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Effect of Paracetamol and Ibuprofen on Morphological Parameters and Chlorophyll Content of *Vigna radiata* (Green Gram)

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Abstract

Seed germination performance is an essential development stage in plants. The present study was designed to assess the effect of Paracetamol (PCM) and Ibuprofen (IBU) on seed germination of *Vigna radiata* (Common name: Green Gram; Moong). The uniformly weighted seeds raised in petriplates treated with increasing concentrations of PCM and IBU (control, 10%, 20%, 40%, 80% and 100%) for seven days. On the seventh day, various growth parameters such as germination%, vigour index (VI), root and shoot length, dry and fresh weight and chlorophyll (Chl) content were evaluated. The presence of pharmaceutical after seven days of cultivation significantly influenced some growth and physiological parameters. The values of germination%, VI, root length, fresh weight and dry weight was found to be more at 80% concentration of PCM however photosynthetic pigments (chlorophyll *a*, chlorophyll *b*) shown a reducing trend with an increase in concentration. On the other hand, in case of IBU highest germination%, vigour index, root length were observed at 40%, shoot length and fresh weight at 80% and chlorophyll a and b pigments at 0% concentration (control), respectively. Therefore, the results indicate that most of the growth parameters of seeds studied was not affected by the introduction of pharmaceuticals under laboratory conditions except the dry weight and Chl content.

Introduction

Pulses and its products are a rich source of vital nutrients like proteins, minerals and vitamins. They grow quickly, generate good earnings for farmers, and contribute to agricultural and environmental sustainability. India is accounting for 22% of world production of pulses. The United Nations Food and Agricultural Organization (UNFAO)

have declared 2016 as the "International Year of Pulses". Pulses play a major role not only in food security but also contribute to increasing soil health by adding organic manure in the soil as well as by fixing atmospheric nitrogen. In India, the per capita availability of pulses has fallen by less than half since independence. The average per capita availability of wheat and rice together has increased appreciably during this period. The requirement for pulses as per physiological norms set by the Indian Council of Medical Research (ICMR) is 43 gms/day/capita. Green Gram is one of the major pulses growing mainly in Andhra Pradesh, Maharashtra and Orissa, provides 1229 Kilo Joule and 22.53 gm protein content per 100g of edible portion (National Institute of Nutrition, 2017). Consumption pattern of green gram in rural India is 3.33 gm/day/person (NSSO, 2002).

Many antibiotics and personal care products (PCPs) used on large scale and are released into the environment as residue or left over products as well as effluents from the various industries. These may affect the growth and quality of crops, cereals, and pulses growing in the fields as well as soil fertility. Pharmaceuticals and PCPs in the environment came to the attention in the late 1990s (Daughton and Ternes, 1999), and since that time research relating to the concentrations, fate, and effects of PCPs has increased significantly. A large amount of pharmacologically active substances are used annually in human and animal medicines for treatment and prevention of illness (Dı´az-Cruz *et al.,* 2003; Sarmah *et al.,* 2006). Antibiotics are specifically designed to treat infections in humans and animals by either directly killing bacteria or inhibiting their growth, to protect the health. Most antibiotics are excreted from the treated body, either unaltered or as metabolites, and some are still in bioactive form (Sarmah *et al.,* 2006). Antibiotics used to treat animals are poorly absorbed in their gut and as much as 90 % of some antibiotics may be excreted (Kumar *et al.,* 2005; Jjemba 2002b) in the environment as manure or by grazing animals.

