

**Online Publication Date: 15<sup>th</sup> May, 2012**

**Publisher: Asian Economic and Social Society**

Journal of Asian Scientific Research



**Toxic Effects of Cadmium (Cd) and Lead (Pb) on Growth and Productivity of *Arachis Hypogaea (L)* and *Glycine Max (L)***

**Paul O. Fatoba., Clement O. Ogunkunle and Bolaji Z. Salihu**

(University of Ilorin, Department of Plant Biology, Ilorin, Nigeria)

**Citation:** Paul O. Fatoba., Clement O. Ogunkunle and Bolaji Z. Salihu (2012) "Toxic Effects of Cadmium (Cd) and Lead (Pb) on Growth and Productivity of *Arachis Hypogaea (L)* and *Glycine Max (L)*", Journal of Asian Scientific Research, Vol. 2, No. 5, pp. 254-259.



### Author(s)

**Paul O. Fatoba, Clement O. Ogunkunle and Bolaji Z. Salihu.**  
University of Ilorin,  
Department of Plant Biology,  
Ilorin, Nigeria  
E-mail:[ogunkunle.co@unilorin.edu.ng](mailto:ogunkunle.co@unilorin.edu.ng)

## Toxic Effects of Cadmium (Cd) and Lead (Pb) on Growth and Productivity of *Arachis Hypogaea* (L) and *Glycine Max* (L)

### Abstract

A study was conducted to determine the effects of different concentrations of cadmium and lead on the growth and productivity of *Arachis hypogaea* and *Glycine max*. Seeds were sown on soils irrigated with 0, 10, 20, 30 and 40 ppm concentrations of cadmium and lead. Growth and productivity indices were recorded at the 8<sup>th</sup> week of growth and the elemental contents of the seeds produced were determined using Atomic Absorption Spectrophotometer. The data generated were subjected to One-Way Analysis of Variance and Duncan Multiple Range Test. The results showed that both lead and cadmium treatments had toxic effects on various growth indices and productivity of *Arachis hypogaea* and *Glycine max*. Increasing the concentrations of the metals (lead and cadmium) to 40 ppm, significantly ( $p<0.05$ ) decreased seed germination and number of leaves in both *Arachis hypogaea* and *Glycine max* as compared to Control. The filling potentials of the two plants were greatly reduced with increase in concentrations of cadmium and lead regimes. The seeds produced accumulated a lot of cadmium and lead and their concentrations in the seeds increased as the regime concentrations increased. This study has been able to show the risks/hazards of the consumption of groundnuts and cowpeas that are irrigated with effluents or wastewaters. It is recommended that soils polluted with Pb and Cd should not be used to raise these crops.

**Keywords:** Bio-accumulation, Biodegradability, Filling potential, Germination, Growth indices, Inhibition

### Introduction

Groundnut and soybeans are important economic crops in most countries around the world because of their high protein contents (Baohui, 2007). Owing to numerous economic importance of these crops (groundnut and soybeans) and increasingly international concern on the soil contaminations due to cadmium, and lead (Fargasova, 1994). It is very important to investigate the impacts of heavy metals on the growth and productivity of these crops. The presence of heavy metals in the environment is of great ecological concern owing to their toxicity at certain levels in living organisms and their non-

biodegradability attribute which is responsible for their bio-accumulation in living organisms (Manila & Amallaha, 2008). Several studies have been conducted in order to evaluate the effects of different heavy metals on plants (Reeves & baker, 2000). Heavy metals have been reported to interfere in biochemical reactions of plant and induce physiological disorders like reduction in leaf chlorophyll (Heale *et al.*, 1985; Khan *et al.*, 1988). Adverse effects of nickel, copper and cobalt have been reported on growth characters, biomass, yield, etc. of celery, tomato and lentil plants both in ambient and artificial treatment conditions (Bisessar *et al.*, 1983; Heale *et al.*, 1985). Stunted growth, chlorosis and necrosis were

reported by Vassilev *et al.* (1998) to be symptoms of severe phytotoxicity.

