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# Toxicity Evaluation of Crop Plants Irrigated with Treated Municipal Wastewater

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## ABSTRACT

In the present investigation, tomato plants irrigated with treated wastewater were evaluated for their toxicity using short term (acute) animal studies. Male wistar rats fed with tomato plants at the dosage of 150 and 300 mg kg$^{-1}$ b.wt. for 14 consecutive days showed no symptoms of toxicity. Assessment of haematological parameters such as RBC, WBC, haemoglobin, platelet count showed no significant changes. Plasma and serum analysis also indicated no significant differences in parameters such as urea, AST, ALT, ALP, bilirubin in all the treatments. The present investigations suggested that short term wastewater irrigation in plants do not pose any toxicity to animals. The toxicity depends upon the level of composition of treated wastewater and its transfer to the soil and then plants. Further studies are required to assess toxic effect of long term irrigation with treated wastewater.

Key words: Acute toxicity, haematology, rats, serum, tomato plants, treated wastewater

## INTRODUCTION

Freshwater scarcity has generated the need for reuse of treated wastewater. Use of domestic and industrial wastewater for irrigation of agricultural and horticultural plants has been practiced in various countries of the world (Angelakis et al., 1999; Lubello et al., 2004; McNeill et al., 2009; Khurana and Singh, 2012), but is constrained because of health associated risks (McNeill et al., 2009). Reuse of wastewater for agriculture can prove to be a resource because its application can improve the physical properties and nutrient content of soils. Since, wastewaters contain many toxic substances such as metals and organic chemicals that can have hazardous impact on human health and environment, therefore toxicity studies need to be carried out to assess the associated risks. The World Health Organization (WHO) and the US Environmental Protection Agency (USEPA) have issued guidelines for water quality parameters for waters to be used in crop irrigation.

In India, treated wastewater from Common Effluent Treatment Plants (CETPs) has been used for watering horticultural crops. Irrigation with treated wastewater might add heavy metals and other contaminants to plants, which might pose a health risk to the animals and humans therefore it becomes mandatory to examine the toxicity of plants (Bartholomaeus et al., 2013). Moreover, if we wish to use the treated wastewater for irrigation of crop plants (which are edible), toxicity assessment is required. The present studies were conducted to evaluate toxic effects (if any) of tomato plants irrigated with treated municipal wastewater using male wistar rats as the animal model system.

## MATERIALS AND METHODS

**Treatment process:** The final treated wastewater was collected from a Common Effluent Treatment Plant (CETP) located at Mayapuri, New Delhi, India. *Solanum lycopersicum* (tomato) plants were raised in different soil beds and irrigated with treated wastewater. The plants irrigated with groundwater were taken as control. The experiment was continued for three months. The plants harvested after fruiting stage were dried, powdered and used for the animal studies.

Six to eight week old male wistar rats weighing around 150-200 g were taken and kept in animal care facility at room temperature 25±1°C with 12 h light/dark cycle and fed with standard pellet diet and tap water. Rats were left for seven days to acclimatization. The CPCSEA guidelines were followed for animal handling and treatment. The animals were categorized into four groups depending upon the type and amount of dose. Each group contained 6 male wistar rats. The rats in each group were fed with standard diet (Hindustan Lever Limited, New Delhi, India) along with dose of powdered plant material as given below:

- Group I was given A at lower dose (D1) 150 (mg kg$^{-1}$ b.wt.) dissolved in distilled water for 14 consecutive days
- Group II was given A at higher dose (D2) 300 (mg kg$^{-1}$ b.wt.) dissolved in distilled water for 14 consecutive days
- Group III was given B at lower dose (D1) 150 (mg kg$^{-1}$ b.wt.) dissolved in distilled water for 14 consecutive days
- Group IV was given B at higher dose (D2) 300 (mg kg$^{-1}$ b.wt.) dissolved in distilled water for 14 consecutive days
- Plants A raised with groundwater (control)
- Plants B raised with treated wastewater

The treatment continued for 14 days and animals were sacrificed on 15th day.

