*Eco. Env. & Cons. 29 (April Suppl. Issue) : 2023; pp. (S261-S282)*

*ISSN 0971–765X*

**DOI No.: http://doi.org/10.53550/EEC.2023.v29i02s.045**

# Red Amaranth (*Amaranthus tricolor* L.) Irrigated with Domestic Sewage Wastewater as a Source of Irrigation: Environmental Food Security and Safety Assessment Perspectives through Acute and Sub-Acute Toxicity Study

**Siddhuraju Perumal*, Dhanya Viswanathan, Haritha Thulaseedharan Nair and Roopika Sivaraman**

***Bioresource Technology Lab, Department of Environmental Sciences, School of Life Sciences, Bharathiar University, Coimbatore 641 046, Tamil Nadu, India***

(Received 6 July, 2022; Accepted 5 December, 2022)

**ABSTRACT**

Water deficiency is one of the main factors for limiting sustainable agricultural development in most arid and semi-arid regions. There is a gradual decline in the availability of fresh water to be used for irrigation in developing countries like India. Sewage farming is quite common in all urban areas in India. The effect of its direct and long-term use for irrigation needs a thorough study. However, there is increasing concern about food safety and health risks, hence, a case study was undertaken to understand the long-term effect of domestic sewage wastewater irrigation on heavy metal concentrations in soil and plants. The study reveals the mineral and heavy metal composition of irrigated domestic sewage wastewater and it has been compared with the well water irrigated in the rural area, soil parameters and also the mineral and heavy metal composition of a cultivated plant, *Amaranthus tricolor* L. Transfer factor (TF) was calculated to understand the extent of risk and associated hazard due to wastewater irrigation and the consequence of heavy metals accumulation in the edible portion of experimented vegetables. The present study was carried out to assess the potential toxicity of acetone extract of *A. tricolor* with some essential parameters such as haematological and biochemical parameters, liver and kidney weight and their histopathological study. After conducting *in vivo* acute and subchronic toxicity experiments using the rat model, there was no toxicity or mortality observed between domestic sewage water and well water irrigated to red amaranth, *A. tricolor.*

***Key words :*** *Domestic wastewater irrigation, Amaranthus tricolor, Semi-urban agriculture, Micro-macro elements, Heavy metals, Acute and Sub-chronic toxicity.*

## Introduction

Currently, the reuse of wastewater in urban and peri-urban agriculture is already a widespread practice in different parts of the world. It is estimated that at least 10% of the global population consumes foods produced by irrigation with wastewater (WHO, 2006) and more than 20 million hectares are irrigated with untreated, partly treated/diluted or treated wastewater around the world. To a large extent, wastewater can be considered a reliable source of water and nutrients that is available all year round. Its availability and nutrient properties are important factors that make it a valuable re-source, particularly in arid and semi-arid zones (Elgallal *et al*., 2016). Raw sewage is a rich source of organic and inorganic nutrients for plant growth; sewage farming is quite common in all urban areas in India.

Improved management of wastewater use can offer positive-sum solutions in human welfare and the environment. Reliable estimates of future wastewater supply and demand are needed for better planning and risk management, but the limited information available on wastewater use and the informal agriculture that uses it makes future projections difficult. The fact that wastewater continues to be excluded from water accounting also adds to this difficulty (Munir *et al.,* 2012). Recently, in India, effective implementation of Swachh Bharat Mission (SBM), Swachh Bharat Abhiyan, or Clean India Mission, Smart Cities Mission, Jal Shakti Abhiyan (JSA), Jal Jeevan Mission's urban track (JJM-U) and Waste Management programmes at Town Panchayat, Municipalities and Corporation levels through government and non-governmental organisations, participation of volunteers and public along with State Pollution Control Board (SPCB) and Central Pollution Control Board (CPCB) have taken immense efforts on controlling and releasing of much lesser amount of pollutants in the environment particularly hazardous contaminants in the domestic sewage wastewater which is coming out from the residential area, small retail vegetable markets, food industries etc. in around Coimbatore semi-urban area. Such sewage wastewater is found to be a rich source of organic nutrients and mineral composition which are essential for crop productivity as well as soil fertility improvement without further accumulating considerable pollutants in the soil and crop.

Red amaranth (*A. tricolor*) belongs to the family Amaranthaceae which originated in Asia. This crop is grown commonly as a food or an ornamental crop throughout the tropics. It is also known as 'Lalshak' and is a popular leafy vegetable grown both in the winter and summer seasons in Bangladesh. For the economic production of red amaranth leafy vegetable, a good seed germination percentage and high plant growth requires frequent and proper irrigation (Biswas *et al*., 2015).

