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Research Article

Evaluation of phytotoxic effects of varying copper and zinc concentrations on seed germination, early growth and biomass yield of sesame (*sesamum indicum* linn.) and grain amaranth (*amaranthus cruentus*) under different growing media

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Abstract

Laboratory and green house experiments were conducted at the Teaching and Research Farms, Ladoke Akintola University of Technology, Ogbomoso, Nigeria, in the year 2012, to evaluate the effect of varying concentrations of copper and zinc on germination, early growth and biomass production of two selected arable crops. The two sets of experiment were factorial with two (2) levels of heavy metal (copper and zinc) and six (6) levels of their concentrations in ppm (0, 50, 100, 200, 400 and 500). The experiments were arranged in completely randomized design (CRD), replicated six (6) times. Data collected were subjected to analysis of variance (ANOVA), while the significant means were separated using Duncan Multiple Range Test (DMRT). Application of copper and zinc at varying concentrations significantly ($p<0.05$) influenced seed germination, growth and biomass yield, under both laboratory (filter paper) and green house (soil) conditions. Seeds of both crops tested failed to germinate at zinc concentrations ≥400ppm in the laboratory and green house. However, total seed germination failure was observed at copper and zinc concentrations ≥400ppm in the laboratory for grain amaranth. However, zero germination was only recorded at 500ppm concentrations of copper and at ≥400ppm concentration of zinc, for sesame in the laboratory. In the greenhouse, there were no records on germination for both tested crops at 500ppm concentrations of copper and zinc. Also, under green house conditions, sesame was observed to be more tolerant to the heavy metals' effects as significantly higher cumulative germination percentages were recorded at 400ppm, compared to those of grain amaranth which were not significantly different from 0.0% recorded for 500ppm concentrations for the assayed heavy metals. In addition, higher the dosages of heavy metals significantly affected growth and yield of the tested crops, particularly at above 200ppm concentrations. Both copper and zinc are therefore adjudged as being highly phytotoxic at relatively high dosages. Thus, accumulation of heavy metals (particularly copper and zinc) in soils, is undesirable for sustainable arable crop production and should therefore be totally discouraged in the study.

Keywords: Copper, Zinc, Germination, Biomass yield, Pollution, Sesame, Grain amaranth

INTRODUCTION

Pollution of tropical farmlands with heavy metals is not an uncommon phenomenon. It could be easily traced to incessant application of pesticides and other agro-chemicals, as deemed necessary by arable crop farmers from time to time. Abusive application of these chemicals in heavy dosages particularly without recommendations is apparently the major reason for their persistence in cultivated fields (Clark and Mahanty, 1991; Sobulo, 2000; Tilman *et al.,* 2002; Anon, 2006).

Copper and zinc are equally ranked amongst the most commonly persisting toxic heavy metals in the tropical soils like cadmium and nickel, which are now imposing a serious threat to man, animals and general agricultural activities (Gupta *et al.,* 2001; Allen and Gretchen, 2002; Singh *et al.*, 2007). Although, these heavy metals are ideally required in minute quantities (like other micro nutrients), for effective plant growth and development (Hall, 2002), hence, availability of these nutrient elements in high dosages turn them toxic and undesirable to crop plants (Doran and Zeiss, 2000; Kramer *et al*., 2007). Availability of heavy metals in high concentrations had been reported to influence general crop performance (particularly seed germination and emergence which are the most vulnerable stages in the life of every green plant), and that the severity of the toxicity on the concerned plants may be dependent on some factors, such as genetic variations, amounts of nutrients taken up, geological origin of the soil, source(s) of pollution / contamination, type of heavy metal concerned, soil CEC etc. (Vojtechava and Leblova*,* 1991; Blaylock and Huang, 2000; Hajiboland *et al.,* 2006).

