* L.) yield. The reduction in yield for pots receiving higher loads of Cu may be explained by the decrease in soil pH, as the lowest 18-day soil pH values were associated with the highest Cu application rates (Table 4.2). It has been widely reported (e.g., Merry et al.,1986; Mulch et al.,1987) that pH plays an important role in the uptake of Cu/Zn and other heavy metals. Gupta and Aten (1993) also observed that soil pH greatly influences Cu content in plant parts, with Cu contents increasing under more acidic soil pH conditions. Hinsely et al. (1984) reported a decrease in uptake of heavy metals by plants with soil pH values over 6.0.

4.4.2 Cu and Zn uptake into leaves

The content of both Cu and Zn in leaves, on a dry weight basis, increased with time, e.g. day 6, 12, 18 (Fig.4.2, 4.3 and 4.4). Thus, prolonged irrigation with heavy metals can lead to greater leaf heavy metal content. Furthermore, Cu/Zn uptake was more pronounced in plants grown under the high transpiration rate. With no additions of Zn in the irrigation solution, Cu uptake increased with Cu application rate, for both transpiration rates. As compared to the absence of Zn, a Zn level of 25 mg L^{-1} had little effect on Cu uptake by leaves. In contrast, Cu at concentrations over 5 mg L^{-1} decreased the uptake of Zn by the leaves, indicating that Cu has an antagonistic effect on Zn uptake to the leaves. Nevertheless, leaves had higher concentrations of Zn compared to Cu for both transpiration rates, indicating that Zn is more mobile than Cu.

Because of the absence of research reports on buckwheat, other crops will be used to compare the results obtained. Adriano (1986) reported similar results where Cu interacted antagonistically with Zn on plant growth. Nevertheless, Reboredo (1994) concluded that the uptake and accumulation of Cu by *Halimione* was independent of soil Zn level. In contrast, Nan and Cheng (2001) found that Cu/Zn acted synergistically on root absorption of metals under field conditions. Thus, plant uptake and accumulation of heavy metals can vary depending on which parts (leaf, stem, seed and root) of the crop is examined, the level and type of heavy metal applied, and the physico-chemical properties of the soil and crop species (Adriano,1986).

4.4.3 Cu and Zn uptake to stems and roots

Copper and Zn levels in plant stems at 6, 12 and 18 days shown in Figures 4.5, 4.6 and 4.7, respectively. Over the whole experimental period (18 days) the Cu and Zn content of plant stems varied significantly with heavy metal treatment for both low and high transpiration rates. The uptake of Cu and Zn by buckwheat stems followed a pattern similar to that of leaves, but each day the stem accumulated, on a dry weight basis, more Cu than did the leaves. As for the leaves, a Cu level in the irrigation water as low as 5 mg Cu L^{-1} decreased Zn uptake to the stem.

The greatest levels of Cu and Zn were found in the roots (Fig. 4.8). Plants under the high transpiration rate showed higher Cu and Zn contents. The Zn content of buckwheat roots was greater than that of Cu. Large amounts of Cu and Zn accumulated in the roots. When compared to levels found in the stems and leaves, these higher root levels indicate that translocation of Cu and Zn towards the plant shoots is restricted. This phenomenon has been observed in many plant species. Adriano (1986), for example, noted that the translocation rate from plant roots is generally slow for essential elements.

The percentages of Cu and Zn absorbed by the plant with respect to that applied in irrigation water and the total mass of Cu and Zn absorbed by the plant, are presented in Table 4.5. Under both transpiration rates, and irrespective of Zn level, plants absorbed the greatest percentage of Cu under the 5 mg Cu L^{-1} treatment and the least under the 30 Cu mg L^{-1} treatment. Thus, the lowest concentration of Cu in irrigation water contributed to the highest percentage uptake under both transpiration rates. The percentage of Zn absorbed at 25 mg L^{-1} ranged form 1.5 to 7%. The higher the plant growth rate, the lower the percentage of heavy metal absorbed (Table 4.5).

4.5 Conclusions

After 3 weeks of irrigation with irrigation solutions bearing Cu and Zn, singly or in combination, both Cu and Zn concentrations in leaves and stems did not exceed the standard safety limit (Table 2.3) for crops and livestock. We therefore conclude that treated municipal wastewater should not impose a threat in terms of Cu/Zn levels in plant shoots. Copper concentrations in the root system greatly exceeded the safety limit for both crops and animals.

We propose that the mechanisms of interaction among metals in buckwheat be researched further in order to set up some proper guidelines for wastewater usage on the buckwheat crop.

4.6 Acknowledgements

This research was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC).

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Table 4.1: Selected chemical and physical properties of the soil.

Property	Value
Particle size distribution [% (w/w)]	
>1.0 mm	0
1.0-0.50 mm	5
0.50-0.25 mm	55
0.25-0.10 mm	38
< 0.10 mm	2
Chemical properties	
Available nutrients (mg kg^{-1})	
P	173
K	190
Mg	4
Ca	90
Al	120
Zn	0.73
Cu	0.92
B	3
Mn	1.4
Fe	104
Other characteristics	
Total Cd [mg kg^{-1}]	0.5
pH	7.00
Organic matter [% (w/w)]	0.4
CEC [cmol kg^{-1}]	2.02

Table 4.2: pH of the soil after harvest in buckwheat plant.

Treatments (mg/Kg)	Low transpiration rate	High transpiration rate
Cu_0/Zn_0	6.79	6.86
Cu_0/Zn_{25}	6.60	6.63
Cu_5Zn_{25}	6.50	6.55
$Cu_{15}Zn_{25}$	5.56	5.82
$Cu_{30}Zn_{25}$	5.45	5.72
Cu_5Zn_0	6.36	6.40
$Cu_{15}Zn_0$	6.80	6.23
Cu_{30}/Zn_0	6.05	6.00

Table 4.3: Leaf dry weight for buckwheat (g $plant^{-1}$).

Treatments (mg/L)	Low transpiration rate			High transpiration rate		
	Day 6	Day 12	Day 18	Day 6	Day 12	Day 18
Cu_0/Zn_0	0.520	0.705	0.812	0.528	0.709	0.828
Cu_0/Zn_{25}	0.581H	0.762H	0.864H	0.590H	0.770H	0.875H
Cu_5/Zn_{25}	0.552H	0.719H	0.820H	0.560H	0.725H	0.829H
Cu_{15}/Zn_{25}	0.501L	0.676L	0.765L	0.509L	0.681L	0.770L
Cu_{30}/Zn_{25}	0.460L	0.608L	0.713L	0.468L	0.610L	0.736L
Cu_5/Zn_0	0.521	0.708	0.812	0.531	0.712	0.816
Cu_{15}/Zn_0	0.532H	0.717H	0.828H	0.538H	0.720H	0.839H
Cu_{30}/Zn_0	0.488	0.628	0.736	0.492	0.631	0.741

Note: L = significantly lower than control
H = significantly greater than control

Table 4.4: Stem dry weight for buckwheat (g $plant^{-1}$).