Studies also showed that lead inhibit root growth and elongation at levels between 3 and 10  $\mu\text{g/g}$  of soil (Bartuska, 1985) while Farooqi *et al.* (2009) and Padmaja *et al.* (1990) reported that lead and cadmium toxicities affected seed germination, root and shoot seedling length, root-shoot ratio and dry biomass of *Albizia lebbeck* L and reduction of biomass through inhibition of chlorophyll synthesis and photosynthesis respectively. Excess amount of cadmium was reported to cause decreased uptake of nutrient elements, inhibition of various enzymes activities and induction of oxidative stress by Sandalio *et al.* (2001).

High concentrations of cadmium in soils represent a potential threat to human health because it is incorporated in the food chain mainly by plant uptake (Alvarez-Ayizo, 2008). Heavy metals (Pb, Cd, Cr, Ni) even at low concentration may impair a range of cellular activities and reduce the uptake of other essential nutrients (Kadar & Kastori, 2003). Groundnut and soybeans are common legumes planted in Nigeria and consumed by all. The poor waste management coupled with release of pollutants and the use of agrochemicals pose problem to the safety of these crops. This is because groundnut can be consumed raw and half-cooked before consumption. It is therefore necessary to investigate the impact of these heavy metals on these plants so as to reduce the hazards associated with them. In the present paper, we report the growth performance of *Arachis hypogaea* and *Glycine max* grown in soil artificially treated with 0, 10, 20, 30 and 40 ppm of cadmium and lead.

### Materials and Methods

Seeds of *Glycine max* L (SAM Soya type 1) and *Arachis hypogaea* L. (local red peanut) were obtained from Ministry of Agriculture, Ilorin, North-Central Nigeria in 2010. Top soils were collected and treated for two weeks with varied concentrations of cadmium chloride and lead nitrate. The

experiment was carried out at the Screen house, Biological Garden, University of Ilorin, North-Central Nigeria between September 2010 and March, 2011.

The seeds were treated with 3% formaldehyde for five minutes to remove fungal contamination and later washed in deionized water and dried. 20 seeds of each study plants were sown in two replicates in 20cm-diameter pots containing pretreated soils of varying concentrations (0, 10, 20, 30 and 40 ppm) of cadmium chloride and lead nitrate. The percentage germination was calculated for each treatment. After the emergence of the seeds, the seedlings were thinned out to two and three replicates of each treatment were carried out and growth indices were recorded after the eight week. After the 8<sup>th</sup> weeks, the plants' seeds were harvested and digested using aqua regia according to ISO Standard 11466 (1995). The digestion of the spiked soils followed the procedure of the nitric-perchloric acid digestion method of AOAC (1990), adopted by Hseu (2004) and the digestates from the seeds and soils were analyzed for cadmium and lead using atomic Absorption Spectrophotometric method (Allen, 1989). Data generated from seed germination, growth indices and heavy metal loads of the seeds were statistically analyzed by one-way Analysis of Variance (ANOVA) (Steel and Torrie, 1984) and Duncan's Multiple Range Test (DMRT) (Duncan, 1955) and tested for significance at  $P<0.05$ .

### Results

Seed germination and other growth indices of *Arachis hypogaea* and *Glycine max* highly decreased with the treatments of cadmium and lead at 10, 20, 30 and 40 ppm as compared to the Control (Table 1&2). Percentage germination of both *A. hypogaea* and *G. max* were lowest at regimes of 40 ppm of cadmium and lead but the effect of cadmium was more pronounced in the two studied plants.

Cadmium treatment at 10 ppm concentration produced significant ( $p<0.05$ ) effect on seed

germination in *A. hypogaea* and *G. max*. Increased concentration to 30 ppm significantly affected the numbers of leaf, leaf area, number of pods per plant and number of seeds per plant (Table 1&2). Further increase in concentration of cadmium to 40 ppm drastically reduced all the growth indices significantly as compared to the Control. Lead concentration in *G. max* at 20ppm greatly affected the leaf area, number of pod per plant and number of seeds per plant while significant effect was recorded in *A. hypogaea* at 10ppm regime in number of leaf, height, leaf area and number of seeds per plant (Table 2). There was a great reduction in the leave sizes produced in *G. max* and *A. hypogaea* in the regimes of 30ppm of both cadmium and lead and the least number of leaves was found in the treatment regime of cadmium (Table 1 & 2).