Sesame (*Sesamum indicum*), which belongs to the family Pedaliaceae and the genus sesamum, is an erect, flowering annual plant which grows 50 to 250 cm tall or more, depending on varieties, soil or environmental conditions (Bedigian, 2006; Babajide *et al.,* 2012). About thirty six species exist currently in nature, out of which *Sesamum indicum L.,* popularly known as beniseed in Nigeria, is the most commonly recognized (Akpan-Iwo *et al.,* 2005). It is widely naturalized in the tropical region around the world and cultivated primarily for its tiny edible seed containing 25 % protein and about 50 % oil (Weiss, 2000; Langham *et al.*, 2006). Sesame is usually propagated by seeds and, depending on the varieties, maturity may be reached in 70 - 150 days after sowing (Ashri, 1998; Anon., 2000). Flowering commences and stops at 38-45 and 70-120 days after sowing respectively (Akpan-Iwo *et al.,* 2005). Although sesame is ranked relatively amongst the drought-tolerant crops, but can die in standing water (Weiss, 2000; Anon., 2007). The precise natural origin of the species is unknown but numerous wild relatives are found mostly in Africa while a smaller number also found in India (Bedigian, 2006; Babajide *et al.,* 2012). It is believed to have originated from the tropical Africa where the greatest genetic diversity exists but was believed to have been introduced to India at a very early date, where a secondary center of diversity is well developed (Bedigian, 2006). Cultivation of sesame is now successfully extended from the tropical and subtropical zones to temperate and sub-temperate zones of the world (Ali *et al.,* 2000; Anon.*,* 2007). Sesame utilization includes human consumption, health treatments, beautification, livestock feeding and industrial uses (Chang *et al.,* 2002; Babajide *et al.,* 2012). As a result of the growing awareness about its desirable versatility and successful adaptability to various eco-regions, it is now widely cultivated in the derived, northern and southern guinea, Sudan and Sahel savannas of Nigeria (Olowe *et al.,* 2003; Akpan-Iwo *et al.,* 2005). Although sesame is commonly grown as a sole crop, it is also successfully well intercropped with other arable crops e.g. millet, guinea corn, maize, soybean (Olowe *et al.,* 2003).

Grain Amaranth (*Amaranthus cruentus L.)* is an annual dicotyledonous plant belonging to the family Amaranthaceae. It is a broadleaf pseudo-cereal with an upright growth habit mainly cultivated for both its edible grains and leaves. Grain Amaranth is probably originated from America but now widely cultivated in the tropical and subtropical Africa, Asia, the Pacific Islands, the Caribbean and Central America (Olaniyi, 2007). Some of the most frequently cultivated species are *Amaranthus blitum; A. lividus; A. dubius; A. spinosus; A. Thunbergii; A. graecizans* (vegetables); *A cruentus and A. hypochondriacus* (grain) (Schippers, 2000). *Amaranthus cruentus L.* is a vegetable of high dietary value commonly grown and consumed in many parts of Nigeria. *Amaranthus cruentus* is best recognized by its leaves that are twice or three times as long as wide, which are often known to have pointed leaf tips and plume-like inflorescence with raceme diameter of 10mm or more (Schippers, 2000). Although all African species are used for their leaves, they can also be grown for their seeds. It contains relatively high levels of vitamin A and C, carotene, iron, calcium, potassium, protein and lysine (Akanbi and Togun, 2002). The seeds contain 1.5 – 3.0 times higher oil content than any cultivated cereal (Olaniyi, 2007). Leaves are processed into many food items, supplements and additives (Ojo and Olufolaji, 1997). Uses of amaranth include; grains used in breakfast cereals or as an ingredient in confectioneries. As a snack, the tiny grains are popped and taste like nutty-flavored popcorn, or it is mixed with honey. The leaves (which are high in protein, vitamins and minerals) are boiled and eaten as greens. They are the most commonly grown vegetable of the lowland tropics in Asia and Africa. Amaranth seeds are occasionally used in Ethiopia to brew the alcoholic beverage “tala”, a kind of beer. It is especially rich in protein and lysine, an essential amino acid that is lacking in most of the cereal-based diets and could be processed into animal feed (Breene, 1991). Apart from its nutritional value, amaranthus is extensively cultivated in Nigeria for its relatively drought tolerant quality and wide range of climatic adaptability. It could be grown in areas where grains like sorghum, maize and millet can thrive well and could compete effectively with some other arable crops, under mixed cropping conditions (Denton, 1992). Green leaves of amaranth were reported to contain 2.6mg iron and 654µg vitamin A per 100g which make it suitable forage, for livestock feed management (Olaniyi, 2007).