Treatments (mg/L)	Low transpiration rate			High transpiration rate		
	Day 6	Day 12	Day 18	Day 6	Day 12	Day 18
Cu_0/Zn_0	0.611	0.785	0.822	0.625	0.799	0.829
Cu_0/Zn_{25}	0.680H	0.824H	0.885H	0.687H	0.826H	0.899H
Cu_5/Zn_{25}	0.652H	0.798H	0.859H	0.659H	0.810H	0.879H
Cu_{15}/Zn_{25}	0.591L	0.729L	0.795L	0.599L	0.735L	0.815L
Cu_{30}/Zn_{25}	0.526L	0.669L	0.756L	0.529L	0.708L	0.795L
Cu_5/Zn_0	0.615	0.789	0.829	0.62	0.802	0.84
Cu_{15}/Zn_0	0.619H	0.790H	0.832H	0.624H	0.808H	0.844H
Cu_{30}/Zn_0	0.559L	0.694L	0.776L	0.568L	0.712L	0.808L

Note: L = significantly lower than control
H = significantly greater than control

Table 4.5: Cu/Zn absorbed in the plant based on the total of heavy metal irrigation (Cu/Zn % absorbed).

Treatments (mg/L)	Low transpiration rate						High transpiration rate					
	Day 6		Day 12		Day 18		Day 6		Day 12		Day 18	
	Cu	Zn	Cu	Zn	Cu	Zn	Cu	Zn	Cu	Zn	Cu	Zn
Cu_0/Zn_0	-	-	-	-	-	-	-	-	-	-	-	-
Cu_0/Zn_{25}	-	7.0 (4.5)	-	5.1 (9.0)	-	4.8 (12.5)	-	5.79 (6.0)	-	5.5 (10.2)	-	4.7 (15.0)
Cu_5/Zn_{25}	12.8 (0.9)	6.5 (4.4)	9.8 (1.8)	4.50 (8.9)	9.97 (2.5)	4.4 (12.3)	11.5 (1.2)	5.16 (5.9)	10.6 (2.0)	5.0 (10.1)	10.8 (3.0)	4.30 (14.9)
Cu_{15}/Zn_{25}	4.8 (2.7)	4.3 (4.5)	3.7 (5.3)	3.30 (9.0)	3.97 (74)	3.4 (12.5)	4.0 (3.6)	3.74 (6.0)	4.0 (6.1)	4.6 (10.2)	4.1 (8.9)	3.2 (15.0)
Cu_{30}/Zn_{25}	2.6 (5.3)	1.5 (4.3)	2.2 (10.6)	2.48 (9.0)	2.20 (4.8)	2.5 (12.5)	2.2 (7.1)	2.74 (6)	2.4 (12.1)	2.8 (10.3)	2.2 (18.0)	2.7 (14.8)
Cu_5/Zn_0	13.6 (0.8)	-	10.5 (1.7)	-	10.24 (2.5)	-	11.2 (1.1)	-	11.0 (2.0)	-	11.0 (2.9)	6.91
Cu_{15}/Zn_0	5.40 (2.7)	-	4.2 (5.4)	-	4.43 (7.5)	-	4.5 (3.6)	-	4.5 (6.2)	-	4.4 (9.0)	6.02
Cu_{30}/Zn_0	2.9 (5.2)	-	2.3 (10.7)	-	2.34 (15)	-	2.4 (7.2)	-	2.5 (12.2)	-	2.3 (18)	5.65

Values in parenthesis are the total mass of Cu or Zn absorbed by the plants.

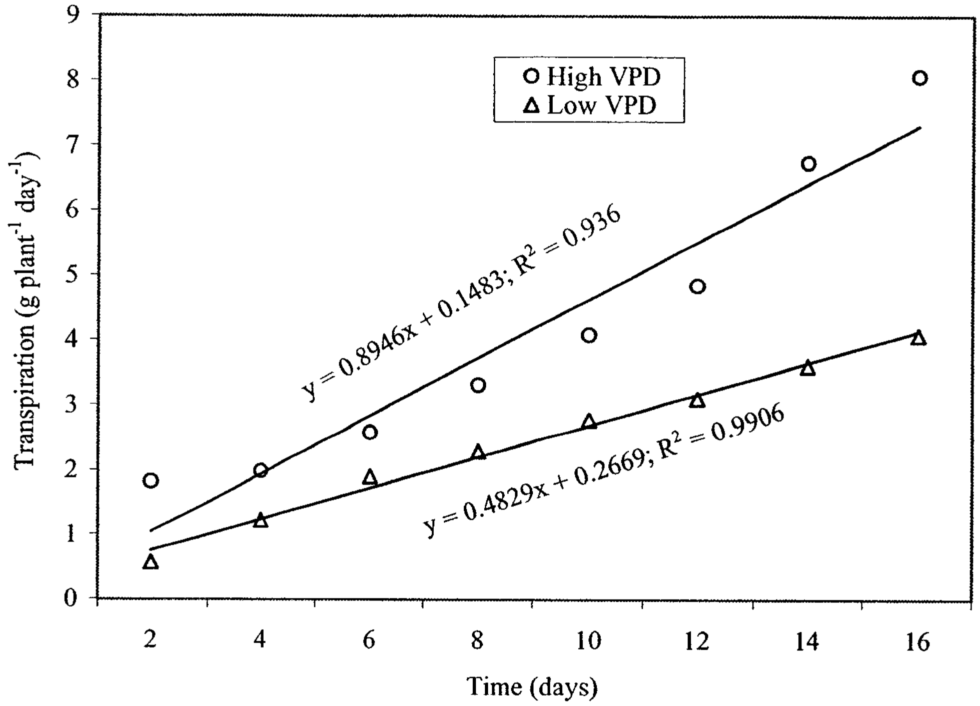


Fig. 4.1: Transpiration rate of buckwheat plants in growth chambers with imposed low (70%) and high (90%) relative humidity regimes (I.e. high and low vapour pressure deficits, respectively).