The effects of metal treatments on the filling potentials were reported in Table 3. Filling potentials in the two studied plants reduced with increase in concentration of the treatments. It is also noted that toxic effect of metals was pronounced in the various treatments of cadmium than that of lead. Table 4 showed the elemental content of cadmium and lead in the seeds of *G. max* and *A. hypogaea*. Generally, cadmium and lead were present in all the seeds produced by different treatments. The seeds of both the control and 10ppm regime of both plants had the same concentration of these heavy metals statistically but different numerically. It is noted that elemental concentration of cadmium and lead increased alongside the increased treatment concentrations.

## Discussion

Cadmium and lead toxicity have become an important issue due to their constant proliferation in the environment. Cadmium and lead treatments significantly ( $P<0.05$ ) decreased seed germination percentage as compared to control. Seed germination inhibition by heavy metals has been reported by many other workers (Azmat et al., 2005). The reduced percentage germination of these plants could be related to the negative effects of metals on water uptake and

production of  $GA_3$  in the embryo (Mukherji & Ganguly, 1974). Several biochemical processes are affected by cadmium and lead as put by Athar & Ahmed (2002) by altering several enzymes activities; thus reducing germination rate (Al-Rumaih, 2001).

Significant reduction in the growth of *Arachis hypogaea* and *Glycine max* with the increase in concentrations of cadmium treatment were observed as compared to the Control. Cadmium is known to be a highly toxic contaminant that affects many plants' metabolic processes (Li et al., 2008). Servilia et al. (2005) revealed that heavy metals contamination reduced leaves production in plant while Poschenreider et al. (1989) reported that excess levels of cadmium and other heavy metals can decrease the concentration of chlorophylls which in turn can lead to growth reduction.

The reduced growth indices in *A. hypogaea* and *G. max* could be related to the findings of Tuna et al. (2002), Oncel et al. (2000) and Farooqi et al. (2009) that heavy metals affect plant physiological and biochemical processes. However, cadmium and lead showed concentration-dependent inhibition on the growth of the plants which was in tandem with the findings of Farooqi et al. (2009). Investigations into the productivity of *A. hypogaea* and *G. max* under the influences of cadmium and lead revealed reduction in productivity. Cadmium showed more adverse effects on seeds and pods production.

There is high content of elemental cadmium and lead in the seeds of *Arachis hypogaea* and *Glycine max* and the concentrations correspond to the contents in the treated soils. The high elemental contents in the seeds were attributed to the high level of cadmium and lead in the soils; plants absorb metals based on their availabilities in the soil. This relationship is referred to as linear by Benzarti et al. (2008). Consumption of plants with high levels of metals usually lead to bio-accumulation in the body over time, they remain in an unchanged state and are continuously accumulated during the life

of an organism leading to bio-magnification that could cause various health problems.

William & Robertson (1999) reported that lead poisoning remain a serious problem in India while cadmium contamination has been proved to damage liver, placenta, kidney and the brain.

### **Conclusion**

Pollution by heavy metals has been of great concern in the last decades because of their health hazards to man and other organisms when accumulated within a biological system. Therefore, the use of wastewater and effluent water as irrigation water should be discouraged because of the tendency of edible crop plants to accumulate these heavy metals in their tissues which will eventually get up the food chain through consumption and bio-accumulation.

