

# Effect of pharmaceutical effluent on the growth of crops in Nigeria

## Abstract

Environmental pollution constitutes a great health hazard to human, animals and plants with local, regional and global implications. Pollution has adverse effects on land, water and its biotic and abiotic components. Effluents from industries are normally considered as the main industrial pollutants containing organic and inorganic compounds.

This experiment was conducted under laboratory condition to investigate the effect of different heavy metals in pharmaceutical effluent on germination and growth of okro (*Abelmoschus esculentus*) and tomato (*Lycopersicon esculentum*) seed. The effect of these effluents was compared with control water. The soil on which the plants were grown was analyzed. A control sample watered with de-ionized water was also analyzed. The plant samples were divided into stem, root and leaf prior to digestion and analyzed. The soil and plant samples were digested by wet-oxidation technique and analyzed for heavy metals by atomic absorption spectrophotometer (AAS). Lead, Cadmium, Chromium, Zinc, Copper, Nickel and Iron concentrations were found in tomato (*Lycopersicon esculentum*) and okro (*Abelmoschus esculentus*) plants watered with different concentrations of pharmaceutical effluent. The results stated that the industrial effluents significantly affect germination; root, stem and shoot elongation of the investigated crops with highest concentration found in the root of the investigated plants when compared to the stem and leaf. Hence, it can be concluded that effluents from pharmaceutical companies is toxic to life.

## Introduction

Pollution of the biosphere by heavy metals due to industrial, agricultural and domestic activities has been considered to be a global problem owing to its serious effects on all forms of life and exposed materials [1, 2]. Pollution of the land occurs from various degradable and non degradable materials. These materials may be solid waste, trash or chemicals. Heavy

metal pollution serves as a great threat to the biosphere due to the fact that they cannot be degraded, rather they persist and are accumulated, hence pose severe effects on humans, animals and plants. They can cause adverse toxic effects on the plants growing in the affected area leading to a decrease in agricultural productivity. Moreover, due to high cost and scarcity of chemical fertilizers, the land disposal of agricultural, municipal and industrial waste is widely practiced as a major and economic source of nutrients and organic matter for growing cereal crops by poor farmers in Pakistan [3, 4]. The use of waste water in irrigation system definitely provide some nutrients to enhance the fertility of soil, it also deposits toxicants that change soil properties in the long run. This necessitates a detailed scientific study before any specific waste can be used for irrigation for a particular crop and environmental conditions. Different crop species may have different tolerance to various pollutants. Seed germination and plant growth bioassays are the most common techniques used to evaluate phytotoxicity [5]. Present study was designed to assess the impact of heavy metals in pharmaceutical effluent on germination and growth of okra (*Abelmoschus esculentus*) and tomato (*Lycopersicon esculentum*).

## Materials and Methods

The soil on which the studied plant was grown was taken from egbejila village, airport road, Ilorin with co-ordinates latitude 08°25.535' N and longitude 004°29.885'. The soil samples were air dried to remove moisture content. After drying, the samples were crushed with a clean, dry mortar and pestle then sieved through a 2-mm sieve to fineness. Three ( replicates) of tomato, okra and pepper were grown in small buckets and watered with ordinary water for 5 weeks before wetting with different concentration (0%, 1%, 5%, 10%, 20%, 30%, 40%) of untreated effluent gotten from a pharmaceutical company. The vegetables were planted between December, 2014 and March, 2015. The different plant samples were harvested and sliced into chips using knife rinsed with nitric acid and air dried for 3-4 days. The samples were ground and sieved with a 2mm sieve prior to digestion [6].

## Digestion of soil and plant samples

1g of sieved soil samples were weighed into digestion flask. 10ml of 1:1 HNO<sub>3</sub> was added to the digest. The sample was heated to 95<sup>0</sup>C ± 5<sup>0</sup>C and refluxed for 10-15 minutes without boiling. The sample was allowed to cool and 5mL of concentrated HNO<sub>3</sub> was added and refluxed for another 30 minutes. The step was repeated by addition of 5ml of conc. HNO<sub>3</sub>. The solution was evaporated to approximately 5ml without boiling by heating for two hours,

the sample was cooled, 2ml of water and 3ml of 30% H<sub>2</sub>O<sub>2</sub> were added. The vessel was covered with a watch glass and the peroxide reaction was initiated. 1-ml of 30% H<sub>2</sub>O<sub>2</sub> was continuously added with warming until the effervescence was minimal. The sample was covered with a ribbed watch glass device and the acid peroxide digestate continued until the volume was reduced to approximately 5ml. After cooling, the digestate was diluted to 100ml with water. 10ml conc. HCl was added to the sample digest and covered with a watch glass. The sample was placed on the heating source and refluxed at 95<sup>0</sup>C ± 5<sup>0</sup>C for 15 minutes. The digestate was filtered through Whatman No. 41 filter paper and the filtrate collected in a 100-ml volumetric flask, made to volume before analyzing by FLAA [7].