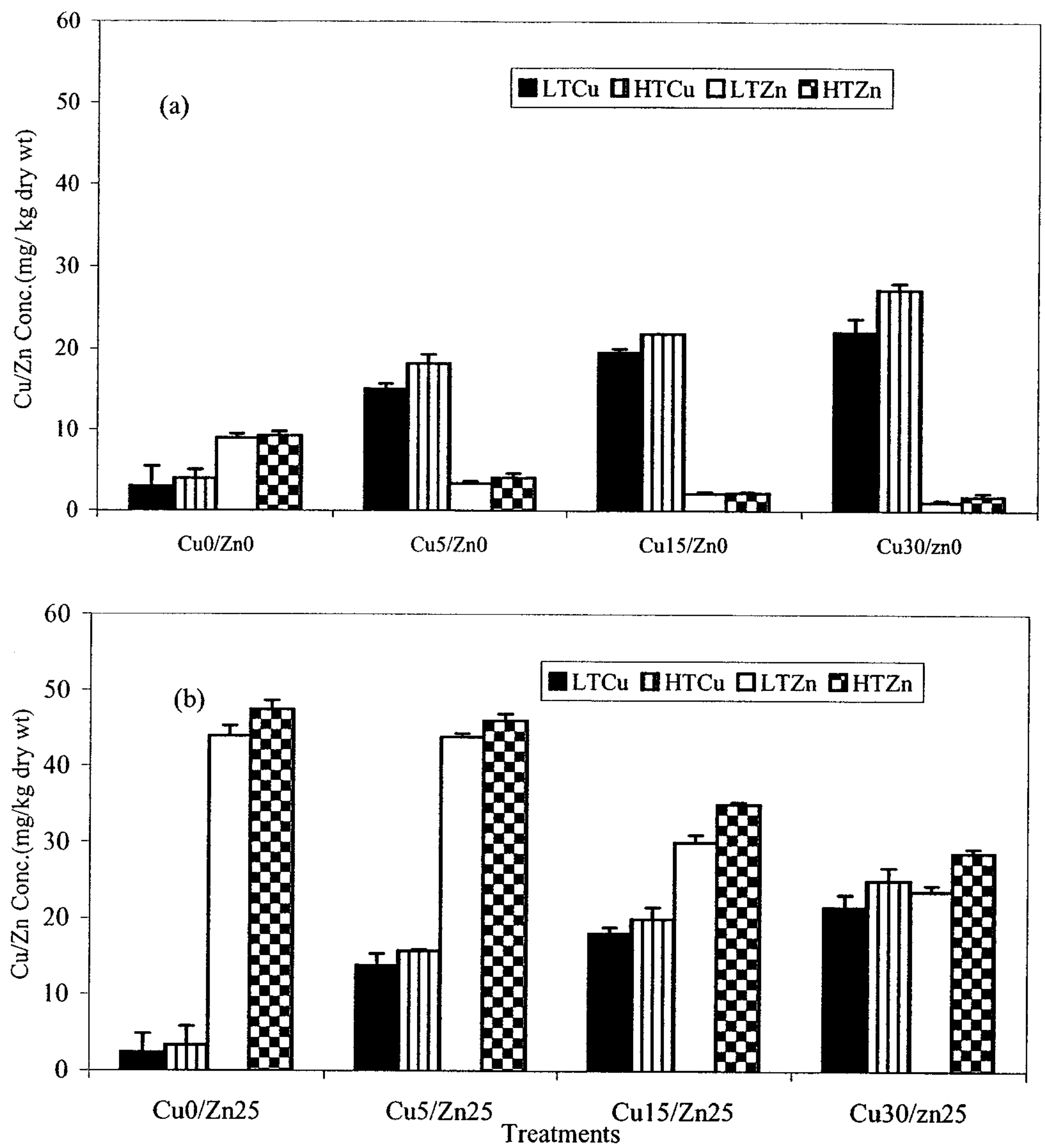
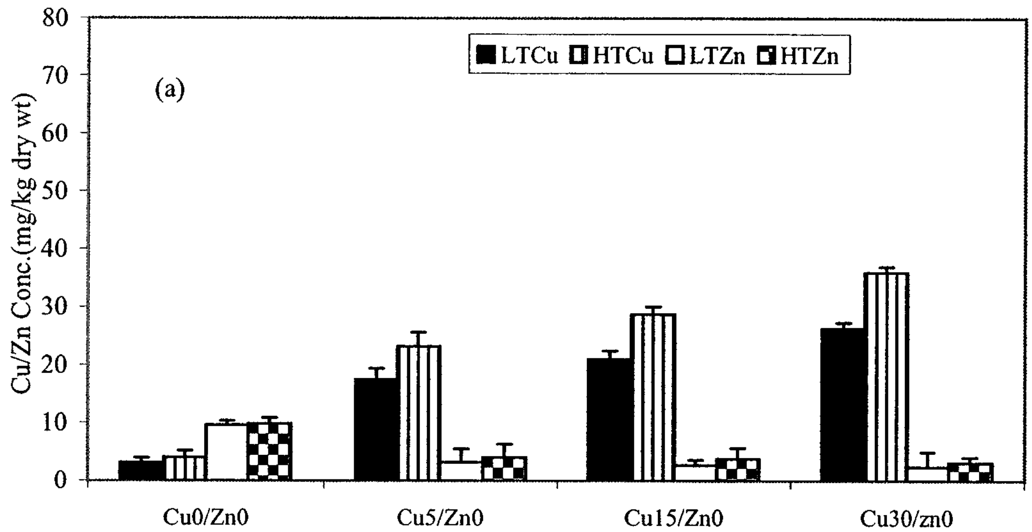


Fig. 4.2: Day 6 concentration of Cu/Zn in buckwheat leaves, for both growth chambers, in response to irrigation with water bearing different concentrations of Cu, (a) no Zn, (b) 25 mg L^{-1} Zn in irrigation water. Bars are standard error (n=3)