**Haematological analysis:** Blood samples collected from anesthetized animals were stored in vials for haematology and serum biochemistry. Blood collected in a sterile centrifuge tube was left undisturbed (37°C) for 1h till the formation of clot. Haematological studies were carried out following standard methods. Thin blood film smears stained with Leishman's stain were used for measuring RBC and WBC. Around 200 RBC in 20 oil immersion microscopic fields (10 x×100 x) were observed and their morphological abnormalities (poikilocytosis), were closely observed in each treatment.

Non-haemolysed serum was separated and serum enzyme levels were estimated within 24 h. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, creatinine, bilirubin, glucose, cholesterol, total protein, albumin and globulin were estimated using auto-analyzer (ERBA-Smart lab SL-10304) and biomedical kits (Transasia Mumbai, India) (Nasim *et al.*, 2009). Serum glucose, lipid profile (total cholesterol, LDL-and VLDL-cholesterol, HDL-cholesterol, total lipids, triglycerides) and serum enzyme levels (ALP, AST and ALT) were analyzed using assay kits (Span Diagnostic Ltd., Surat, India) (Wright *et al.*, 1972; IFCC., 1980).

**Biochemical measurements:** Liver and kidney tissues were homogenized in chilled phosphate buffer (0.1 M, pH 7.4) and suspension was centrifuged at 3000 rpm for 10 min at 4°C. The aliquot was centrifuged at 12000 rpm for 20 min at 4°C to obtain PMS (source of enzymes). The activity of enzymes namely aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were

determined by the method of Reitman and Frankel (1957). Blood Urea Nitrogen (BUN) was estimated by method of Kanter (1975). Creatinine was estimated by the method of Hare (1950). Membrane lipid peroxidation LPO was done following the modified protocol of Wright *et al.* (1981). Reduced glutathione was measured by the method of Jollow *et al.* (1974).

The data obtained as Mean±SEM (Standard error of mean) was statistically analyzed using Student's t-test and ANOVA variance analysis using SYSTAT program version 5.0.

## RESULTS AND DISCUSSION

Municipal wastewater from domestic and industrial sources mainly contains suspended and dissolved solids, heavy metals, nutrients and organic contaminants (Matouq, 2008). Treated municipal wastewater used in the present study did not show presence of heavy metals except Fe, Cr which was detected at low levels. Other parameters, such as Electrical Conductivity (EC), Total Dissolved Solids (TDS), phosphate and nitrate was recorded in high levels. The values of the parameters such as chloride, sulphate, alkalinity and hardness (total) were high in both groundwater and treated wastewater samples (Table 1). Generally it is assumed that crops irrigated with wastewater show metal toxicity which might pose potential health risks to the consumers (Khan *et al.*, 2008; Shad *et al.*, 2013; Satpathy *et al.*, 2014). Since, the wastewater does not contain high level of heavy metals, therefore no significant transfer of metals to soil and hence to crop plants was reported (Table 2). The possibility of toxicity arising from any other contaminant (nutrients or other physicochemical parameters) present in the treated wastewater cannot be ruled out, therefore toxicity assessment of wastewater irrigated plants was done using animal studies. Use of animal models systems such as *Daphnia* (water flea), fish, mice for toxicity evaluation has been reported earlier (Moriarty, 1999; Ghazy *et al.*, 2013).

Table 1: Composition of groundwater and treated wastewater collected from CETP

| Parameters | Ground water | Treated wastewater |
|---|---|---|
| pH | 6.3±0.9 | 6.5±0.8 |
| EC (mS cm$^{-1}$) | 0.17±0.09 | 3.08±0.67 |
| TDS (mg L$^{-1}$) | 818±45 | 1510±67 |
| DO (mg L$^{-1}$) | 5.31±0.9 | 4.99±0.8 |
| Chlorides (mg L$^{-1}$) | 500±64 | 534±66 |
| Sulphates (mg L$^{-1}$) | 200±19 | 178±16 |
| Phosphate (mg L$^{-1}$) | 0 | 25±2 |
| Nitrate (mg L$^{-1}$) | 0 | 67±4 |
| Alkalinity (mg L$^{-1}$) | 180±19 | 188±16 |
| Hardness (total) (mg L$^{-1}$) | 1021±67 | 1000±71 |
| Fe (mg L$^{-1}$) | 0 | 3±0.3 |
| Cr (mg L$^{-1}$) | 0 | 6±0.5 |