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## 75 Results and Discussion

The mean concentrations of heavy metals (chromium, cadmium, iron, copper, nickel, lead and zinc) in *Abelmoschus esculentus* stem, leaf and root are presented in tables 1 to 3. The samples gave a range concentration value of the 7 elements. The concentrations of the heavy metals in the various samples from the sites were detected using Atomic Absorption Spectrophotometer.

81 **Table 1:** Mean Concentration of heavy metals in *Abelmoschus esculentus* Stem

Sample	Concentration (Mean±SD)						
	Zn(mg/kg)	Cu(mg/kg)	Fe(mg/kg)	Cr(mg/kg)	Ni(mg/kg)	Pb(mg/kg)	Cd(mg/kg)
5% OS	16.50±0.13	26.25±0.25	201.00±0.04	3.33±0.31	11.00±0.31	7.50±0.13	2.88±0.13
10% OS	32.88±0.34	54.50±0.34	319.50±0.34	8.00±0.13	10.75±0.55	9.25±0.13	3.63±0.34
20% OS	52.88±14.33	76.38±0.34	393.50±0.21	8.63±0.13	13.02±0.34	11.25±0.25	3.88±0.34
30% OS	69.38±0.125	84.38±0.125	578.88±0.13	10.38±0.34	14.50±0.13	12.63±0.13	5.00±0.13

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83 **Table 2:** Mean Concentration of heavy metals in *Abelmoschus esculentus* Leaf

Sample (Mean±SD)	Concentration						
	Zn(mg/kg)	Cu(mg/kg)	Fe(mg/kg)	Cr(mg/kg)	Ni(mg/kg)	Pb(mg/kg)	Cd(mg/kg)
5% OL	105.75±0.34	133.25±0.21	325.63±0.13	3.88±0.33	13.75±0.44	11.50±0.21	6.38±0.13
10% OL	163±0.45	138.75±0.13	477.63±0.13	8.50±0.13	14.75±0.34	12.75±0.34	7.87±0.45
20% OL	238.50±0.45	254.25±0.74	545.25±0.31	8.77±0.13	15.75±0.34	13±0.25	8.58±0.15
30% OL	376.75±0.21	355.00±0.76	851.75±0.25	9.13±0.34	26.25±0.25	13.75±0.13	9.13±0.34

84 **Table 3:** Mean Concentration of heavy metal in *Abelmoschus esculentus* Root

Sample Concentration (Mean±SD)							
	Zn(mg/kg)	Cu(mg/kg)	Fe(mg/kg)	Cr(mg/kg)	Ni(mg/kg)	Pb(mg/kg)	Cd(mg/kg)
5% OR 13	112.63±0. 13	120.50±0.1 2	314.75±0. 10	4.13±0.21	15.21±1.55	12.25±0.25	7.75±0.34
10% OR	162.75±0. 34	205.5±0.13	343.25±0. 13	8.5±0.21	15±0.25	13±0.34	8.5±0.21
20% OR	251.38±0. 13	196±0.13	701.25±0. 13	9.5±0.13	16±0.13	13.75±0.13	8.875±0.3 4
30% OR	451.25±0. 21	492.75±0.1 3	917.75±0. 21	10.25±0.2 6	17±0.26	14.458±0.2 6	9.75±0.25

86 Generally, it is observed that the concentration of all the metals was increasing with  
 87 increasing concentration of untreated pharmaceutical effluent (table 1-3). Considering the  
 88 control plant (table 4), the concentration of heavy metals was significantly low.

90 **Table 4:** Mean Concentration of heavy metal in control plant.