The effect of domestic sewage wastewater irrigated crops on the yield, quality of the yield and effects of the environmental issues on consumers was studied and analysed in the semi-urban areas of Coimbatore, and compared against the similar crops cultivated and irrigated with well water in rural areas of the same place with similar microclimatic conditions. The soil and water quality of the selected fields in which the particular crops were cultivated was also analysed for assessing such environmental issues. The domestic wastewater may also carry trace toxic metals and its long-term application on agricultural lands contributes significantly to the build-up of elevated concentrations of toxic metals (Heavy metals) in irrigated soil and plants. Plants have the property to bioaccumulate heavy metals. The *in vitro* and mineral analysis found that trace of heavy metals was reported in *A. tricolor.* Animal models are commonly used to assess preliminary toxicity because the early identification of undesirable effects is usually predictive of the toxicity in humans and can save time, resources and efforts (Araujo *et al*., 2017). In this study, several parameters were evaluated after the *in vivo* acute and sub chronic administration of *A. tricolor* raw and boiled samples. Therefore, Sub-chronic toxicity studies on *A. tricolor* irrigated with domestic sewage water was studied *in vivo.* Despite its widespread use, very little toxicological data and information are available regarding safety. As part of a safety evaluation of *A. tricolor*, a toxicological study was thus carried out to investigate its potential toxicity after single and 28-day repeated oral dosing of an acetone extract of *A. tricolor* in Wistar albino rats.

## Materials and Methods

All the chemicals, reagents and solvents used in the study were procured from Sigma-Aldrich, E-Merck, Sisco (SRL), BDH, ThermoFisher Scientific and HI Media. The diagnostic kits were obtained from Agappe Diagnostics Ltd., Ernakulum, Kerala, India for the studies.

### Study Area

Coimbatore, also known as Kovai, is a major city in the Indian state of Tamil Nadu which is located on the banks of the Noyyal River surrounded by the Western Ghats and it is the sixteenth largest urban agglomeration in India. Coimbatore lies at 11°1263N 76°582213E in south India at 411 meters (1349 ft.) above sea level on the banks of the Noyyal River, in south-western Tamil Nadu. It covers an area of 642.12 km$^2$ (247.92 sq. mi.). The annual mean temperature is 26 °C; monthly mean temperatures range from 18 °C to 35 °C. The average annual rainfall is approximately in between 650-700 mm and the average wind speed is 2.5 m/s with a maximum wind speed of about 8.0 m/s. The average Relative Humidity is around 73%. The Noyyal River forms the southern boundary of the city, which has an extensive tank system fed by the river and rainwater. Madhukkarai, Kinathukadavu were selected for study as semi-urban areas in Coimbatore and the rural areas selected and used for the study were Ettimadai and Kannamanaickanur in Coimbatore.

**Plant samples**

*Amaranthus tricolor* L. commonly used as a leafy vegetable in Coimbatore district in Tamil Nadu, India were used for this study. The samples were collected from rural and urban areas of Coimbatore district in Tamil Nadu, in August 2017. The plant was authenticated (No. BSI/SRC/5/23/2017/Tech./1242) in the Botanical Survey of India, Southern Regional Centre, Coimbatore. Samples were cleaned by removing foreign particles, immature and damaged parts were removed. The samples were divided into two portions. The first portion was taken as raw and the second portion was taken for boiling at 100 $^{o}$C for 20min. After discord the boiled water, the samples were dried at 45 °C in a hot air oven until getting constant weight. After drying, all the raw and processed samples were ground to a fine powder (particle size of about 0.25nm) and stored in separate screw-capped bottles at room temperature for further analysis.

**Soil Samples**

Domestic sewage wastewater irrigated and well water irrigated soil samples were collected from the respective area for the analysis of physicochemical parameters. The surface litter was scraped away to obtain a homogeneously thick slice of soil from the surface to the plough depth from each spot. The V-shaped cut was made with a spade to remove a 1–2-cm slice of soil and the soil sample on the blade of the spade was collected and put in a clean bucket. In this way, the collected soil samples from all the spots were marked for one sampling unit and now the soil samples were transferred from the bucket onto a piece of clean paper or cloth, and it was mixed thoroughly. Now the soil was spread evenly and divided into quarters. Two opposite quarters were rejected and the rest of the soil portion was mixed once more (Motsara and Roy, 2008). The process was repeated until left with about 0.5 kg of the soil. Further, such a way prepared soil sample, after removal of fragment stone, pebbles and organic residues, was air-dried at room temperature and thus obtained respective soil sample was ground, screened through 2-mm sieve and stored in a separate screw capped glass bottle at room temperature until further analysis. The various physicochemical parameters of the soil samples such as lime status, soil texture, soil type, pH, EC, TOC, N, P and K were analysed according to the methods described by Motsara and Roy (2008).

