

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 (*abelmoschusesculentus*) and tomato (*lycopersiconesculentum*) 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 by 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 by 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.

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

32 degraded, rather they persist and are accumulated, hence pose severe effects on humans,
33 animals and plants. They can cause adverse toxic effects on the plants growing in the affected
34 area leading to a decrease in agricultural productivity. Another point of view is that due to
35 high cost and scarcity of chemical fertilizers, the land disposal of agricultural, municipal and
36 industrial waste is widely practiced as a major and economic source of nutrients and organic
37 matter for growing cereal crops by poor farmers in Pakistan [3, 4]. The use of such waste
38 water in irrigation system definitely provide some nutrients to enhance the fertility of soil but
39 also deposit toxicants that change soil properties in the long run. This necessitates a detailed
40 scientific study before any specific waste can be used for irrigation for a particular crop and
41 environmental conditions. Since different crop species may have different tolerance to
42 various pollutants. Seed germination and plant growth bioassays are the most common
43 techniques used to evaluate phytotoxicity [5]. Present study was designed for the assessment
44 and impact of heavy metals in pharmaceutical effluent on germination and growth of okro
45 (*Abelmoschus esculentus*) and tomato (*Lycopersicon esculentum*).

46 **Materials and Methods**

47 Effluent samples were collected from a pharmaceutical company in Ilorin, Kwara State. They
48 were subjected to various physico-chemical tests using the standard methods of Eaton et al.,
49 [6]. For the germination experiments, certified seeds of *Abelmoschus esculentus* and
50 *Lycopersicon esculentum* were obtained from NARC, Islamabad. Experimental setup was the
51 same as described by Nawaz et al. [7]. Healthy and equally sized seeds of *Abelmoschus*
52 *esculentus* and *Lycopersicon esculentum* were sterilized with 0.1% HNO₃. After repeated
53 washings with sterilized distilled water, seeds were soaked in the same water for 4hrs. Then
54 20 sterilized seeds were arranged in sterilized Petri dishes, lined with double layer of
55 Wattman filter paper No 1. Plates were labeled as per type, concentration of the effluent and
56 variety of lentil. Different concentrations (0, 1, 5, 10, 20, 30, and 40%) of each effluent were
57 made with distilled water. Respective effluent concentration were provided and incubated for
58 three days at 26 ±2°C for germination. Daily observations were made for the germinated
59 seeds. On third day they were shifted to light (10kLux) for next seven days. Before shifting,
60 10 ml of nutrient solution was provided with the same concentration of effluents. After seven
61 days, seedlings were harvested; root, shoot & seedling lengths and fresh weights were
62 recorded. For dry weights seeds were incubated at 60°C for 24 hrs. Data was subjected to
63 statistical analysis.

64

65 Results and Discussion

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

71 **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	16.5±0.125	26.25±0.25	201±0.036	3.33±0.313	11±0.313	7.5±0.125	2.875±0.125
10% OS	32.88±0.338	54.5±0.338	319.5±0.338	8±0.125	10.75±0.55	9.25±0.125	3.625±0.338
20% OS	52.88±14.325	76.38±0.338	393.5±0.213	8.625±0.125	13.02±0.338	11.25±0.25	3.875±0.338
30% OS	69.38±0.125	84.38±0.125	578.875±0.125	10.375±0.338	14.5±0.125	12.625±0.125	5±0.125

72

73 **Table 2:** Mean Concentration of heavy metals in *Abelmoschus esculentus* 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% OL	105.75±0.338	133.25±0.213	325.625±0.125	3.875±0.338	13.75±0.438	11.5±0.213	6.375±0.125
10% OL	163±0.45	138.75±0.125	477.625±0.125	8.5±0.125	14.75±0.338	12.75±0.338	7.875±0.45

20% 238.5±0.45 254.25±0.738 545.25±0.313 8.77±0.125 15.75±0.338 13±0.25 8.583±0.15

OL

30% 376.75±0.213 355±0.763 851.75±0.25 9.125±0.338 26.25±0.25 13.75±0.125 9.125±0.338

OL

74 **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	112.63±0.13	120.5±0.12	314.75±0.12	4.125±0.2	15.21±1.55	12.25±0.25	7.75±0.33
10% OR	162.75±0.338	205.5±0.12	343.25±0.125	8.5±0.213	15±0.25	13±0.338	8.5±0.213
20% OR	251.38±0.125	196±0.125	701.25±0.125	9.5±0.125	16±0.125	13.75±0.12	8.875±0.3
30% OR	451.25±0.213	492.75±0.1	917.75±0.213	10.25±0.2	17±0.263	14.458±0.2	9.75±0.25

75

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

79

80 **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±0.125	23.5±0.125	182.875±0.125	2.625±0.125	10.75±0.25	2.625±0.213	2.25±0.125