Paracetamol (PCM) is a common analgesic and antipyretic drug used by human beings. At therapeutic doses, PCM is considered a safe drug. So in most countries, it can be purchased in retail stores as an over-the-counter preparation, and it is currently the most widely used drug worldwide (An *et al.,* 2009). Acetaminophen (paracetamol) is a widely spread analgesic, anti-inflammatory, and antipyretic agent in human medicine and one of the possible problem compounds in water bodies (Schulte-Oehlmann *et al.,* 2007). On the other hand, Ibuprofen (IBU) is a non-steroidal anti-inflammatory drug of great concern for aquatic environments because of its high consumption worldwide and potential ecological impact (Ferrando-Climent *et al.,* 2012). Man-made antibiotics can enter the environment in many ways, from the production of active pharmaceutical ingredients, through the excretion of residues after usage or through discarding, unused medicines. After intake, many pharmaceuticals applied in human medical care are not completely eliminated by human bodies, and more than 50% of the intake is excreted with feces or urine to raw sewage in an unchanged form or slightly transformed (Heberer, 2002). There are also reports of major industrial discharges from India and China that serve much of the world with bulk drugs, along with the Europe and the US (Larsson, 2014). When these antibiotics get into the cultivable land, they could possibly affect growth and development of vegetation as well as soil microbial activity (Jjemba, 2002a &

b). Literature proves that significant amounts of the active compounds can be detected throughout the world at concentrations in the ng/l to µg/l range, even in the best available treatment systems (Kolpin *et al.,* 2002; Schröder *et al.,* 2007). The contamination of water with pharmaceuticals is also of growing concern, as water treatment plants are not able to fully remove these chemicals from drinking water sources, and their effects on aquatic organisms and humans are unknown (Cole, 2014). The main pathway for antibiotics release in the terrestrial environment is through the application of animal manure and biosolids containing excreted antibiotics to agricultural land as fertilizers (Kemper, 2008). Much of the antibiotics accumulate in sludge that subsequently may be spread on farmland with the intent to recycle nutrients. The effects of antibiotics on plants in soils were found different between compounds and between plant species (Jjemba, 2002a; Farkas *et al.,* 2007). For example Tetracyclines increased radish yields, but decreased pinto bean yields (Batchelder, 1982). If antibiotic effects are indeed largely species-specific, effects of concentrations typically found in real (agricultural) environments could be either negative in some species (i.e. antibiotic induced retardation of germination) or neutral. In this case, less sensitive species may experience a competitive advantage, which might trigger changes in species composition in natural communities (Minden *et al.,* 2017). Therefore, in this research, the effect of PCM and IBU on seed germination of a major pulse *Vigna radiata* (Green Gram; Moong) has been studied.

Materials and Methods

The five different concentrations of each Paracetamol and Ibuprofen viz 0% (control), 10%, 20%, 40%, 80% and 100% were used in the study. Healthy seeds of *Vigna radiata* were sterilized with 0.1% $HgCl_2$ for 5 minutes and then were washed thoroughly with distilled water. The seeds were then allowed to germinate for 24 hrs at 28°C. Five healthy and germinated seeds were then transferred to petriplates lined with Whatman filter paper no. 42. Dilution was added in 3 ml quantity to moisten filter paper in each petriplates at the first day of the experiment and then 2 ml of respective dilution was added for consecutive 6 days. Three replicates in each dilution were maintained along with the control for comparison. On the seventh day (Figure 1a, b), various growth parameters and chlorophyll content were evaluated as follows:

Germination percentage: The formula given by Rehman *et al.,* (1998) was used to estimate germination percentage.

Germination % = no. of seeds germinated/ total no. of seeds × 100

Root and shoot length: Length of root and shoot of seedlings were calculated using the standard centimeter (cm) scale.

Vigour index (VI): The formula suggested by Abdul-Baki and Anderson (1973) was used to calculate vigour index as: Vigour index = germination % × (root length +shoot length)*

(* indicate that length of root and shoot is in cm).

Fresh and dry weight: Five seeds of each treatment were weighed in order to determine the fresh weight and then dried in an oven at 80°C for 24 hrs to obtain dry weight. Fresh weight and dry weight were recorded in grams (gm).