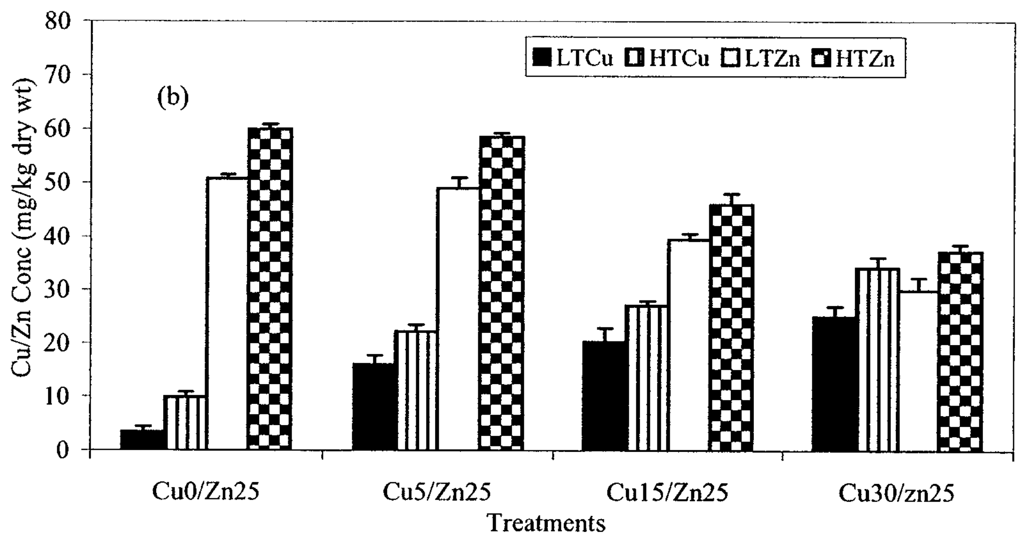


Fig 4.3: Day 12 concentration of Cu/Zn in buckwheat leaves, for both growth chambers, in response to irrigation with water bearing different concentrations of Cu, (a) no Zn , (b) 25mg L^{-1} Zn in irrigation water.Bars are standard error (n=3) .

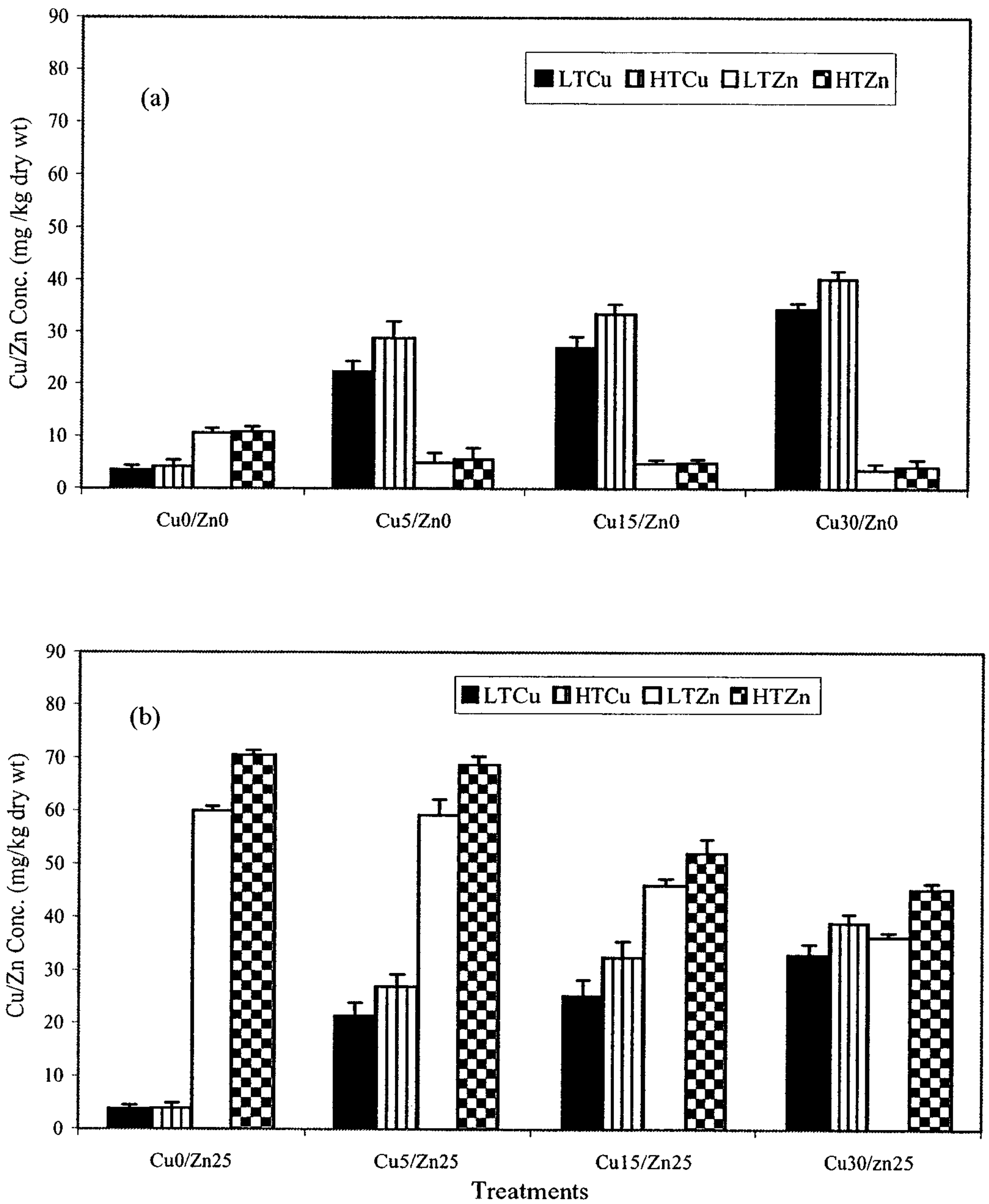


Fig 4.4: Day 18 concentration of Cu/Zn in buckeheat leaves, for both growth chambers , in response with water bearing different concentrations of Cu, (a) no Zn, (b) 25 mg L^{-1} Zn in irrigation water. Bars are standard error (n=3)