However, this research was conducted to investigate the response of these versatile arable crops, which equally possess relatively high climatic adaptability, to the rapidly advancing crop production threat (i.e. heavy metal pollution), so as to recommend clues for improved sustainable crop production.

MATERIALS AND METHODS

Laboratory and green house experiments were conducted at the Teaching and Research Farms, Ladoke Akintola University of Technology, Ogbomoso, Nigeria, in the year 2012, to evaluate the effect of varying concentrations of copper and zinc on germination, early growth and biomass production of two selected arable crops, under different growth media.

The two sets of experiment were factorized with two (2) levels of heavy metal (M^1=copper and M^2=zinc) and six (6) levels of their concentrations in ppm (C^0=0, C^1=50, C^2=100, C^3=200, C^4=400 and C^5 = 500). A total of twelve treatment combinations were obtained. The trials were arranged in completely randomized design (CRD), replicated four (4) times. Data collected were subjected to analysis of variance (ANOVA), while the significant means were separated using Duncan Multiple Range Test (DMRT).

Sesame seeds of variety E8 (early maturing type), obtained from the National Cereal Research Institute (NCRI) at Badeggi in Niger State, Nigeria, and grain amaranth seeds of variety NHAC3, obtained from the National Institute of Horticultural Research (NIHORT), Ibadan, Nigeria were used for the experiments. The seeds were surface sterilized by using 95% ethanol for 10 seconds and were later rinsed six (6) times with sterile water after shaking for three to five minutes in 3% hydrogen peroxide (H_2O_2).

The laboratory experiment: twenty sterilized seeds were placed in each corresponding Petri dish (9.5cm diameter) containing well-lined filter paper moistened with 10ml of the respective heavy metal solutions and ordinary deionized water (control). The petri dishes were well-covered immediately with their lids and kept at a room temperature. Re-moistening was maintained at an interval of 48 hours according to the procedures of Naveed *et al*. (2001). Counting of germinated seeds commenced at 24 hours (a day) after seed placement in the petri-dishes (as evident by protruding radicle length of 2mm) and lasted fourteen days. After 14 days germination percentage was determined according to ISTA (1999).

$$\text{Germination percentage} = \frac{\text{No. of germinated seeds}}{\text{Total No. of seeds sown}} \times 100$$

The green house experiment: twenty sterilized seeds were placed in each corresponding plastic pot (having 4.0cm height and 25.6cm circumference), containing 0.5 kg well-drained sandy loam alfisols belonging to Egbeda soil series (Smyth and Montgomery, 1962). Application of 500ml each of the respective concentration of the tested heavy metal solutions and ordinary deionized water (control) was carried out. Re-moistening was maintained at an interval of 48 hours according to the procedures of Naveed *et al*. (2001). Counting of germinated seeds commenced at 24 hours after seed sowing. The germinated seeds counted were those possessing protruding radicle length of at least 2mm. The counting lasted fourteen days. After 14 days, germination percentage was determined according to ISTA (1999).

Data collection and analyses: The experiments were terminated after 14 days after seed placements in the growth media. Growth parameters such as shoot and root lengths were determined using a meter rule. Then, after harvesting, all shoots and roots were oven-dried at a temperature of 80 ^{0}C to a constant weight for five days, for dry weight determination of the total biomass yield (Babajide *et al.,* 2012). The yield parameters such as shoot and root dry weights were determined using sensitive electronic weighing balance (Citizen MP600H), and recorded accordingly. Data collected were subjected to analysis of variance (ANOVA), using SAS, (2012), while the significant means were separated using Duncan Multiple Range Test (DMRT).