Chlorophyll estimation: The estimation of chlorophyll content was done according to the method given by Sadasivam and Manickam (1992). Chlorophyll is extracted in 80% acetone and the absorbance at 663nm and 645nm are read in a digital spectrophotometer. Using the absorption coefficients, the amount of chlorophyll is calculated.

$$\text{Chlorophyll (a) in mg/gm tissue} = 12.7(A_{663}) - 2.69(A_{645}) \times V/1000\times W$$

$$\text{Chlorophyll (b) in mg/gm tissue} = 22.9(A_{645}) - 4.68(A_{663}) \times V/1000\times W$$

$$\text{Total chlorophyll (mg/gm tissue)} = 20.2(A_{645}) + 8.02(A_{663}) \times V/1000\times W$$

Results and Discussions

The effect of different concentrations of PCM and IBU on germination %, vigour index, roots and shoot length, fresh and dry weight, total chlorophyll, chlorophyll a and chlorophyll b in *Vigna radiata* are shown in Table 1 and 2, respectively.

Germination % in untreated seedlings (0% concentration) was 99.04% in both PCM and IBU while the maximum (100%) germination was found in seeds treated with 80 and 100% concentration of PCM. Highest vigour index (2796), root length (13.58 cm), fresh weight (0.41gm) and dry weight (0.017gm) were found at 80% concentration of PCM. Shoot length (15.34cm) was observed maximum at 10% concentration. Maximum content of chl a (0.0106 mg/gm) and chl b (0.0148 mg/gm) were observed in untreated seedlings (control) i.e. treated with distilled water. The maximum reduction in most of the parameters like germination%, vigour index (2269.8) and fresh weight (0.33gm) was found at 20% and dry weight (0.013gm), total chl (0.0179 mg/gm) and chl b (0.0148 mg/gm) at 10% concentration except fresh weight (0.33gm) and chl a (0.009mg/gm) at 100% concentration whereas lowest root length (10.12cm) and shoot length (13.12cm) were found at 40% concentration.

In case of IBU, maximum germination (99.04%) was observed at 0%, 10%, 40% and 100% concentration. Vigour index (3196.02) and root length (18.28 cm) were found maximum at 40% concentration whereas shoot length (16.91cm) and fresh weight (0.80gm) were observed high at 80% concentration. Dry weight (0.029gm), total chl (0.148mg/g), chl a (0.009mg/gm) and chl b (0.157mg/gm) were found maximum in untreated seedlings i.e. at 0% concentration of IBU pharmaceutical. Highest reduction in root length (6.66cm) and shoot length (10.29cm) was observed at 0% concentration. Vigour index (1678.72), fresh weight (0.34gm), dry weight (0.014gm), total chl (0.014mg/gm), chl a (0.005mg/gm) and chl b (0.009mg/gm) content was found minimum at concentration of 100% in IBU. Therefore, not much effect on germination% of seeds was observed despite the highest concentration of antibiotics. However, a decrease in root and shoot lengths occurred at several concentrations (Minden *et al.,* 2017). Also, if compared with the shoot and total length measurements, root length was consistently the