Sample	Concentration(Mean±SD)						
	Zn(mg/kg)	Cu(mg/kg)	Fe(mg/kg)	Cr(mg/kg)	Ni(mg/kg)	Pb(mg/kg)	Cd(mg/kg)
0%BR	16.25±0.13	23.50±0.13	182.88±0.13	2.63±0.13	10.75±0.25	2.63±0.21	2.25±0.13

91 The use of many plants for food is often limited by the composition of heavy metals in them  
 92 as they pose dangerous effects in both man and animals [8]. Heavy metals are environmental  
 93 pollutants, and their toxicity is a problem of increasing significance for ecological,  
 94 nutritional, evolutionary, and environmental reasons. The term “heavy metal” refers to any  
 95 metallic element which has a relatively high specific gravity (typically five times heavier than  
 96 water) and is often toxic or poisonous even at low concentrations. This group of heavy metals  
 97 includes lead (Pb), cadmium (Cd), nickel (Ni), cobalt (Co), iron (Fe), zinc (Zn), chromium  
 98 (Cr), arsenic (As), silver (Ag), and the platinum group elements [9]. Some of the heavy  
 99 metals (Fe, Cu, and Zn) are known to be essential for plants and animals [10]. Other heavy  
 100 metals such as Cu, Zn, Fe, Mn, Mo, Ni, and Co are essential micronutrients [11], excess  
 101 uptake of which by plants results in toxic effects [12, 13].

102 Cu is an essential micronutrient, exposure to excess Cu has a detrimental effect on plant  
 103 growth. In the table above, the concentration of Cu was high in the root compared to the stem  
 104 and leaf of *Abelmoschus esculentus*. Marschner [14] reported that Cu tends to accumulate in  
 105 the root tissue of plants and simultaneously translocate to the shoots. The concentration of Cu  
 106 in the root tissue of *Abelmoschus esculentus* ranges between 120.5 to 492.75 mg/kg which is  
 107 far above the range of 80-100 mg/kg suggested by Marschner [14] as a general critical  
 108 concentration for Cu toxicity. The effect of Cu toxicity on root morphology is similar to that  
 109 of chromium toxicity [15]. Both affect root proliferation and reduce root hair formation and  
 110 hence, affect nodulation. Similarly, iron is an essential micronutrient and the third most  
 111 limiting nutrient for plant growth and metabolism, primarily due to the low solubility of the  
 112 oxidized ferric form in aerobic environments [16, 17]. The concentration of iron in  
 113 *Abelmoschus esculentus* was generally high in the root compared to the stem and leaf and

consequently above optimal level [18]. Iron toxicity promote the formation of reactive oxygen-based radicals, which are able to damage vital cellular constituents (e.g., membranes) by lipid peroxidation which are often characterized by bronzing (coalesced tissue necrosis), acidity, and/or blackening of the roots [19].

Tables 5 to 7 presents the mean concentrations of heavy metals (chromium, cadmium, iron, copper, nickel, lead and zinc) in *Lycopersicon esculentum* stem, leaf and root.

Table 5: Mean Concentration of heavy metal in *Lycopersicon esculentum* stem

Sample							
Concentration (Mean±SD)							
	Zn(mg/kg)	Cu(mg/kg)	Fe(mg/kg)	Cr(mg/kg)	Ni(mg/kg)	Pb(mg/kg)	Cd(mg/kg)
5% TS	8.50±0.13	21.75±0.13	175.13±0.21	2.25±0.25	9.75±0.13	8.38±0.13	2.38±0.13
10% TS	67.13±0.21	46±0.13	279.50±0.25	5.25±0.21	12.92±0.53	10.13±0.13	2.63±0.13
20% TS	35.25±0.21	63.88±0.13	367.50±0.13	6.00±0.21	13.63±0.21	11.88±0.33	3.63±0.13
30% TS	39.25±0.50	70.25±0.13	455.96±0.38	7.00±0.34	14±0.25	13.29±0.26	3.88±0.21

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Table 6: Mean Concentration of heavy metal in *Lycopersicon esculentum* leaf

Sample							
Concentration (Mean±SD)							
	Zn(mg/kg)	Cu(mg/kg)	Fe(mg/kg)	Cr(mg/kg)	Ni(mg/kg)	Pb(mg/kg)	Cd(mg/kg)
5% TL	112.63±0.13	101.38±0.19	147.75±0.21	3.75±0.13	12.50±0.25	12.25±0.13	5.25±0.34
10% TL	155.38±0.19	127.63±0.21	251.25±0.13	4.88±0.45	13.88±0.13	12.88±0.34	6±0.13