**Water Samples**

Both domestic sewage wastewater and well water samples were collected separately from respective and different fields in pre-cleaned 100ml polythene bottles. The collected water samples were kept in an insulated field kit containing ice and transported to the laboratory and were analyzed as per APHA (2005).

**Mineral and Heavy Metal Analysis**

Mineral profiling of respective well water, sewage wastewater, soil samples and raw and processed *A. tricolor* leaf samples were performed, using Perkin Elmer inductively coupled Argon plasma quadrupole mass spectrometer (ICP-Q-MS) model NexIon 300X, (PerkinElmer, USA) according to the method previously described by Mastro *et al.* (2015) with slight modifications. Samples were digested using tri-acid. For the tri-acid preparation, Nitric acid, Sulphuric acid and Perchloric acid were mixed in the ratio of 9:2:1 respectively. 200mg of sample was mixed with 10 ml of tri-acid and digested at 80 $^{o}$C. After digestion, the samples were made up to 100 ml using HPLC grade water (Thermo Fisher Scientific, India) and they were used to analyse all the 17 minerals through the ICP-Q-MS (NexIon 300X, Perkin Elmer, USA) (Dhanya *et al*., 2019). Ar gas of purity 99.99% (minimum purity) was used for ICP-MS determination. Commercially available 100 mg/l standard solutions (PerkinElmer pure and pure plus) of the elements were used. Diluted working solutions were prepared daily by serial dilutions of the stock solution using the standards. All samples and standards were stored in polyethylene bottles under 4 °C until further analysis.

**Transfer Factor**

The transfer factor expresses the bioavailability of a metal at a particular position on a species of plant

(Khan *et al.,* 2009). Transfer factor (TF) was calculated to understand the extent of risk and associated hazard due to wastewater irrigation and consequent heavy metals accumulation in the edible portion of test vegetables (Cui *et al*., 2004). The Transfer factors of, Al, Fe, Pb, Mn, Cr, Cd, Zn and Cu are calculated by the equation below:

$$TF = \frac{MP}{MS}$$

Where: TF – Transfer Factor

MP – Metal content in Plant (mg $kg^{-1}$)

MS – Metal content in Soil (mg $kg^{-1}$)

The ratio ">1" means a higher accumulation of metals in plant parts than soil (Barman *et al*., 2000). But according to Khan *et al*. (2009), if the transfer coefficient of metal is greater than 0.50, the plant will have a greater chance of metal contamination by anthropogenic activities. TF is also called a bioconcentration factor (BCF). If the ratio>1, the plants have accumulated elements, the ratios around 1, indicate that the plants are not influenced by the elements, and ratios <1 show that plants exclude the elements from the uptake (Olowoyo *et al*., 2010).

**Solvent Extraction**

Powdered samples of both raw and processed *A. tricolor* (each 15 g) were extracted with 80% acetone for all the samples in the ratio of 1:8 by occasional stirring at room temperature for 48 h and filtered through Whatmann No.1 filter paper. The residues were re-extracted with 80% acetone in the ratio of 1:5 for another 24 h. The respective solvent extracts thus obtained were pooled and solvents were removed at 55 °C using a rotary vacuum evaporator under reduced pressure and sample extracts were recovered. Further, the residual moisture content of the recovered extracts was removed at 40 °C using an incubator. Thus the respective dried extract samples were stored in screw-capped vials at 4 °C until further use.

**Animals**

The experimental animals were male Wistar albino rats (150–200 g), obtained from the Small Animal Breeding station, Agricultural University, Mannuthy, Kerala, India. The animals were lodged in the animal house at Nandha College of Pharmacy, Perundurai, Erode, Tamil Nadu, India. The animals were housed in polypropylene cages and maintained under standard conditions (12 h light and dark cycles at an ambient temperature of 25±1ºC). They were fed with commercially available rat feed (Amrut Rat and Mice Feed Pvt. Ltd., Sangli, India) with access to water and libitum. The animals were allowed to acclimatize to the environment for one week before the commencement of experiments. The experimental protocol followed the International Principles for the Biomedical Research Involving Animal (CIOMS/WHO) and was performed after prior approval procured from the Institutional Animal Ethics Committee, obliging strictly to the guidelines of the Committee for Control and Supervision of Experiments on Animals (CPCSEA) constituted by the Animal Welfare Division, Government of India (Protocol registration number: 688/PO/Re/S/02/CPCSEA).