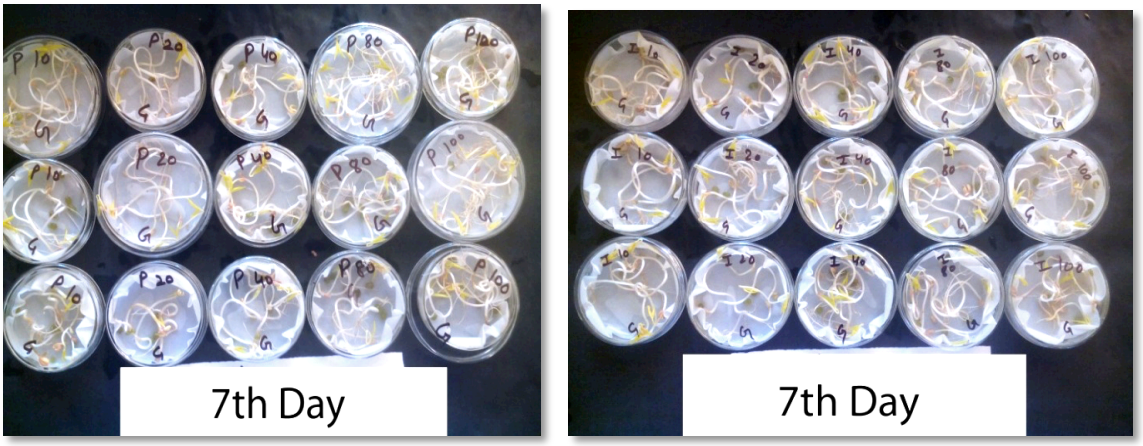
most sensitive point (Hillis *et al.,* 2010). Out of the two antibiotics applied, the maximum concentration of IBU (100%) has affected the seed growth more than PCM.

Table 1 Effect of Paracetamol (PCM) on seed germination of *Vigna radiata* (Green Gram)

Sr. No	Parameters studied	Concentration of PCM (%)					
		0%	10%	20%	40%	80%	100%
1	Germination %	99.04 ±2.52	95.23 ±12.6	95.23 ±12.6	98.09 ±5.4	100 ±0.00	100 ±0.00
2	Vigour Index	2561 ±587.06	2453 ±523.1	2269.8 ±463.05	2324 ±386.02	2796 ±483.6	2539 ±489.06
3	Root length (cm)	11.21 ±2.15	10.62 ±3.18	10.31 ±3.78	10.12 ±4.74	13.58 ±4.02	11.34 ±3.81
4	Shoot length (cm)	14.18 ±3.05	15.34 ±2.68	14.01 ±3.99	13.12 ±2.71	14.37 ±1.81	14.27 ±1.39
5	Fresh Weight (gm)	0.39 ±0.11	0.36 ±0.05	0.33 ±0.14	0.34 ±0.05	0.41 ±0.02	0.33 ±0.06
6	Dry weight. (gm)	0.013 ±0.01	0.008 ±0.001	0.01 ±0.001	0.012 ±0.001	0.017 ±0.002	0.015 ±0.001
7	Total Chl (mg/gm)	0.0254 ±0.005	0.0179 ±0.001	0.0226 ±0.002	0.0233 ±0.002	0.0246 ±0.0031	0.024 ±0.002
8	Chl a (mg/gm)	0.0106 ±0.003	0.00941 ±0.004	0.0100 ±0.003	0.0101 ±0.004	0.0102 ±0.006	0.009 ±0.002
9	Chl b (mg/gm)	0.015 ±0.005	0.0085 ±0.004	0.0126 ±0.003	0.0131 ±0.006	0.0143 ±0.005	0.0148 ±0.006

Table 2 Effect of Ibuprofen (IBU) on seed germination of *Vigna radiata* (Green Gram)

Sr. No	Parameters studied	Concentration of IBU (%)					
		0%	10%	20%	40%	80%	100%
1	Germination %	99.04 ±2.52	99.04 ±2.52	98.09 ±2.52	99.04 ±3.25	98.09 ±5.04	99.04 ±2.52
2	Vigour Index	2714.68 ±208.9	2226.41 ±159.03	3163.4 ±210.3	3196.02 ±126.7	2858.34 ±217.4	1678.72 ±201.2
3	Root length (cm)	6.66 ±1.65	12.69 ±6.79	8.51 ±3.57	18.28 ±2.83	15.9 ±4.14	13.74 ±5.34
4	Shoot length (cm)	10.29 ±3.47	14.72 ±2.89	13.97 ±1.96	14.74 ±1.53	16.91 ±2.78	15.33 ±1.51
5	Fresh Weight (gm)	0.78 ±0.07	0.35 ±0.08	0.36 ±0.03	0.41 ±0.02	0.80 ±0.37	0.34 ±0.07
6	Dry weight. (gm)	0.029 ±0.00	0.013 ±0.00	0.014 ±0.00	0.014 ±0.00	0.019 ±0.00	0.014 ±0.00
7	Total Chl (mg/gm)	0.148 ±0.01	0.026 ±0.00	0.027 ±0.01	0.028 ±0.02	0.017 ±0.01	0.014 ±0.01
8	Chl a (mg/gm)	0.009 ±0.00	0.008 ±0.00	0.009 ±0.01	0.009 ±0.01	0.005 ±0.01	0.005 ±0.01
9	Chl b (mg/gm)	0.157 ±0.00	0.018 ±0.01	0.018 ±0.01	0.018 ±0.01	0.011 ±0.01	0.009 ±0.01