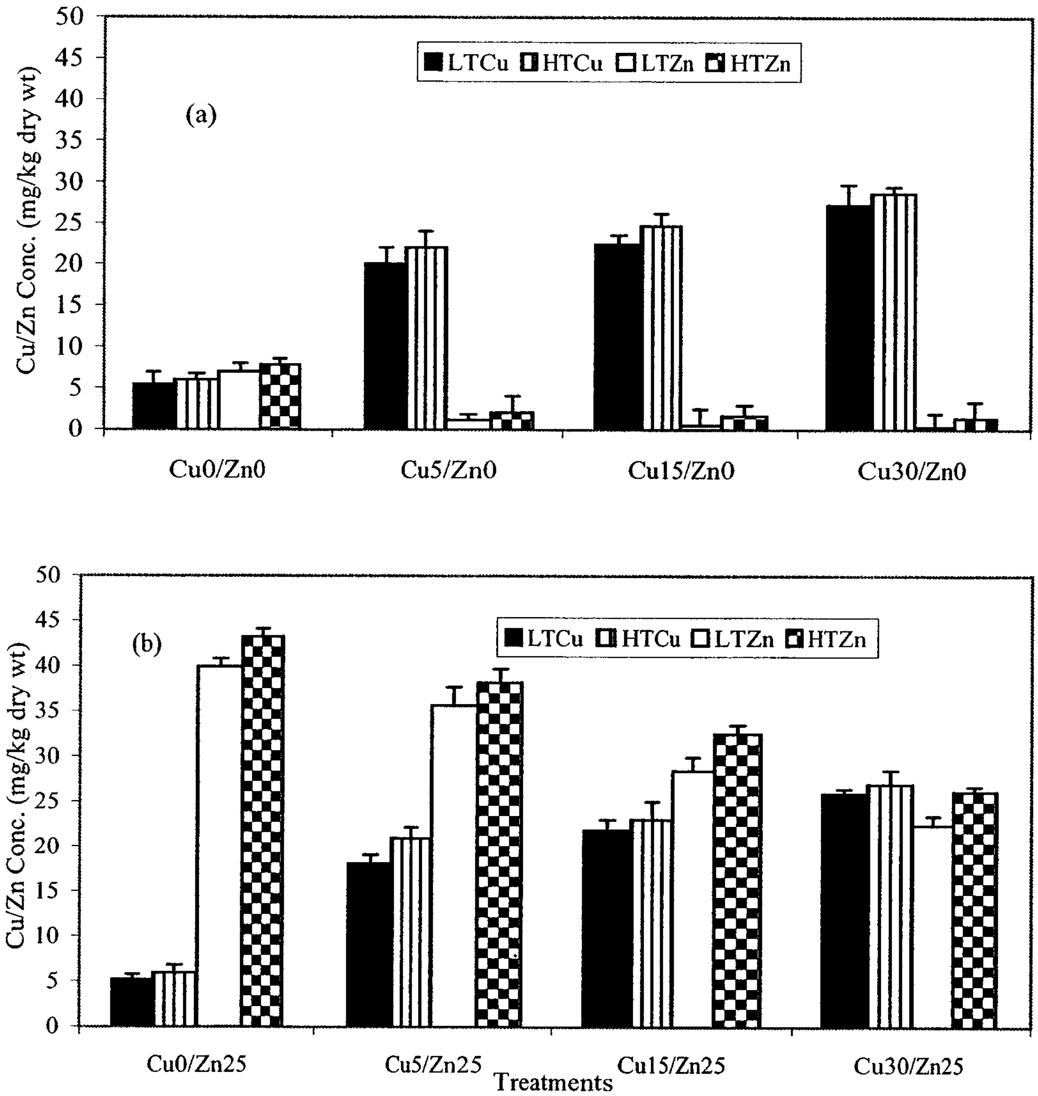


Fig. 4.5: Day 6 concentration of Cu/Zn in buckwheat stems, for both growth chambers, in response to irrigation with water bearing different concentrations of Cu, (a) no Zn, (b) 25 mg L^{-1} Zn in irrigation water. Bars are standard error (n=3)

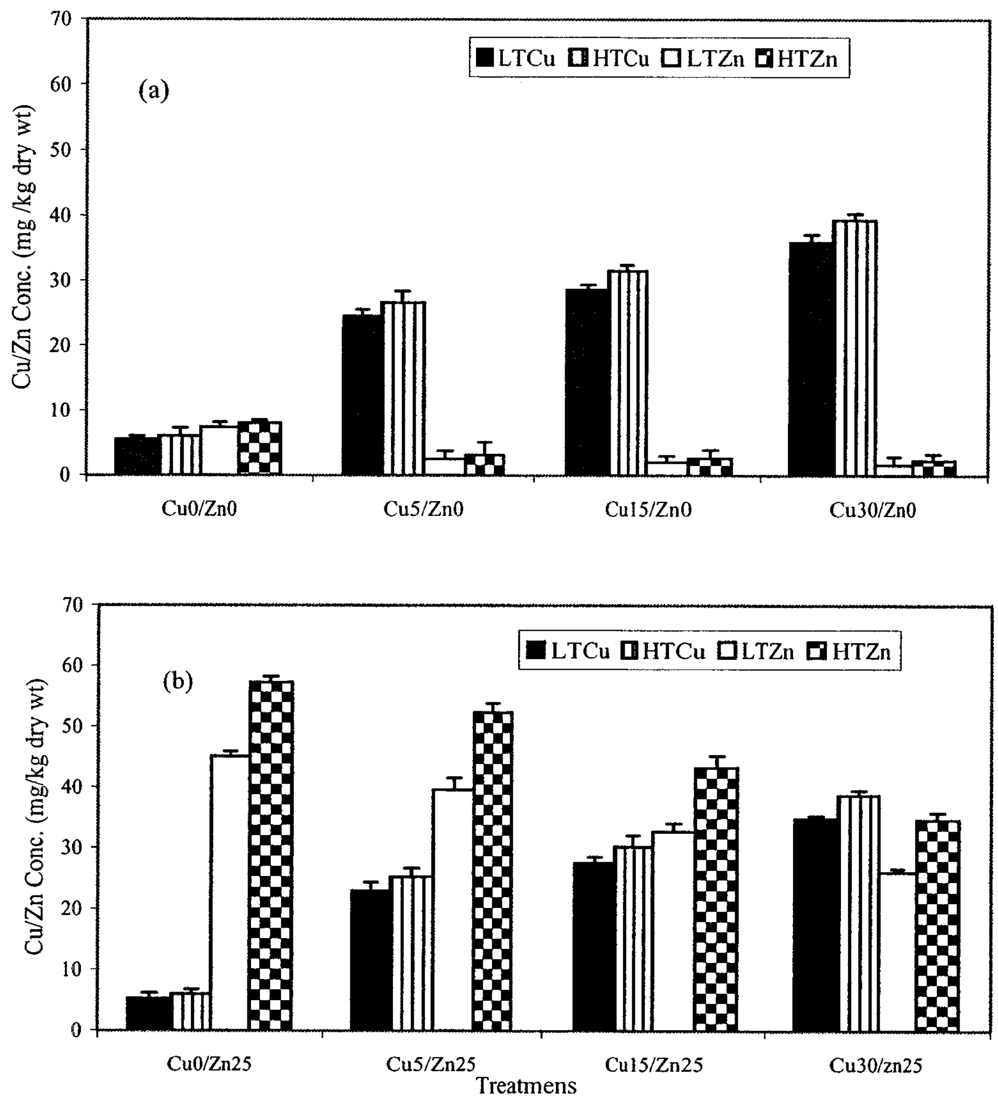


Fig. 4.6: Day 12 concentration of Cu/Zn in buckwheat stems, for both growth chambers, in response to irrigation with water bearing different concentrations of Cu, (a) no Zn, (b) 25 mg L^{-1} Zn in irrigation water. Bars are standard error (n=3).