THE RESULTS AND DISCUSSION

The results from the physicochemical analyses (I.I.T.A., 1982) of the soil showed that the soil was mildly acidic with pH (H_20) (6.20), texturally sandy-loam with sand (77.18 %), silt (12.10 %) and clay (10.72 %), grossly low in the essential nutrient concentrations with total N (0.06 %), extractable P Bray 1 (1.40 $mgkg^{-1}$), Organic carbon = 3.72 (%) and the exchangeable bases (in Cmol kg^{-1}) were K^+ (0.26), Ca^+ (8.14), Mg^{2+} (2.78) and Na^+ (0.21). These results are in support of earlier researchers (Akanbi *et al.*, 2005; Babajide *et al.,* 2012), who reported that the soils in the study area are majorly low in essential nutrient concentrations and thereby require adequate supply of organic manures to meet plants requirements. Application of heavy metals at varying concentrations significantly ($p<0.05$) influenced seed germination of both arable crops tested, irrespective of the growth medium used (Table1). Germination reduced significantly with the increasing dosages of the heavy metals (particularly from above 100ppm). Considering the grain amaranth, total seed germination failure was observed at Cu and Zn concentrations greater than 200ppm in the laboratory (Table 2). Although significantly lower values were recorded for copper and zinc concentrations at 400ppm in the green house, the values were not significantly different from 0.0% values obtained at 500ppm concentrations for Cu and Zn (Table 2). Seed germination of sesame was significantly affected by the applied heavy metal concentrations. Increasing dosages of the heavy metals (particularly at ≥ 200ppm) induced significant germination reduction (Table 2). Application of either Cu or Zn at ≥ 400ppm concentration, seed germination of sesame seized totally in the laboratory. Germination of sesame seed failed totally when Cu and Zn applications were at 500ppm concentrations only, in the green house. Sesame resisted the negative effects of applications of the heavy metals on germination better than grain amaranth, particularly in the green house (Table 2). Although Stoinova *et al.* (2007) as well as Souguir *et al.* (2008) had earlier reported the possible diverse genetic variability-based responses expected from different cultivated crop species regarding copper ion load in the soil, the facts surrounding the poor active break down of starch by low amylase activities in seeds particularly at relatively high concentrations of copper in the soil solution, cannot be adjudged as irrelevant (Kabata-Pendias and Pendias, 1973; Olowolafe *et al.,* 2012). Generally, the osmotic effects in relation to ionic toxicity of the heavy metal salts which manifested on seed germination of some crop plants had been earlier reported (Alloway, 2008; Jadi and Fulekar, 2008; Muhammad *et al*., 2008; Hema and Subramani, 2013). These results however corroborated the findings of Shaukat *et al.* (1999); Mahmood *et al.* (2005); Singh *et al.* (2007) and Muhammad *et al.* (2008), who reported the toxic effects of heavy metals on germination.

Growth parameters of the tested arable crops were significantly affected under both growing media conditions (Table 3). Shoot and root lengths of sesame and grain amaranth decreased significantly with increasing dosage / concentration of Cu and Zn particularly at > 200ppm, under laboratory conditions (Table 3). Under green house conditions, sesame significantly tolerated the effects of Cu and Zinc applications at 400ppm compared to grain amaranth (Table 3). At 500ppm, no values were recorded for both crops (since 0.0. % germination was earlier recorded). Excessive accumulation of the tested heavy salts in the cell wall of the roots may alter and modify the metabolic activities (Alloway, 2008). In addition, an interference with cell division may occur, leading to possible chromosomal aberrations as well as defective mitosis (Munzuroglu and Geckil, 2002; Olowolafe et al., 2012). Such malformations and disorders may limit regular massive flow of the cell wall's substances as well as its elasticity. These may induce significant effects on root formation, elongation and development. These are in line with the reports of Kabata-Pendias and Pendias (1973); Naseer *et al.* (2001); Alloway (2008) and Muhammad *et al.* (2008) who reported poor crop performance in relation to toxic nutrition.