### **References**

- Allen, S. E. (1989)** "Chemical Analysis of Ecological Materials". Second Editions, Blackwell Scientific Publications, Oxford.
- Alvarez-Ayuso, E. (2008)** "Cadmium in Soil-Plant Systems: An Overview". International Journal of Environment and Pollution Vol. 33, No. 2&3, pp. 275-291.
- Al-Rumaih, M.M. (2001)** "Interactive Effect of Cadmium and Gibberellic Acid on Cowpea (*Vigna unguiculata*)". Ph.D thesis; King Saud University, Riyadh, Saudi Arabia
- AOAC (1990)** "Official Methods of Analysis". Association of Analytical Chemists (15th edition). USA, VA. Pp. 1-34
- Athar, R. and A. Ahmad. (2002)** "Heavy Metals Toxicity Effects on Plant Growth and Metal Uptake by wheat and on free-living Azobacter". Water, Air, Soil Pollut. Vol. 138, pp. 165-180
- Azmat, R., A. Zill-e-Huma, A. Hayat, T. Khanum, and R. Talat (2005)** "The Inhibition of Bean Plant Metabolism by Cadmium Metal: Effects of Cadmium Metal on Physiological Process of Bean Plant and Rhizobium Species". Pakistan Journal of Biological Sciences Vol. 8, No. 3, pp. 401-404
- Baohui, S. (2007)** "Market Power and Competitive Analysis of China's Soybean Import Market". International Agricultural Trade Research Consortium (IATRC)
- Bartuska, A. (1985)** "Assessment of Acid Deposition and Its Effects". NAPAP Draft Distribution, Washington, DC
- Benzarti, S., S. Nohri, and Y. Ono (2008)** "Plant Response to Heavy Metal Toxicity: Comparative Study between the Hyper Accumulator *Thlaspi caerulescens* (Ecotype gauge) and Non Accumulator Plants, Lettuce, Radish and Alfalfa". Environ. Toxicol. Vol. 25, No. 5, PP. 607-616
- Bisessar, S., R.J. Rinne, and J.W. Potter (1983)** "Effect of Heavy Metal and Meloidogyne hapla on Celery grown on Organic Soil near Nickel Refinery". Plant Diseases Vol. 67, No. 1, PP. 11-14.
- Duncan, D.B. (1955)** "Multiple ranges and multiple F-test". Biometric Vol. 11, PP. 1-42
- Fargasova, A. (1994)** "Effect of Pb, Cd, Hg, As and Cr on Germination and Root Growth of *Sinapis alba* seeds". Bull. Environ. Contam. Toxicol. Vol. 52, PP. 452-456
- Farooqi, Z.R., I.M. Zafar, M. Kabir, and M. Shafiq (2009)** "Toxic Effects of Lead and Cadmium on Germination and Seedling Growth of *Albizia lebbeck* L". Pakistan. Journal of Botany Vol. 41, No. 1, pp. 27-33
- Heale, E.L., D.P. Ormrod, P.J. Laffey, and O.B. Allen (1985)** "Effect of Nickel and Copper Mixtures on Tomato in Sand Culture". Environmental Pollution Vol.39, pp. 53-69.
- Hseu, Z. Y. (2005)** "Evaluating Heavy Metal Content in Nine Compost using Four Digestion Methods". Bioresource Technology. Vol. 95: 53-59
- ISO 11466 (1995)** "Soil Quality: Extraction of Trace Elements Soluble in Aqua Regia". International Organization for Standardization. Geneva
- Kadar, I., and R. Kastori (2003)** "Effects of Microelement Loads on Rape Grown on Calcareous Chernozem soil". Agrokemia Talajtan Vol. 52, PP. 331-346
- Khan, M. R., S.K. Singh and M. W. Khan (1988)** "Response of Lentil to Cobalt as Soil Pollutant". TAC 9 Ann Appl Biol (Suppl.) Vol. 112, PP. 104-105.

- Li, M., L. Zhang, L. Tao, and W. Li (2008)** "Ecophysiological Responses of *Jussiaea rapens* to Cadmium Exposure". Aquatic Botany Vol. 88, No. 4, PP. 347-352
- Manilla, P.N. and I. Amallaha (2008)** "Niger Delta Flood Plain Lakes II. Chemical Assessment of the Sediment of Lake Apkide, Besini, Bayelsa State, Nigeria". Journal of Nigerian Environment Society Vol. 4, pp. 50-60.
- Mukherji, S. and B.Gangulay (1974)** "Toxic Effects of Mercury in Germinating Rice (*Oryza sativa*) and their Reversal". India J. Exp. Biol. Vol. 12, pp. 432-434
- Oncel, I., Y. Kele and A.S. Ustun (2000)** "Interactive Effects of Temperature and Heavy Metal Stress on the Growth and some Biochemical Compounds in Wheat Seedlings". Environ. Pollut. Vol.107, pp. 315-320
- Padmaja, K., D.D.K. Prasad and A.R.K. Prasad (1990)** "Inhibition of Chlorophyll Synthesis in *Phaseolus vulgaris* Seedlings by Cadmium Acetate" Photosynthetica Vol. 24, pp. 399-405.
- Poschenreider, C.H., B. Gunse and J. Barcelo (1989)** "Influence of Cadmium on Water Relations, Stomatal Resistance and Abscisic acid Content in Expanding Bean Leaves". Plant Physiol. Vol. 90, pp.1365-1371
- Sandalio, L.M., H.C, Dalurzo, M. Gomez, M.C, Romero-Puertas and L.A. del Rio (2001)** "Cadmium-induced Changes in the Growth and Oxidative Metabolism of Pea Plants". J. Exp. Bot. Vol. 52, pp. 2115-2126.
- Servilia, O., N. Foca and A. Airinei (2005)** "Effects of Heavy Metals on Plant Growth and Photosynthetic Activity". Not. Bio. Sci, pp. 107-110
- Steel, R.G.D. and J.H. Torrie (1984)** "Principles and Procedures of Statistics". McGraw Hill Book Inc., Singapore. Pp. 172-177.
- Tuna, L.A.,B. Burun, I. Yokas and E. Coban (2002)** "The Effects of Heavy Metals on Pollen Germination and Pollen Tube Length in the Tobacco Plant". Turk J. Biol. Vol. 26, pp. 109-113
- Vassilev, A., M. Berova and Z. Zlatev (1998)** "Influence of Cd on Growth, Chlorophyll Content and Water Relations in Young Barley Plants". Biol. Plant. Vol. 41, No, 4, pp. 601-606
- William O, and B. Robertson (1999)** "The Role of Heavy Metals in Human Metabolism". Environmental Pollution. Vol. 42, pp. 1-5.