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

92 Cu is an essential micronutrient, exposure to excess Cu has a detrimental effect on plant
 93 growth. In the **table above**, the concentration of Cu was high in the root compared to the stem
 94 and leaf of *Abelmoschus esculentus*. Marschner [14] reported that Cu tends to accumulate in
 95 the root tissue of plants and simultaneously translocate to the shoots. The concentration of Cu
 96 in the root tissue of *Abelmoschus esculentus* ranges between 120.5 to 492.75 mg/kg which is
 97 far above the range of 80-100 mg/kg suggested by Marschner [14] as a general critical
 98 concentration for Cu toxicity. The effect of Cu toxicity on root morphology is similar to that
 99 of chromium toxicity [15]. Both affect root proliferation and reduce root hair formation and
 100 hence, affect nodulation. Similarly, iron is an essential micronutrient and the third most
 101 limiting nutrient for plant growth and metabolism, primarily due to the low solubility of the
 102 oxidized ferric form in aerobic environments [16, 17]. The concentration of iron in
 103 *Abelmoschus esculentus* was generally high in the root compared to the stem and leaf and
 104 consequently above optimal level [18]. Iron toxicity promote the formation of reactive
 105 oxygen-based radicals, which are able to damage vital cellular constituents (e.g., membranes)
 106 by lipid peroxidation which are often characterized by bronzing (coalesced tissue necrosis),
 107 acidity, and/or blackening of the roots [19].

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

110 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	8.5±0.125	21.75±0.125	175.125±0.213	2.25±0.25	9.75±0.125	8.375±0.125	2.375±0.125
10% TS	67.13±0.213	46±0.125	279.5±0.25	5.25±0.213	12.92±0.525	10.125±0.125	2.625±0.125
20% TS	35.25±0.213	63.88±0.125	367.5±0.125	6±0.213	13.63±0.213	11.875±0.325	3.625±0.125
30% TS	39.25±0.5	70.25±0.125	455.96±0.375	7±0.338	14±0.25	13.292±0.263	3.875±0.213

111

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

Sample	Zn(mg/kg)	Cu(mg/kg)	Fe(mg/kg)	Cr(mg/kg)	Ni(mg/kg)	Pb(mg/kg)	Cd(mg/kg)
Concentration (Mean±SD)							
5% TL	112.63±0.125	101.38±0.188	147.75±0.213	3.75±0.125	12.5±0.25	12.25±0.125	5.25±0.338
10% TL	155.38±0.188	127.63±0.213	251.25±0.125	4.875±0.45	13.88±0.125	12.875±0.338	6±0.125
20% TL	225.5±0.213	81±0.125	362.625±64.84	5.25±0.25	14.25±0.213	12.75±0.338	7.75±0.213
30% TL	360.25±0.025	298.25±0.213	418.25±0.125	6.625±0.65	14.88±0.125	13.875±0.125	8.25±0.125

113

114 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% TR	113.13±0.213	102±0.125	148.5±0.213	3.875±0.125	12.63±0.213	12.625±0.125	5.375±0.125
10% TR	157.875±0.125	129.5±0.213	262.625±0.125	4.75±0.25	14.25±0.438	14.58±0.188	6.125±0.34
20% TR	220.25±0.375	251.75±0.575	252.417±0.263	5.625±0.125	14.75±0.125	14.375±0.213	7±0.125
30% TR	363.75±0.338	298.25±0.125	455.5±0.125	7.25±0.21	15.13±254.3	14.75±0.25	8.625±0.125

115

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

120 Lead is a major environmental pollutant of world-wide concern that accumulates in soils [20].
 121 It is known to exert its toxic effect on plants by causing a rapid inhibition of root growth,
 122 probably due to the inhibition of cell division in the root tip [21]. It has been reported to have
 123 similar mechanism in several plants species, including *Triticum aestivum* [22, 23], *Z. mays* L.
 124 [24], *Pisum sativum* [25]. This effect on root growth has been shown to be similar to that of
 125 nickel toxicity [26, 27].

126 Conclusion

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

133

134

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). Muncipal 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, 20th edition.
7. Nawaz, S., Ali, S.M., and Yasmin, A. (2006). Effect of industrial effluents on seed germination and early growth of *Cicer arientum*. *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.
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* (Raskin I, Ensley BD, eds.). 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* (Raskin I, Ensley BD, eds.). 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 (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.
23. Kaur, G., Singh, H. P., Batish, D. R. and Kohli, R. K. (2013). Lead (Pb)-induced biochemical and ultrastructural changes in wheat (*Triticum aestivum*) roots. *Protoplasma* 1:53-62.
24. Kozhevnikova, A. D., Seregin, I. V., Bystrova, E. I., Belyaeva, A. I., Kataeva, M. N. and Ivanov, V. B. (2009). The effects of lead, nickel, and strontium nitrates on cell division and elongation in maize roots. *Russ. J. Plant Physiol.* 56:242–250.
25. Malecka, A., Piechalak, A. and Tomaszewska, B. (2009). Reactive oxygen species production and antioxidative defense system in pea root tissues treated with lead ions: the whole roots level. *Acta Physiol. Plant*. 31:1053–1063.

- 201 26. Munzuroglu, O. and Geckil, H. (2002). Effects of metals on seed germination, root
202 elongation, and coleoptile and hypocotyl growth in *Triticum aestivum* and *Cucumis*
203 *sativus*. *Arch. Environ. Contam. Toxicol.* 43:203–213.
- 204 27. Verma, S. and Dubey, R. S. (2003). Lead toxicity induces lipid peroxidation and
205 alters the activities of antioxidant enzymes in growing rice plants. *Plant Sci.* 164:645-
206 655.