Cd, Pb, Ni, Cu, Zn-not detectable, CETP: Common effluent treatment plants, EC: Electrical conductivity, TDS: Total dissolved solids, Fe: Iron, Cr: Chromium

Table 2: Heavy metal content in tomato plants under different treatments

| Metals (mg kg$^{-1}$) | Treatments | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | A | | | | B | | | |
| | Stem | Root | Leaf | Fruit | Stem | Root | Leaf | Fruit |
| Cr | 2.80±0.9 | 5.80±0.8 | 5.20±0.9 | 2.50±0.8 | 1.80±0.8[NS] | 5.40±0.7[NS] | 4.80±0.7[NS] | 2.50±0.8[NS] |
| Fe | 13.00±2 | 18.60±0.5 | 24.00±0.7 | 16.00±1 | 12.70±2[NS] | 18.00±0.3[NS] | 22.00±0.9[NS] | 16.00±1[NS] |
| Cd | 0.18±0.06 | 0.25±0.05 | 0.34±0.06 | 0.22±0.09 | 0.19±0.08[NS] | 0.22±0.04[NS] | 0.31±0.07[NS] | 0.20±0.08[NS] |
| Pb | 0.99±0.09 | 0.78±0.08 | 1.56±0.08 | 1.32±0.08 | 1.09±0.09[NS] | 0.88±0.08[NS] | 1.67±0.07[NS] | 1.10±0.08[NS] |
| Ni | 0.10±0.09 | 0.07±0.01 | 0.15±0.07 | 0.12±0.05 | 0.17±0.08[NS] | 0.09±0.02[NS] | 0.19±0.08[NS] | 0.12±0.05[NS] |
| Cu | 9.90±1 | 12.20±1 | 15.80±0.9 | 11.20±0.9 | 10.30±0.9[NS] | 12.80±1[NS] | 14.60±0.08[NS] | 10.70±0.8[NS] |
| Zn | 26.00±3 | 36.00±3 | 45.00±4 | 33.00±2 | 25.30±3[NS] | 35.70±4[NS] | 43.80±4[NS] | 32.30±2[NS] |

A: Plants raised with groundwater (control), B: Plants raised with treated wastewater, NS: Non-significant, Cr: Chromium, Fe: Iron, Cd: Cadmium, Pb: Lead, Ni: Nickle, Cu: Copper, Zn: Zinc

Table 3: Haematological parameters measured in blood of different groups of rats

| Parameters | Treatments | | | |
|---|---|---|---|---|
| | Group I | Group II | Group III | Group IV |
| WBC ($10^3$ µL) | 14.40±0.05[NS] | 13.41±0.03[NS] | 15.10±0.03[NS] | 14.10±0.06[NS] |
| RBC ($10^6$ µL) | 8.71±0.11[NS] | 7.99±0.13[NS] | 9.70±0.11[NS] | 8.79±0.11[NS] |
| Eosinophil (%) | 9.11±1.10[NS] | 8.86±1.17[NS] | 11.11±1.18[NS] | 9.10±1.15[NS] |
| Lymphocyte (%) | 44.00±0.50[NS] | 43.50±0.57[NS] | 42.10±0.51[NS] | 41.11±0.50[NS] |
| Neutrophil (%) | 53.00±0.51[NS] | 51.30±0.51[NS] | 50.50±0.58[NS] | 49.20±0.18[NS] |
| Monocyte (%) | 3.50±0.19[NS] | 2.99±0.20[NS] | 3.80±0.29[NS] | 2.99±0.27[NS] |
| Basophil (%) | 2.22±0.01[NS] | 2.10±0.03[NS] | 2.98±0.05[NS] | 2.70±0.03[NS] |
| Haemoglobin (g $dL^{-1}$) | 147.33±0.32[NS] | 145.31±0.39[NS] | 146.33±0.63[NS] | 144.33±0.33[NS] |
| Haematocrit (%) | 45.46±2.10[NS] | 44.44±2.00[NS] | 43.46±2.12[NS] | 42.96±2.20[NS] |
| MCV (µm³) | 58.10±0.42[NS] | 57.50±0.45[NS] | 58.05±0.48[NS] | 57.30±0.44[NS] |
| MCH (pg) | 22.87±1.20[NS] | 21.81±1.11[NS] | 19.87±1.21[NS] | 18.87±1.27[NS] |
| Platelet (×$10^3$ µL) | 616.67±4.51[NS] | 606.67±4.41[NS] | 596.68±4.42[NS] | 576.67±4.21[NS] |