20%	225.5±0.21	81±0.13	362.63±64.84	5.25±0.25	14.25±0.21	12.75±0.34	7.75±0.21
TL							
30%	360.25±0.03	298.25±0.21	418.25±0.13	6.63±0.65	14.88±0.13	13.88±0.13	8.25±0.13
TL							

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124 Table 7: Mean Concentration of heavy metal in *Lycopersicon esculentum* root

Sample							
Concentration (Mean±SD)							
	Zn(mg/kg)	Cu(mg/kg)	Fe(mg/kg)	Cr(mg/kg)	Ni(mg/kg)	Pb(mg/kg)	Cd(mg/kg)
5%	113.13±0.21	102.00±0.13	148.50±0.21	3.88±0.13	12.63±0.21	12.63±0.13	5.38±0.13
TR							
10%	157.88±0.13	129.50±0.21	262.63±0.13	4.75±0.25	14.25±0.44	14.58±0.19	6.13±0.34
TR							
20%	220.25±0.38	251.75±0.58	252.42±0.26	5.63±0.13	14.75±0.13	14.38±0.21	7±0.13
TR							
30%	363.75±0.34	298.25±0.13	455.50±0.13	7.25±0.21	15.13±4.30	14.75±0.25	8.63±0.13
TR							

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126 From the tables (5-7) above, it is observed that the concentration of the metals increased with  
127 increasing concentration of the effluents in the entire samples (stem, leaf and root) with the  
128 root being highest. All the investigated heavy metals are significantly higher than the  
129 tolerable values for plants [18].

130 Lead is a major environmental pollutant of world-wide concern that accumulates in soils [20].  
131 It is known to exert its toxic effect on plants by causing a rapid inhibition of root growth,  
132 probably due to the inhibition of cell division in the root tip [21]. It has been reported to have  
133 similar mechanism in several plants species, including *Triticum aestivum* [22, 23], *Z. mays* L.  
134 [24], *Pisum sativum* [25]. This effect on root growth has been shown to be similar to that of  
135 nickel toxicity [26, 27].

## Conclusion

From the present study it could be concluded that the effluent from pharmaceutical companies are toxic to life and consequently affect the growth of plants. All the studied metals are significantly higher in concentration compared to the control group and as well higher than the permissible limit by world health organization. The increase in the concentrations of these elements from the control might be as a result of their presence in soil and pharmaceutical effluent as well as the plant itself.

## References

1. Igwe, J.C., Nnorom, I.C. and Gbaruko. B.C.G. (2005). Kinetics of radio nuclides and heavy metals behaviour in soils: Implications for plant growth. *Afr. J. Biotechnol.* 4: 1541-1547.
2. Srivatava, R., Kumar, D. and Gupta, S.K. (2005). Municipal sludge-induced phytotoxicity. *ALTLA*. 33: 501-508
3. Younas, M. and Shahzad, F. (1998). Assessment of Cd, Ni, Cu and Pb pollution in Lahore. *Pakistan Environ. Intern.* 24: 761-766
4. Jamal, A., Ayub, N., Usman, M. and Khan. A.G. (2002). Arbuscular mycorrhizal fungi enhance Zn and Ni uptake from contaminated soil by soybean and lentil. *Int. J. Phytorem.* 4: 205-221.
5. Kapanen, A. and Itawara. M. (2001). Ecotoxicity tests for compost application. *Ecotox. Environ. Safety.*, 49: 1-16.
6. Eaton, A.D., Clesceri, L.S., Rice, E.W., Greenberg, A.E. and Franson, M.A. (1998). *Standard Methods for the Examination of Water and Wastewater*. American public health Association, 20<sup>th</sup> edition. 22-29.
7. Nawaz, S., Ali, S.M., and Yasmin, A. (2006). Effect of industrial effluents on seed germination and early growth of *Cicer arietum*. *J. Biosci.*, 6: 49-54.
8. Kubmarawa, D., Andenyand, I.F.H. and Magomya, A.M. (2008). Amino acid of two non conventional leafy vegetables: *Sesamum* and *Balanitesaegyptical*. *Afr. J. Biotechnol.* 7(19): 3502-3504.
9. Farlex, I. (2005). *Definition: Environment*, the Free Dictionary. Farlex Inc. Publishing, USA. 12-15.