Application of Cu and Zn at ≥ 200ppm significantly affected the biomass yields of the two crops tested (Figure 1). Sesame was observed to be more tolerant to the heavy metals effects in both growing media and produced significantly higher biomass yield at 400ppm, compared to the grain amaranth, except for under green house conditions when grain amaranth also produced significantly higher biomass at 400ppm (Figure 1). The results from this study also confirmed that both zinc and copper are toxic at higher concentrations (Figure 1 and Figure 2). Although, these results partially disagreed with the findings of Kabata-Pendias and Pendias (1973) which did not recognize zinc as being hyper toxic to crop plants, hence, the results are in agreement with those findings reported by Sing *et al.* (2007); Gardestedt *et al.* (2009); Manivasagaperumal *et al.* (2011) and Mohammadi *et al.* (2013), which established phyto-toxicity of heavy metals (zinc inclusive).

CONCLUSION

Both heavy metals tested were found to be toxic (particularly at higher concentrations) to the arable crops irrespective of the growing medium. Effects of the two heavy metals were demonstrated on germination, growth and yield of the two arable crops tested under the two growing media conditions experimented. Improvement in soil organic matter content of the growing medium may possibly reduce the phyto-toxic effects of heavy metals, as observed in the green house experiment (where seeds were buried inside the soil containing the corresponding heavy metal solutions), compared to the laboratory experiment (where seeds were only placed on the filter moistened with the corresponding heavy metal solutions). Sesame is more tolerant to the toxic effects of the heavy metals, compared to the grain amaranth. However, any farming activities that can promote undesirable accumulation of heavy metals should be totally discouraged. Meanwhile, such farming operations that will promote proper maintenance of soil organic matter and moisture conservation should be encouraged. In addition, soil testing excess is also recommended on regular basis, so as to know the nutrient status of the soil from time to time, in order to improve seed germination and general crop performance, which will maximally compensate farmers' labours and facilitate sustainable arable crop production in the tropics, where soils are majorly marginal.

Table 1. Pre-cropping Chemical and Physical Properties of the Soil Sample Used

PROPERTIES	VALUES
pH (H_20)	6.20
Organic C (%)	3.72
Total N (%)	0.06
Extractable P Bray 1(mg kg^{-1})	1.40
Fe (mg kg^{-1})	14.10
Cu (mg kg^{-1})	3.12
Zn (mg kg^{-1})	2.92
Exchangeable K^+ ($cmolKg^{-1}$)	0.26
Exchangeable Na^+ ($cmolKg^{-1}$)	0.21
Exchangeable Ca^{2+} ($cmolkg^{-1}$)	8.14
Exchangeable Mg^{2+} ($cmolkg^{-1}$)	2.78
Sand (%)	77.18
Silt (%)	12.10
Clay (%)	10.72
Textural class	Sandy loam

Table 2. Seed germination of grain amaranth (*Amaranthus cruentus*) and sesame (*Sesamum indicum*) as influenced by heavy metal concentrations under different growing media

Treatment combinations	LABORATORY Cumulative Germination percentage (%)		GREEN HOUSE Cumulative Germination percentage (%)	
	Amaranth	Sesame	Amaranth	Sesame
M^1C^0	98.6a	96.0a	97.8a	96.0a
M^1C^1	94.5a	94.5a	95.6a	94.5a
M^1C^2	96.7a	88.3a	95.0a	87.3a
M^1C^3	68.3b	73.0b	71.8b	74.0b
M^1C^4	0.0c	8.2c	4.0c	25.2c
M^1C^5	0.0c	0.0c	0.0c	0.0c
M^2C^0	98.2a	96.8a	97.4a	96.8a
M^2C^1	96.4a	94.0a	96.2a	94.0a
M^2C^2	96.9a	86.4a	95.2a	86.4a
M^2C^3	67.2b	72.5b	62.5b	72.5a
M^2C^4	0.0c	0.0c	6.2c	28.1c
M^2C^5	0.0c	0.0c	0.0c	0.0d

Means followed by the same letters within the same column are not significantly different at $p \leq 0.05$, using DMRT. M^1=copper and M^2=zinc. C^0= application of deionized water only; C^1= application @ 50ppm; C^2= application @ 100ppm; C^3= application @ 200ppm; C^4= application @ 400ppm and C^5 = application @ 500ppm.