a **b**

Figure 1 (a, b) Growth of seedlings in different concentrations of PCM and IBU in *Vigna radiata* on seventh day *(note: P at left side petriplates means PCM and I at right side petriplates means IBU)*.

The increase in germination percentage might be due to the declining level of toxic metabolites by dilution and better usage of nutrients found in the effluent or pharmaceuticals (Kannan 2001; Kaushik *et al.,* 2005). On the evaluation of seed quality with *Vigna radiata*, it was found that at higher concentration (above 50%) of industrial effluent, the seed germination percentage was retarded but diluted effluent (up to 50%) favored seedling growth (Augusthy and Mani, 2001). According to Ghava *et al.,* (2015) none of the antibiotics caused a significant decrease in seed germination for different types of wheat plant species studied. According to previous studies, *Trigonella foenum-glaceum* (Fenugreek) and *Hordeum vulgare* (Barley) showed the highest seed germination in pharmaceutical industrial and domestic waste waters. It seems that there are some essential organic compounds in waste waters which may reduce some part of negative impacts (Huma *et al.,* 2012). Other researchers also reported that waste water contain some essential organic compound which increase growth of crop (Lubello *et al.,* 2004, Nagajyothi *et al.,* 2009). Suthar *et al.,* (2005) also studied the impact of distillery effluent on seed germination and seedling growth of some plants. They reported that the percent of seed germination in *Vigna radiata* (Moongbean), *Cyamopsis tetragonoloba* (Guar), *Vigna aconitifolia* (Moth bean) and *Trigonella foenum-graecum* (Fenugreek) was found maximum with treatment of 60% ($P<0.001$), 80% ($P<0.005$), 40% ($P<0.0005$) and 100% ($P<0.005$), respectively as compared to control. Similarly, root and shoot length also showed maximum values with the range of 20-40% distillery effluent concentration as compared to control ($P<0.001$).

Nevertheless, increased concentration of distillery effluent significantly inhibited the plant development. Balashouri and Prameeladevi (1994) studied the effect of tannery effluent on germination and growth of selected pulse and cereal crop plants. The study was carried out to assess the impact of tannery effluent on seedling growth of *Vigna radiate* (Moongbean)*, Cajanus cajan* (Pigeon Pea) and *Sorghum bicolor* (Sorghum)

plants under laboratory conditions. The values of germination percentage, seedling growth, chlorophyll content and phytomass accumulation increased over control set with a corresponding increase of effluents concentrations. David *et al.,* 2015 studied various growth parameters in lady's finger and found that parameters such as plumule and radical length and tolerance index of seedlings were minimum at 100% and maximum at 25% of effluent concentration. Therefore, no particular trend has been followed by the seeds in varying concentrations of industrial and pharmaceutical effluents. In the present study also, we have observed that seed growth is favored at high concentration of the antibiotics applied. Hence, it can be concluded that diluted effluent (above 50%) can favor seedling growth.