10. Wintz, H., Fox, T. and Vulpe, C. (2002). Responses of plants to iron, zinc and copper deficiencies. *Biochem. Soc. Trans.*, 30: 766-768.
11. Reeves, R. D. and Baker, A.J.M. (2000). Metal-accumulating plants. In: *Phytoremediation of Toxic Metals: Using Plants to Clean Up the Environment*. 193-229. Wiley. New York.
12. Blaylock, M.J. and Huang, J.W. (2000). Phytoextraction of metals. In: *Phytoremediation of Toxic Metals Using Plants to Clean Up the Environment*. 53-70. New York.
13. Monni, S., Salemma, M. and Millar, N. (2000). The tolerance of *Empetrum nigrum* to copper and nickel. *Environ. Pollut.*, 109: 221-229.
14. Marschner, H. (1995). 'Mineral nutrition of higher plants.' (Academic Press: London).
15. Hecht-Buchholz, C.H., Brady, D. J., Asher, C. J. and Edwards, D. G. (1990). Effects of low activities of aluminium on soybean (*Glycine max*). II. Root cell structure and root hair development. Plant nutrition - physiology and applications. M. L. van Beusichem. Dordrecht, Kluwer Academic Publishers: 335-343.
16. Zuo, Y. and Zhang, F. (2011). Soil and crop management strategies to prevent iron deficiency in crops. *Plant Soil*. 339: 83-95.
17. Samaranayake, P., Peiris, B.D. and Dissanayake, S. (2012). Effect of excessive ferrous ( $\text{Fe}^{2+}$ ) on growth and iron content in rice (*Oryza sativa*). *Int. J. Agri. Biol.*, 14: 296-298.
18. Laan, P., Smolders, A. J. P. and Blom, C.W. P. M. (1991). The relative importance of anaerobiosis and high iron levels in flood tolerance of *Rumex* species. *Plant Soil*. 136:153-161.
19. Cesco, S., Neumann, G., Tomasi, N., Pinton, R. and Weiskopf, L. (2010). Release of plant borne flavonoids into the rhizosphere and their role in plant nutrition. *Plant Soil*. 329: 1-25.
20. Traunfeld, J. H. and Clement, D. L. (2001). Lead in Garden Soils. Home and Garden. College Park, MD: Maryland Cooperative Extension, University of Maryland.
21. Eun, S. O., Youn, H. S. and Lee, Y. (2000). Lead disturbs microtubule organization in the root meristem of *Zea mays*. *Physiol. Plant*. 110:357-365.
22. Dey, S. K., Dey, J., Patra, S. and Pothal, D. (2007). Changes in the antioxidative enzyme activities and lipid peroxidation in wheat seedlings exposed to cadmium and lead stress. *Braz. J. Plant Physiol.* 19 53-6010.

- 202 23. Kaur, G., Singh, H. P., Batish, D. R. and Kohli, R. K. (2013). Lead (Pb)-induced  
203 biochemical and ultrastructural changes in wheat (*Triticum aestivum*)  
204 roots. *Protoplasma* 1:53-62.
- 205 24. Kozhevnikova, A. D., Seregin, I. V., Bystrova, E. I., Belyaeva, A. I., Kataeva, M. N.  
206 and Ivanov, V. B. (2009). The effects of lead, nickel, and strontium nitrates on cell  
207 division and elongation in maize roots. *Russ. J. Plant Physiol.* 56:242–250.
- 208 25. Malecka, A., Piechalak, A. and Tomaszewska, B. (2009). Reactive oxygen species  
209 production and antioxidative defense system in pea root tissues treated with lead  
210 ions: the whole roots level. *Acta Physiol. Plant.* 31:1053–1063.
- 211 26. Munzuroglu, O. and Geckil, H. (2002). Effects of metals on seed germination, root  
212 elongation, and coleoptile and hypocotyl growth in *Triticum aestivum* and *Cucumis*  
213 *sativus*. *Arch. Environ. Contam. Toxicol.* 43:203–213.
- 214 27. Verma, S. and Dubey, R. S. (2003). Lead toxicity induces lipid peroxidation and  
215 alters the activities of antioxidant enzymes in growing rice plants. *Plant Sci.* 164:645-  
216 655.