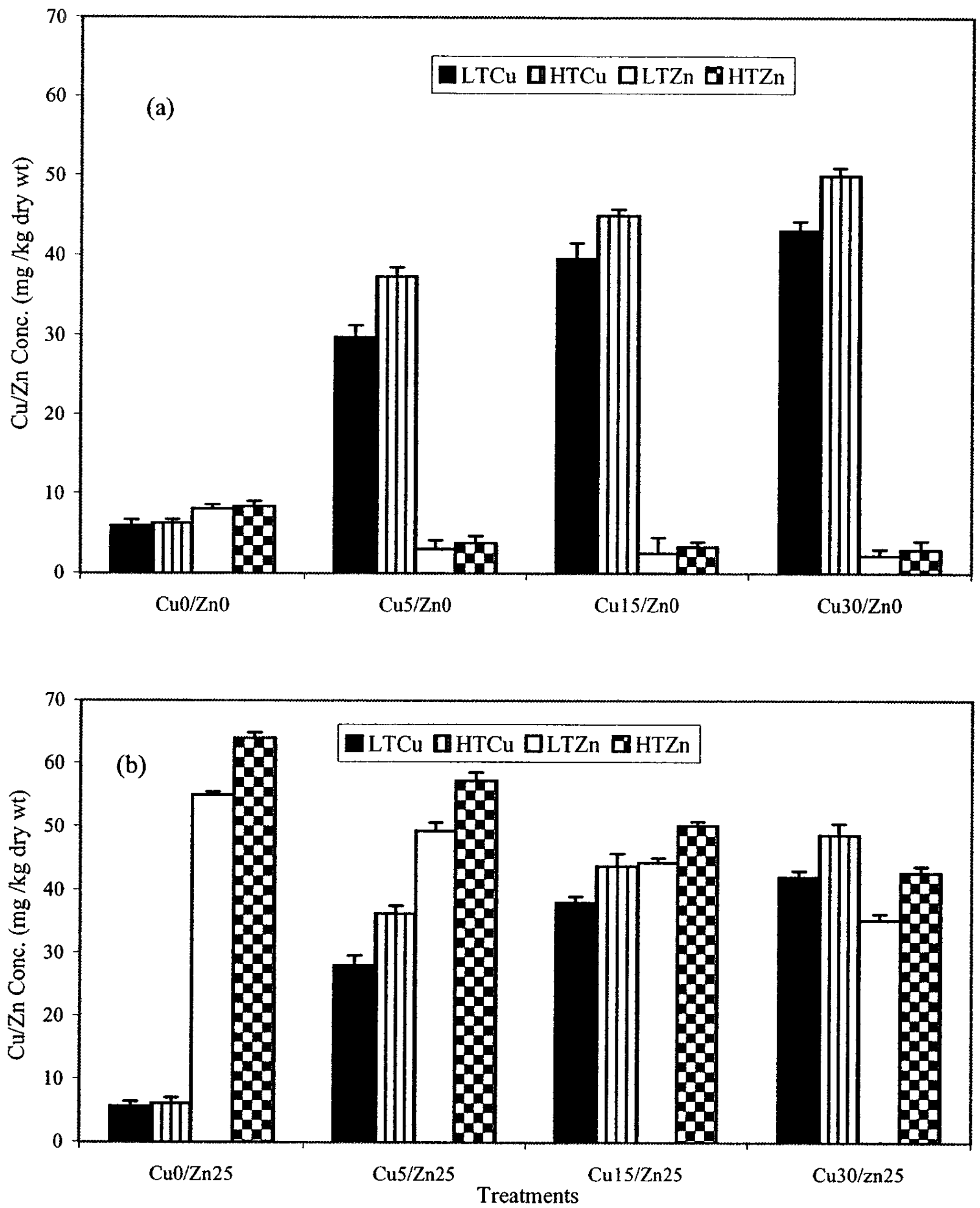


Fig. 4.7: Day 18 concentration of Cu/Zn in buckwheat stems, for both growth chambers, in response to irrigation with water bearing different concentrations of Cu, (a) no Zn, (b) 25 mg L^{-1} Zn in irrigation water. Bars are standard error (n=3).