Correlation matrix

Correlation analysis depicts the relationship between different variables. If the correlation coefficient is nearer to +1 or –1, it shows the perfect linear relationship between the two variables. Parameters showing r>0.7 are considered strongly correlated whereas r between 0.5 and 0.7 shows moderate correlation (Manish *et al.,* 2006). The correlation matrices for germination %, vigour index, root length, shoot length, fresh weight, dry weight, total chlorophyll, chlorophyll a, Chlorophyll b were prepared and illustrated for different concentrations of PCM and IBU in table 3 and 4, respectively.

Table 3 Correlation matrix of parameters at different concentrations of PCM

	Germination %	Vigour index	Root length	Shoot length	Fresh wt	Dry wt	Total chl	Chl a	Chl b
Germination %	1								
Vigour index	.710	1							
Root length	.647	.949**	1						
Shoot length	-.304	.320	.211	1					
Fresh wt	.412	.819*	.773	.253	1				
Dry wt	.937**	.589	.579	-.304	.164	1			
Total chl	.779	.371	.413	-.619	.287	.768	1		
Chl a	.097	.110	.157	-.407	.578	-.116	.489	1	
Chl b	.825*	.376	.409	-.572	.170	.867*	.977**	.291	1
**. Correlation is significant at the 0.01 level (2-tailed).									
*. Correlation is significant at the 0.05 level (2-tailed).									

In seeds treated with PCM, germination % was found highly correlated with vigour index, dry weight, total chl and chl b; Vigour index with root length and fresh weight; root length with fresh weight; dry weight with total chl a and chl b. Total chl showed a very strong correlation with chl b.

Table 4 Correlation matrix of parameters at different concentrations of IBU

	Germination %	Vigour index	Root length	Shoot length	Fresh wt	Dry wt	Total chl	Chl a	Chl b
Germination %	1								
Vigour index	-.489	1							
Root length	.075	.012	1						
Shoot length	-.390	-.126	.772	1					
Fresh wt	-.257	.275	-.167	-.230	1				
Dry wt	.450	.022	-.700	-.970**	.367	1			
Total chl	.320	.134	-.674	-.925**	.568	.967**	1		
Chl a	.196	.582	-.405	-.675	-.160	.507	.471	1	
Chl b	.319	.103	-.675	-.915*	.583	.965**	.999**	.433	1
**. Correlation is significant at the 0.01 level (2-tailed).									
*. Correlation is significant at the 0.05 level (2-tailed).									

Shoot length was in strong correlation with root length in seeds treated with IBU. Shoot length was observed highly correlated with dry weight, total chl and chl b; dry weight with total chl and chl b. A very strong correlation was observed between total chl and chl b.

Conclusion

From the present study it was concluded that the morphological characters like germination%, VI, root length, fresh weight, dry weight were observed maximum at PCM concentration of 80% and 100% while total chl, chl a and b were affected with higher concentration of PCM and favored at lower concentration i.e. 0%. In case of IBU, germination %, VI and root length were found maximum at a concentration of 40% whereas shoot length and fresh weight at 80%, dry weight, total chl, chl a and b at 0% concentration. A particular trend was not observed in the results along the increasing concentration of antibiotics and growth of seeds. However, the different concentrations of antibiotics supported the seedling growth. Many researchers across the world also investigated similar trend from the impact of pharmaceutical effluents from various industries on different crops, depending on the species of crop and the type of effluent used. However, in this study pure antibiotics (of medicinal importance) is used and it was observed that it supported the growth of seeds more than the control. It may be due to the presence of active nutrients in the PCM and IBU, which favored the growth. Therefore, increase in the concentration of antibiotics like paracetamol and ibuprofen, did not affect the germination of seeds and also favors the growth of seedlings. Some morphological parameters like dry weight and the biochemical parameters like chlorophyll content are affected by the higher concentration of antibiotics. Though, it is suggested that introduction of pharmaceuticals in any form should be avoided to be added to soil or water because it will affect the leaf growth and chlorophyll content of the seedlings which is a necessary part for food preparation (photosynthesis) in plants.