Table3: Growth parameters of grain amaranth (*Amaranthus cruentus*) and sesame (*Sesamum indicum*) as influenced by heavy metal concentrations under different growing media

Treatment combinations	LABORATORY (Amaranth)		LABORATORY (Sesame)		GREEN HOUSE (Amaranth)		GREEN HOUSE (Sesame)	
	Shoot length	Root length	Shoot length	Root length	Shoot length	Root length	Shoot length	Root length
M^1C^0	9.8a	2.8a	10.2a	3.6a	12.8a	3.9a	14.1a	4.3a
M^1C^1	8.2a	2.5a	9.6a	3.6a	10.4a	3.6a	12.9a	4.3a
M^1C^2	7.8a	2.2a	9.4a	2.9a	9.2a	3.3a	10.1a	4.2a
M^1C^3	2.1b	0.9b	2.4b	1.1b	4.1b	1.9b	6.9b	2.3b
M^1C^4	0.0c	0.0c	1.2c	0.5b	0.4c	0.2c	3.5c	1.1c
M^1C^5	0.0c	0.0c	0.0c	0.0c	0.0c	0.0c	0.0d	0.0d
M^2C^0	10.1a	3.4a	9.1a	2.7a	11.6a	3.6a	13.7a	4.6a
M^2C^1	7.9a	3.0a	8.9a	2.7a	10.8a	3.5a	13.1a	4.5a
M^2C^2	6.7a	2.9a	8.0a	2.4a	9.4a	3.2a	11.8a	4.1a
M^2C^3	1.8b	1.1b	2.8b	0.8b	5.0b	1.6b	6.2b	2.7b
M^2C^4	0.0c	0.0c	1.3c	0.0c	0.3c	0.2c	2.4c	0.7c
M^2C^5	0.0c	0.0c	0.7c	0.0c	0.0c	0.0c	0.0d	0.0d

Means followed by the same letters within the same column are not significantly different at $p \leq 0.05$, using DMRT. M^1=copper and M^2=zinc. C^0= application of deionized water only; C^1= application @ 50ppm; C^2= application @ 100ppm; C^3= application @ 200ppm; C^4= application @ 400ppm and C^5 = application @ 500ppm.