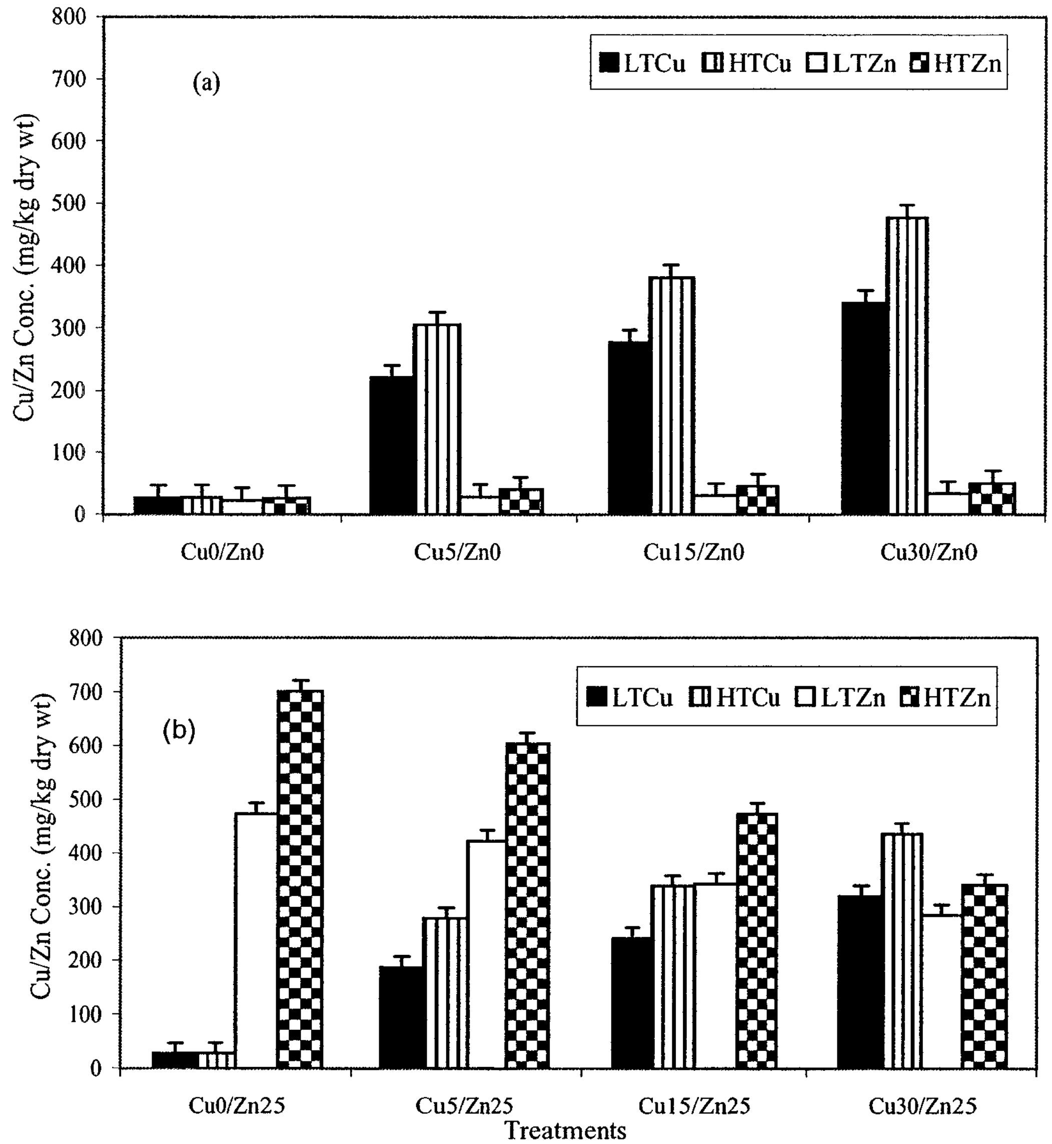


Fig. 4.8: Day 18 concentration of Cu/Zn in buckwheat roots, for both growth chambers, in response to irrigation with water bearing different concentrations Cu, (a) no Zn, (b) 25 mg L^{-1} Zn in irrigation water. Bars are standard error (n=3).

CHAPTER 5.0

General conclusions

Copper and zinc are essential micronutrients. They are easily taken up by plants and translocated to different plant parts. High accumulation of Cu and Zn in plants pose a potential hazard to human and animal health through the food chain. In agricultural soils, heavy metals may accumulate in crops, leading to the contamination of food chain. There are growing concerns that consumption of foods containing high levels of Cu and Zn may lead to chronic toxicity.

Concentrations of Cu and Zn in the roots were always greater than in the tops in both crops. High retention of Cu and Zn (and perhaps other heavy metals) in roots is particularly desirable in cereal crops because these parts are not generally utilized as food. Copper and Zn concentrations in both crops did not exceed standard safety limits for crops and livestock in the tops, but significantly exceeded them in the root system. More research is needed to have a better understanding about the mechanisms controlling the translocation of phytotoxic metals into different plant parts.

The present experiment provided information which is required before the agronomic use of water containing heavy metals can be recommended, in order to avoid the harmful effects on plant growth and contamination of food-chain resulting from Cu and Zn interaction. Buckwheat took up more Cu than Zn, whereas wheat took up more Zn than Cu. Interaction between Cu and Zn in the wheat crop was mainly synergetic where Cu additions enhanced Zn uptake. In contrast, the effect of Cu additions on Zn uptake by the buckwheat crop was antagonistic .

The most noticeable difference in the chemistry of the soil after wheat and buckwheat growth was the changes in soil pH. Buckwheat acidified the soil when exposed to the higher Cu treatments, whereas pH remained close to neutral after wheat was grown. This decrease in rhizosphere pH after buckwheat might have caused a substantial increase in Cu mobility.

APPENDIX A

Table A.1 : Copper/Zinc concentrations in wheat shoots (mg kg^{-1} dry wt)

Treatments ($mg\ L^{-1}$)	Low transpiration rate				High transpiration rate			
	Day 10		Day 20		Day 10		Day 20	
	Cu	Zn	Cu	Zn	Cu	Zn	Cu	Zn
Cu_0/Zn_0	2.20f*	3.00e	2.60e	3.61e	2.40f	3.00e	2.80e	4.00e
Cu_0/Zn_{25}	4.40e	29.5d	3.00e	40.5d	5.00e	36.5d	3.20e	54.7d
Cu_5/Zn_{25}	13.0d	32.0c	22.5d	44.6c	18.2d	39.0c	27.5d	63.0c
Cu_{15}/Zn_{25}	17.4c	37.0b	26.0bc	50.4b	23.0c	45.3b	32.0bc	69.2b
Cu_{30}/Zn_{25}	21.6b	45.0a	28.0b	58.2a	26.8b	50.2a	37.0b	76.3a
Cu_5/Zn_0	16.0c	4.20e	24.8bc	4.01e	21.0c	4.51e	30.1bc	4.30e
Cu_{15}/Zn_0	20.0b	5.01e	28.0b	4.40e	25.4b	5.42e	34.2b	4.61e
Cu_{30}/Zn_0	24.1a	5.20e	31.5a	4.82e	29.0a	5.50e	40.7a	5.20e

*Values within the same column followed by different letters are significantly different (P 0.05).

Table A.2: Copper/Zinc concentrations in wheat grain (mg kg^{-1} dry wt)

Treatments ($mg\ L^{-1}$)	Low transpiration rate				High transpiration rate			
	Day 10		Day 20		Day 10		Day 20	
	Cu	Zn	Cu	Zn	Cu	Zn	Cu	Zn
Cu_0/Zn_0	1.30f*	1.80e	1.60e	2.02e	1.50e	2.00e	1.80e	2.06e
Cu_0/Zn_{25}	1.51f	19.0d	2.02e	31.0d	1.61e	25.0d	2.10e	38.2d
Cu_5/Zn_{25}	3.00e	22.0c	11.2d	34.2c	8.00d	30.0c	17.6d	42.3c
Cu_{15}/Zn_{25}	5.20d	25.1b	15.0c	38.4b	10.5c	31.1b	23.0c	49.0b
Cu_{30}/Zn_{25}	9.00b	31.0a	19.3b	45.1a	15.0b	36.2a	27.5b	53.3a
Cu_5/Zn_0	5.62d	2.40e	13.5c	3.20e	11.0c	2.60e	21.8c	3.41e
Cu_{15}/Zn_0	8.01c	3.10e	17.5b	3.61e	13.1b	3.10e	25.4b	3.60e
Cu_{30}/Zn_0	12.3a	3.20e	21.4a	3.80e	17.6a	3.30e	30.0a	3.80e

*Values within the same column followed by different letters are significantly different (P 0.05).