Authors' contributions: *Dr. Hardeep Rai Sharma (Assistant Professor), corresponding author, designed the research and the manuscript; and Ms. Anjali Malan (Research Scholar) interpretated the data.*

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References

Abdul-Baki, A.A., Anderson, J.D., 1973. Vigour determination of soyabean seeds by multiple criteria. *Crop Science* 13: 630- 633.

An, J., Zhou, Q., Sun, F., Zhang, L., 2009. Ecotoxicological effects of paracetamol on seed germination and seedling development of wheat (*Triticum aestivum* L.). *Journal of Hazardous Materials* 169: 751–757.

Augusthy, P. O., Mani, A. S., 2001. Effect of rubber factory effluent on the seed germination and seedling growth of *Vigna radiates* L. *Journal of Environmental Biology* 22:137-139.

Balashouri, Prameeladevi. 1994. Effect of tannery effluent on germination and growth of selected pulses and cereal crop plants. *Journal of Ecotoxicology and Environmental Monitoring* 4(2): 115-120.

Batchelder, A.R., 1982. Chlortetracycline and oxytetracycline effects on plant growth and development in soil systems. *Journal of Environmental Quality* 11, 675–678.

Cole, E. 2014. The Effects of Tetracycline and Ibuprofen on Common Duckweed, *Lemna minor L*, *ESSAI*: Vol. 12, Article 13. Online from: http://dc.cod.edu/essai/vol12/iss1/13

Daughton C. G., Ternes, T. A., 1999. Pharmaceuticals and personal care products in the environment: agents of subtle change? *Environmental Health Perspectives* 107: 907–938.

David N.S., Rajan, M.R., 2015. Phytotoxic effect of dyeing industry effluent on seed germination and early growth of Lady's finger. *Journal of Pollution Effects Control* 3: 138.

Dı´az-Cruz, M.S., Lo´ pez de Alda, M.J., Barcelo´, D., 2003. Environmental behavior and analysis of veterinary and human drugs in soils, sediments and sludge. *Trends in Analytical Chemistry* 22 (6): 340–351.

Farkas, M.H., Berry, J.O., Aga, D.S., 2007. Chlortetracycline detoxification in maize via induction of glutathione S-transferases after antibiotic exposure. *Environmental Science and Technology* 41, 1450–1456.

Ferrando-Climent, L., Collado, N., Buttiglieri, G., Gros, M., Rodriguez-Roda, I., Rodriguez-Mozaz, S., Barcelo, D. 2012. Comprehensive study of ibuprofen and its metabolites in activated sludge batch experiments and aquatic environment. *Science of the Total Environment* 438: 404–413.

Ghava, K., Rathod, M. C., Dhale, D.A., 2015. Effect of antibiotics on seed germination and root elongation of wheat. *International Journal of Current Microbiology and Applied Sciences* 4(1): 516-527.

Heberer, T. 2002. Tracking persistent pharmaceutical residues from municipal sewage to drinking water. *Journal of Hydrology* 266: 175-189.

Hillis, D. G., Fletcher, J., Solomon, K. R., Sibley, P. K., 2010. Effects of ten antibiotics on seed germination and root elongation in three plant species. *Archives of Environmental Contamination and Toxicology* 60:220–232

Huma, Z., Naveed, S., Rashid, A., Ullah , A., khattak I., 2012. Effects of domestic and industrial waste water on germination and seedling growth of some plants. *Current Opinion in Agriculture* 1(1), 27–30.

Jjemba, P.K., 2002a. The effect of chloroquine, quinacrine, and metronidazole on both soybean plants and soil microbiota. *Chemosphere* 46, 1019–1025.

Jjemba, P.K., 2002b. The potential impact of veterinary and human therapeutic agents in manure and biosolids on plants grown on arable land: a review. *Agriculture, Ecosystems and Environment* 93, 267–278.