**Table-1:** Effects of different concentrations of cadmium on germination and growth parameters of *Arachis hypogaea* and *Glycine max*.

Treatments	Seed germination (%)	No of leaf (cm)	Height (cm)	leaf area (cm <sup>2</sup> )	No of pods/plant	No of seeds/plant
0 ppm	100 <sup>a</sup>	72.0 <sup>a</sup>	36.7 <sup>a</sup>	7.6 <sup>a</sup>	2.7 <sup>a</sup>	7.0 <sup>a</sup>
<i>Arachis hypogaea</i> 10 ppm	85 <sup>b</sup>	47.3 <sup>b</sup>	26.2 <sup>b</sup>	6.1 <sup>b</sup>	1.8 <sup>b</sup>	4.0 <sup>b</sup>
20 ppm	75 <sup>c</sup>	45.0 <sup>b</sup>	23.5 <sup>c</sup>	5.1 <sup>c</sup>	1.3 <sup>c</sup>	3.0 <sup>c</sup>
30 ppm	65 <sup>d</sup>	36.0 <sup>C</sup>	21.0 <sup>D</sup>	4.5 <sup>C</sup>	1.0 <sup>d</sup>	2.0 <sup>d</sup>
40 ppm	60 <sup>e</sup>	30.0 <sup>d</sup>	18.0 <sup>e</sup>	3.7 <sup>d</sup>	1.0 <sup>d</sup>	1.3 <sup>e</sup>
<i>Glycine max</i> 0 ppm	100 <sup>a</sup>	35.7 <sup>a</sup>	58.7 <sup>a</sup>	13.5 <sup>a</sup>	6.7 <sup>a</sup>	13.3 <sup>a</sup>
10 ppm	90 <sup>b</sup>	25.0 <sup>b</sup>	39.0 <sup>b</sup>	9.8 <sup>b</sup>	3.5 <sup>b</sup>	6.7 <sup>b</sup>
20 ppm	80 <sup>c</sup>	16.7 <sup>c</sup>	34.4 <sup>c</sup>	7.6 <sup>c</sup>	2.7 <sup>c</sup>	4.0 <sup>c</sup>
30 ppm	65 <sup>d</sup>	15.7 <sup>C</sup>	30.5 <sup>d</sup>	6.6 <sup>C</sup>	2.0 <sup>d</sup>	3.0 <sup>d</sup>
40 ppm	60 <sup>e</sup>	12.7 <sup>d</sup>	25.5 <sup>e</sup>	5.3 <sup>d</sup>	1.7 <sup>d</sup>	2.0 <sup>e</sup>

Values with the same superscript in the same column are not significantly different at P<0.05 level.

**Table-2:** Effects of different concentrations of lead on germination and growth parameters of *Arachis hypogaea* and *Glycine max*.