Values are expressed as Mean±SD, n = 8 for each treatment group. Non-significantly (NS) different from their respective control group (p<0.001), WBC: White blood cells, RBC: Red blood cells, MCH: Mean corpuscular hemoglobin, MCV: Mean corpuscular volume

Table 4: Level of biochemical parameters in the plasma and the serum of rats

| Parameters | Treatments | | | |
|---|---|---|---|---|
| | Group I | Group II | Group III | Group IV |
| Glucose (mg $dL^{-1}$) | 97.68±8.15[NS] | 93.68±8.84[NS] | 96.61±8.85[NS] | 93.69±7.55[NS] |
| Uric acid (mmol $L^{-1}$) | 0.18±0.18[NS] | 0.14±0.10[NS] | 0.17±0.16[NS] | 0.16±0.11[NS] |
| Urea nitrogen (mmol $L^{-1}$) | 9.84±0.11[NS] | 9.04±0.15[NS] | 9.44±0.10[NS] | 9.14±0.15[NS] |
| AST (UI $L^{-1}$) | 77.94±2.81[NS] | 76.44±2.80[NS] | 75.18±2.81[NS] | 74.94±2.85[NS] |
| ALT (UI $L^{-1}$) | 52.71±5.33[NS] | 51.76±4.83[NS] | 51.61±5.13[NS] | 50.76±5.83[NS] |
| ALP (UI $L^{-1}$) | 106.92±4.44[NS] | 104.98±4.14[NS] | 104.78±4.88[NS] | 103.91±4.10[NS] |
| Triglycerides (mg $dL^{-1}$) | 112.30±3.20[NS] | 111.30±3.10[NS] | 110.50±3.11[NS] | 109.30±3.14[NS] |
| Total cholesterol (mg $dL^{-1}$) | 88.45±5.12[NS] | 87.45±5.18[NS] | 86.75±5.10[NS] | 86.18±5.11[NS] |
| Total bilirubin (mmol $L^{-1}$) | 0.18±0.01[NS] | 0.17±0.02[NS] | 0.15±0.01[NS] | 0.14±0.06[NS] |
| Direct bilirubin (mmol $L^{-1}$) | 0.0017±0.11[NS] | 0.0016±0.11[NS] | 0.0015±0.12[NS] | 0.0014±0.13[NS] |

Values are expressed as Mean±SD, n = 8 for each treatment group. Non-significantly (NS) different from their respective control group (p<0.001), AST: Aspartate aminotransferase, ALT: Alanine amino transferase, ALP: Alkaline phosphate

For toxicity (if any) assessment, male wistar rats were fed with different doses of treated plant material. The animals fed with plant material raised with treated wastewater did not show any visible signs of toxicity. No behavioural changes were noted in animals in any of the dose treatments. The results were confirmed using haematological and serum biochemistry. The haematological system is highly sensitive and responsive to slight level of toxicity in humans and rodent. Moreover, haematological parameters are a good index of physiological and pathological status in man and animals. No significant differences in the hematological parameters, such as RBC, WBC, eosinophil, lymphocyte, neutrophil, monocyte, basophil, haemoglobin, haematocrit, Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) and platelet count was noted within different treatment groups (Table 3). Since, the RBC count was not altered, therefore other parameters like MCV, MCH and MCHC also did not show any significant alteration in any of the treatments (Sharma *et al.*, 2007).