Table A 3: Cu/Zn concentrations (mg kg^{-1} dry wt) in wheat roots at day 20.

Treatments	Low transpiration rate		High transpiration	
(mg L^{-1})	Cu	Zn	Cu	Zn
Cu_0/Zn_0	30.00g	32.02e	30.99g	32.97e
Cu_0/Zn_{25}	28.80g	560.00d	28.90g	720.44d
Cu_5/Zn_{25}	400.00e	634.09c	480.32e	810.23c
Cu_{15}/Zn_{25}	520.00b	730.00b	600.52b	980.12b
Cu_{30}/Zn_{25}	640.14a	820.10a	670.00a	1100.00a
Cu_5/Zn_0	370.10f	33.78e	466.21f	34.71e
Cu_{15}/Zn_0	415.35d	35.54e	512.31d	36.33e
Cu_{30}/Zn_0	480.00c	36.6e	586.00c	37.58e

* Values within the same column followed by different letters are significantly different (P 0.05).

APPENDIX B

Table B.1: Cu/Zn concentration in buckwheat leaves (mg kg^{-1} dry weight)

Treatments	Low transpiration rate						High transpiration rate					
(mg/L)	Day 6		Day 12		Day 18		Day 6		Day 12		Day 18	
	Cu	Zn	Cu	Zn	Cu	Zn	Cu	Zn	Cu	Zn	Cu	Zn
Cu_0/Zn_0	3.00d*	9.00d	3.20d	9.60e	3.52d	10.6e	4.00d	9.30e	4.08d	9.80e	4.20d	10.8e
Cu_0/Zn_{25}	2.40d	44.01a	2.80d	50.8a	3.00d	60.00a	3.33d	47.50a	3.50d	60.00a	3.85d	70.00a
Cu_5/Zn_{25}	13.80c	43.85a	16.05c	48.98a	21.42c	59.23a	15.74d	46.00a	22.24c	58.50a	27.00c	68.85a
Cu_{15}/Zn_{25}	18.00b	30.01c	20.03b	39.5c	25.33b	46.12c	20.00c	35.02c	27.12b	46.02c	32.52b	52.11c
Cu_{30}/Zn_{25}	21.45a	23.52d	25.00a	30.00d	33.00a	36.34d	25.00a	28.58d	34.14a	37.22d	39.00a	45.35d
Cu_5/Zn_0	15.00c	3.37f	17.40c	3.42f	22.33c	4.96f	16.98c	4.08f	23.21c	4.10f	28.85c	5.58f
Cu_{15}/Zn_0	19.56b	2.15gf	21.00b	2.65f	26.85b	4.82f	21.83b	2.22fg	28.71b	3.84f	33.45b	4.96f
Cu_{30}/Zn_0	22.10a	1.08g	26.35a	2.42f	34.44a	3.54f	26.22a	1.75g	36.00a	3.11f	40.22a	4.09f

* Values within the same column followed by different letters are significantly different at P 0.05.

Table B.2 : Cu/Zn concentration (mg kg^{-1} dry weight) in stems buckwheat crop.

Treatments (mg/L)	Low transpiration rate						High transpiration rate					
	Day 6		Day 12		Day 18		Day 6		Day 12		Day 18	
	Cu	Zn	Cu	Zn	Cu	Zn	Cu	Zn	Cu	Zn	Cu	Zn
Cu_0/Zn_0	5.45d*	7.00e	5.60d	7.40e	5.90d	8.01e	6.02d	8.05e	6.10d	7.80e	6.25d	8.32e
Cu_0/Zn_{25}	5.20d	39.39a	5.33d	45.14a	5.60d	55.64a	5.95d	43.22a	6.01d	57.40a	6.10d	64.00a
Cu_5/Zn_{25}	18.11c	35.7b	23.00c	39.59b	28.10c	49.32b	20.95c	38 .19b	25.23c	52.32b	36.33c	57.32b
Cu_{15}/Zn_{25}	21.85b	28.85c	27.51b	32.75c	38.00b	44.30c	23.04b	32.54c	30.21b	43.25c	43.77b	50.12c
Cu_{30}/Zn_{25}	25.95a	22.42d	34.89a	26.00d	42.11a	35.25d	27.00a	26.12d	38.71a	34.62d	48.65a	42.74d
Cu_5/Zn_0	20.00bc	1.12f	24.60c	2.54f	29.72c	2.99f	22.00bc	2.06ef	26.63c	3.12f	37.32c	3.70f
Cu_{15}/Zn_0	22.45b	0.51f	28.63b	2.04f	39.55b	2.48f	24.65b	1.65ef	31.52b	2.62f	45.00b	3.27f
Cu_{30}/Zn_0	27.20a	0.33f	36.01a	1.56f	43.10a	2.18f	28.62a	1.31f	39.33a	2.26f	50.00a	2.90f

*Values within the same column followed by different letters are significantly different at P 0.05.

Table B. 3: Cu and Zn concentrations (mg kg^{-1} dry wt.) in buckwheat roots on day 18.

Treatments (mg/l)	Low transpiration rate		High transpiration rate	
	Cu	Zn	Cu	Zn
Cu_0/Zn_0	26.46g*	24.46f	26.99g	25.97f
Cu_0/Zn_{25}	27.25g	473.72a	28.00g	701.14a
Cu_5/Zn_{25}	187.00f	422.82b	278.21f	604.00b
Cu_{15}/Zn_{25}	241.35d	342.09c	338.31d	473.72c
Cu_{30}/Zn_{25}	319.00b	284.00d	436.00b	340.44d
Cu_5/Zn_0	220.64e	42.98e	301.56e	43.88e
Cu_{15}/Zn_0	277.20c	45.64e	380.52c	46.74e
Cu_{30}/Zn_0	340.14a	46.58e	477.00a	47.56e

*Values within the same column followed by different letters are significantly different (P 0.05).