Treatments	Seed germination (%)	No of leaf (cm)	Height (cm <sup>2</sup> )	leaf area	No of pods/plant	No of seeds/plant
<i>Arachis hypogaea</i>	0 ppm	100 <sup>a</sup>	72.0 <sup>a</sup>	36.7 <sup>a</sup>	7.6 <sup>a</sup>	2.7 <sup>a</sup>
	10 ppm	90 <sup>b</sup>	55.0 <sup>b</sup>	24.0 <sup>b</sup>	5.5 <sup>b</sup>	1.9 <sup>b</sup>
	20 ppm	80 <sup>c</sup>	50.0 <sup>c</sup>	22.8 <sup>c</sup>	5.1 <sup>c</sup>	1.7 <sup>c</sup>
	30 ppm	70 <sup>d</sup>	36.7 <sup>d</sup>	20.5 <sup>d</sup>	4.6 <sup>d</sup>	1.3 <sup>d</sup>
	40 ppm	65 <sup>e</sup>	31.3 <sup>e</sup>	19.8 <sup>d</sup>	3.8 <sup>d</sup>	1.0 <sup>d</sup>
<i>Glycine max</i>	00 ppm	100 <sup>a</sup>	35.7 <sup>a</sup>	58.7 <sup>a</sup>	13.5 <sup>a</sup>	6.7 <sup>a</sup>
	10 ppm	90 <sup>b</sup>	23.0 <sup>b</sup>	42.0	9.0 <sup>b</sup>	5.3 <sup>b</sup>
	20 ppm	75 <sup>c</sup>	20.7 <sup>c</sup>	40.5 <sup>c</sup>	7.1 <sup>c</sup>	4.0 <sup>c</sup>
	30 ppm	70 <sup>c</sup>	16.3 <sup>d</sup>	36.2 <sup>d</sup>	6.5 <sup>c</sup>	3.0 <sup>d</sup>
	40 ppm	60 <sup>d</sup>	15.0 <sup>d</sup>	31.7 <sup>e</sup>	5.0 <sup>d</sup>	2.0 <sup>e</sup>

Values with the same superscript in the same column are not significantly different at P<0.05 level.

**Table-3:** Effects of cadmium and lead on filling potentials of *Arachis hypogaea* and *Glycine max*.

Plants	Concentrations				
	0 ppm	10 ppm	20 ppm	30 ppm	40 ppm
<i>A. hypogaea</i>	Cd	0.95 <sup>a</sup>	0.60 <sup>b</sup>	0.42 <sup>c</sup>	0.40 <sup>c</sup>
	Pb	0.95 <sup>a</sup>	0.60 <sup>b</sup>	0.48 <sup>c</sup>	0.41 <sup>d</sup>
<i>G. max</i>	Cd	0.80 <sup>a</sup>	0.48 <sup>b</sup>	0.48 <sup>b</sup>	0.37 <sup>c</sup>
	Pb	0.80 <sup>a</sup>	0.51 <sup>b</sup>	0.50 <sup>b</sup>	0.43 <sup>c</sup>

Values with the same superscript in the same row are not significantly different at p<0.05 level.

Note: Filling potential =  $\frac{\text{No of seed in a pod}}{\text{Length of the pod}}$

**Table-4:** Accumulation of cadmium and lead in the seeds of *Arachis hypogaea*, *Glycine max* and the spikeed soils.

Plants	Heavy metals	Concentrations				
		0ppm	10ppm	20ppm	30ppm	40ppm
<i>A. hypogaea</i>	Cd	0.0 <sup>d</sup>	1.6 <sup>d</sup>	5.9 <sup>c</sup>	15.7 <sup>b</sup>	22.0 <sup>a</sup>
	Pb	0.0 <sup>d</sup>	1.8 <sup>d</sup>	9.0 <sup>c</sup>	16.0 <sup>b</sup>	23.6 <sup>a</sup>
<i>G. max</i>	Cd	0.0 <sup>d</sup>	1.7 <sup>d</sup>	5.7 <sup>c</sup>	14.3 <sup>b</sup>	21.1 <sup>a</sup>
	Pb	0.0 <sup>d</sup>	2.1 <sup>d</sup>	7.4 <sup>c</sup>	16.0 <sup>b</sup>	22.4 <sup>a</sup>
Soil samples	Cd	0.1 <sup>e</sup>	7.1 <sup>d</sup>	16.8 <sup>c</sup>	28.5 <sup>b</sup>	38.7 <sup>a</sup>
	Pb	0.3 <sup>e</sup>	7.7 <sup>d</sup>	18.0 <sup>c</sup>	28.0 <sup>b</sup>	35.5 <sup>a</sup>

Values with the same superscript in the same column are not significantly different at P<0.05 level