Kannan, J. 2001. Effect of distillery effluent on crop plants. *Advances in Plant Science* 14: 127–132.

Kaushik, P., Garg, V. K., Singh, B., 2005. Effect of textile effluent on growth performance of wheat cultivation. *Bioresource Technology,* 96: 1189-1193.

Kemper, N., 2008. Veterinary antibiotics in the aquatic and terrestrial environment. *Ecological Indicators,* 8: 1–13.

Kolpin, D.W., Furlong, E.T., Meyer, M.T., Thurman, E.M., Zaugg, S.D. et al (2002) Response to comment on, 'Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999–2000: a national reconnaissance'. *Environmental Science and Technology* 36:4007–4008.

Kumar, K., Gupta, S.C., Baidoo, S.K., Chander, Y., Rosen, C.J. 2005. Antibiotic uptake by plants from soil fertilized with animal manure. *Journal of Environmental Quality* 34:2082–2085.

Larsson, D. G. J., 2014. Antibiotics in the environment. *Upsala Journal of Medical Sciences* 119:108–112.

Lubello, C., Gori, R., Paolo, N.F., Ferrini, F., 2004. Municipal-treated waste water reuse for plant nurseries irrigation. *Water Research* 38:2939–2947.

Manish, K., Ramanathan, A., Rao, M.S., Kumar, B. (2006). Identification and evaluation of hydrogeochemical processes in the groundwater environment Of Delhi, India. *Journal of Environmental Geology* 50:1025–1039.

Minden, V., Deloy, A., Volkert, A. M., Leonhardt, S. D., Pufal, G., 2017. Antibiotics impact plant traits, even at small concentrations, *AoB PLANTS* 9 (2).

Nagajyothi, P.C., Dinakar, N., Suresh, S., Udaykiran, Y., Suresh, C., Damodharam, T., 2009. Industrial effluent on the morphological parameters and chlorophyll content of green gram (*Phaseolus aureus* Roxb). *Journal of Environmental Biology* 30, 385–388.

National Institute of Nutrition 2017. *Indian Food Composition Tables 2017.* Indian Council of Medical Research Department of Health Research, Ministry of Health & Family Welfare, Government of India Hyderabad- 500 007, Telangana State, India. Online from: http://www.indiaenvironmentportal.org.in/files/file/IFCT%202017%20Book.pdf

National Sample Survey Organisation (NSSO) 2002. Report No 451. *Level and pattern of consumer expenditure in India, 1999-2000*. New Delhi, India. Online from: mospi.nic.in/sites/default/files/publication_reports/457_final.pdf

Rehman, S., Harris, P.J.C., Bourne, W.F., 1998. Effect of presowing treatment with calcium salts, *Acacia* seeds, *Journal of Plant Nutrition* 21: 277–285.

Sadasivam, S., Manickam, A., 1992. In: Biochemical method for Agricultural sciences Wiley Eastern Ltd, New Delhi, pp. 184–185.

Sarmah, A.K., Meyer, M.T., Boxall, A.B.A., 2006. A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. *Chemosphere* 65: 725–759.

Schroder P. *et al.,* 2007. Using phytoremediation technologies to upgrade waste water treatment in Europe. *Environmental Science and Pollution Research* 14 (7): 490–497.

Schulte-Oehlmann, U., Oehlmann, J., Püttmann, W., 2007. Humanpharmakawirkstoffe in der Umwelt: Einträge, Vorkommen und der Versuch einer Bestandsaufnahme. *Umweltwiss Schadst Forsch* 19(3): 68–179.

Suthar, W. J., Sandhu, M., Rana, S., Kanwal, A., Gupta, D. and Meena, M.S., 2005. Vermicomposting of kitchen waste by using Eisenia foetida. *Asian Journal of Microbiology, Biotechnology and Environmental Sciences* 7: 541-54