Aspartate aminotransferase (AST), alanine amino transferase (ALT), serum alkaline phosphatase (ALP) and bilirubin are the markers for hepatocellular damage. None of these (AST, ALP, ALT) parameters showed alteration within different treatments groups (Table 4). Other plasma and serum parameters viz. glucose, uric acid, urea nitrogen, creatinine, triglycerides, total cholesterol, total bilirubin and AST, ALP, ALT showed no significant alteration within different treatments groups (Table 4). The creatinine and urea are markers of kidney function

Table 5: Levels of SGOT, SGPT, BUN and creatinine in the serum of wistar rats

| Treatments | Parameters | | | |
| | SGPT | SGOT | BUN | Creatinine |
| --- | --- | --- | --- | --- |
| Group I | 81.78±0.60 | 72.93±0.29 | 49.03±0.44 | 3.74±0.11 |
| Group II | 71.34±3.03* | 50.14±2.18* | 38.87±0.80* | 2.47±0.14* |
| Group III | 83.13±0.68[NS] | 81.74±3.22[NS] | 54.83±0.38[NS] | 3.89±0.07[NS] |
| Group IV | 75.64±3.07[NS] | 77.99±3.16[NS] | 47.04±2.55[NS] | 2.70±0.12[NS] |

Values are expressed as Mean±SD, n = 8 for each treatment group. Non-significantly (NS) different among the treatments (p<0.001), *: Significantly different among the treatments, SGPT: Serum glutamate pyruvate transaminase, SGOT: Serum glutamate oxaloacetate transaminase, BUN: Blood urea nitrogen

Table 6: Lipid peroxidation (n moles MDA formed $h^{-1}$ $g^{-1}$ tissue) and glutathione level (n mol CDNB conjugate formed $g^{-1}$ tissue) in the liver and kidney of wistar rats

| Treatments | Parameters | | | |
| | LPO (liver) | LPO (kidney) | GSH (liver) | GSH (kidney) |
| --- | --- | --- | --- | --- |
| Group I | 16.45±1.06 | 21.62±1.06 | 1.03±0.04 | 1.07±0.01 |
| Group II | 12.19±0.53* | 17.89±0.48[NS] | 0.63±0.01* | 0.78±0.02* |
| Group III | 18.18±0.42[NS] | 26.24±0.81[NS] | 1.09±0.01[NS] | 1.16±0.05[NS] |
| Group IV | 15.83±1.04[NS] | 15.83±1.04* | 0.84±0.01[NS] | 0.92±0.02[NS] |

Values are expressed as Mean±SD, n = 8 for each treatment group. Non-significantly (NS) different among the treatments (p<0.001), *: Significantly different among the treatments, LPO: Lipid peroxidation, GSH: Glutathione

(Newman and Price, 1999). Both Serum Glutamate Oxaloacetate Transaminase (SGOT) and Serum Glutamate Pyruvate Transaminase (SGPT) are indicative of deteriorating liver function enzyme. The rise in the level of enzymes SGOT and SGPT are indicative of myocardial and skeletal muscle damages (Sharma *et al.*, 2007). The levels of SGOT and SGPT both did not show any significant difference within different treatment groups except Group II where a decrease was noted (Table 5).

Stress in the body induces generation of reactive oxygen species (Uttara *et al.*, 2009), which leads lipid peroxidation. It is measured via MDA formation. Lipid peroxidation has been implicated in the pathogenesis of various liver and kidney injuries. Lipid peroxidation (LPO) levels showed no significant variation among treatment groups except Group II, where a decrease was noted (Table 6). Glutathione (GSH) play a key role in the detoxification of the toxic metabolites and provides defense against xenobiotic toxicity in animals (Jaiswal *et al.*, 2013). In the present study, the renal and hepatic levels of GSH did not show much variation among different treatment groups except Group II, which showed a decrease (Table 6). Since no lipid peroxidation in liver and kidney was noted, hence no associated decrease in GSH was observed.

## CONCLUSION

The present study established that crop plants irrigated with treated wastewater collected from CETP do not pose any toxicity to rats, hence cannot be a threat to cattle and human beings. The present findings are the outcome of a short term study. Long term studies are required to authenticate the possibility of toxicity (if any). Moreover, response of the animals will also depend upon the composition of treated wastewater, rate of the transfer of the toxicants to soil and hence plants.

## ACKNOWLEDGMENT

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