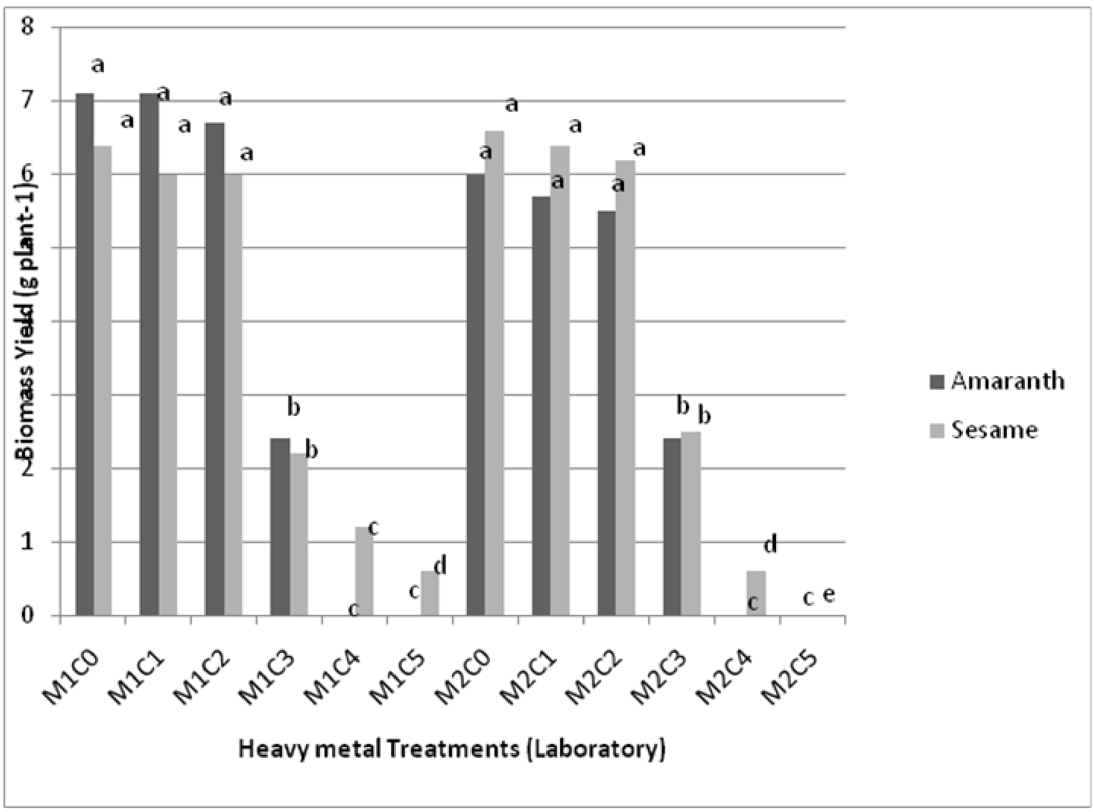

Figure 1. Biomass Yield of grain amaranth (*Amaranthus cruentus*) and sesame (*Sesamum indicum*) as influenced by heavy metal concentrations under laboratory conditions

Means followed by the same letters within the same column are not significantly different at $p \leq 0.05$, using DMRT. M^1=copper and M^2=zinc. C^0= application of deionized water only; C^1= application @ 50ppm; C^2= application @ 100ppm; C^3= application @ 200ppm; C^4= application @ 400ppm and C^5 = application @ 500ppm.

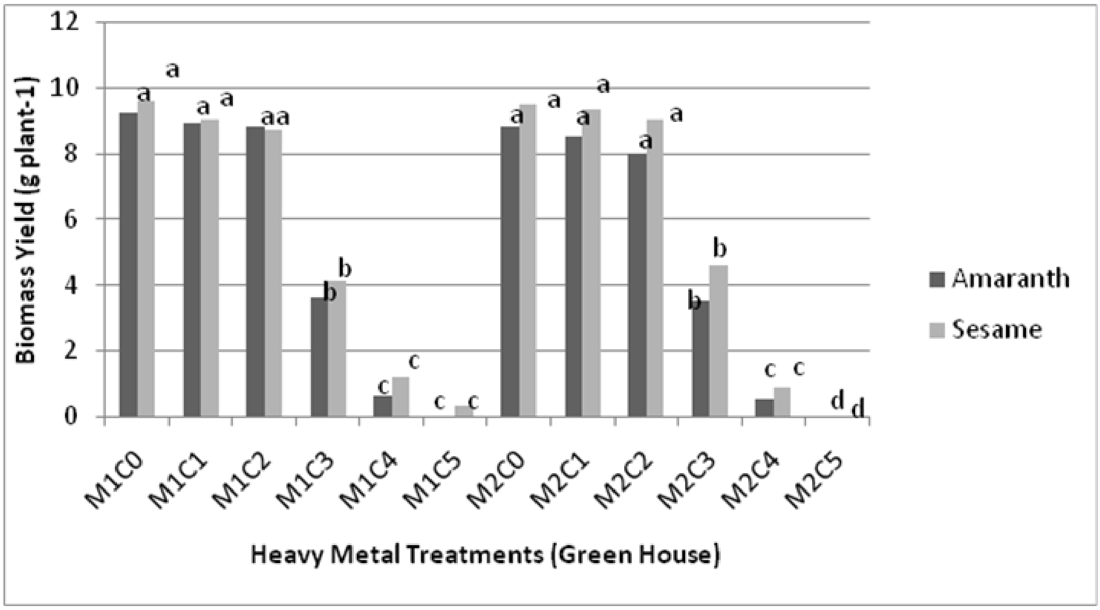

Figure 2. Biomass Yield of grain amaranth (*Amaranthus cruentus*) and sesame (*Sesamum indicum*) as influenced by heavy metal concentrations under green house conditions

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