**Preparation of Test Drugs**

The differently processed edible part of *A. tricolor* were subjected to solvent extraction. Before the extraction, the samples were defatted with petroleum ether. Then the extracted with 80% of acetone (1:7 w/v) for 48 h at room temperature and re-extracted with the same solvent (1:5 w/v) for 24 h. the pooled extracts were filtered, air-dried, lyophilized and stored at 4°C for further analysis.

**Acute Toxicity and Dose Fixation Study**

The acute toxicity study was performed as per the Organization for Economic Co-operation and Development (OECD) 423 guidelines (OECD, 2002). The healthy Swiss albino male mice (n=3/ each dose) fasted for 12h with free access to water only. Acetone extract of different *A. tricolor* (dissolved in distilled water) were administrated orally at a dose range of 5, 50, 300, and 2000 mg/kg body weight (b.w) post esophagus (p.o.) with the control of 0.5% CMC (carboxymethylcellulose). The animals were observed for any sign of toxicity, morbidity and mortality for the first 24h with special attention during the first 4h. They were also analysed for the changes in the behavioral, neurological and autonomic profile, any physical signs of toxicity, such as writhing, gasping, palpitation, decreased respiratory rate, and mortality. Further, they were observed for a period of 72h and till the completion of 14 days (Hor *et al.,* 2011).

**Sub Chronic Toxicity Study in Rats**

The experiment was conducted according to the protocol described by OECD Guideline 407 (OECD, 2008) with minimal modification. Rats of both sexes were randomly assigned into seven groups: a control group and six treatment groups. Wistar rats (30 numbers) weighing 100-150gm were divided into six groups. (n=6/group) as follows. Group I-normal control (NC) rats were fed distilled water 5 ml/Kg b.w.p.o/28 days. All the animals in the groups Gp II-VII were treated with Acetone Extract of *A. tricolor* raw and boiled samples which were dissolved in distilled water and administered orally daily for 28 days at single doses 250, 500, and 1000 mg /Kg b.w.p.o./28days (the extract was administered at a rate of 5 ml/kg), while the control group was given only distilled water. The lyophilized extract was freshly prepared with distilled water daily. The behaviour of the rats was observed daily, and they are weighed once a week. At the end of the experiment (28 days), all rats were anaesthetized and blood samples were collected via cardiac puncture into non-heparinized and EDTA-containing tubes for biochemical and haematological analyses, respectively.

**Histopathology Evaluation**

The animals were sacrificed after blood collection by cervical dislocation and organs were excised and weighed and the relative organ weight was also calculated. Vital organs such as kidneys and liver were preserved in 10% neutral formalin, embedded in paraffin, sectioned at approximately 5 mm, stained with hematoxylin and eosin, and examined with an optical microscope for histopathological evaluation (Wasfi, 1994).

**Statistical Analysis**

The statistical analysis was carried out by one-way ANOVA followed by Dunnet's T-test and significant differences ($p<0.05$) in between the control and treatment groups were analyzed. The Data were expressed as the mean ± S.E.M. All the statistical analyses were performed using the statistical software using SPSS (Version 21.0, SPSS Inc.,Wacker Drive, Chicago, USA.

## Results and Discussion

### Physico-chemical Parameters of Irrigation Water samples of *A. tricolor*

The result of physico chemical properties of the domestic sewage wastewater and well water irrigated *A. tricolor* is represented in Table 1, and this was compared with the FAO (FAO, 1985) standard for irrigation. There were obvious differences in the measured parameters in all places. All the samples were reported with optimum pH for irrigation based on the FAO standard. The heavy metal mobility decreases with increasing soil pH due to the precipitation of hydroxides, carbonates or the formation of insoluble organic complexes (Balkhair and Ashraf, 2016). The pH was found to be range from 7.5 to 8.1. The observed electrical conductivity ranged between 1.17 (KNAW) to 1.78 mS/cm (MAAW). TDS are the total amount of mobile charged ions, including minerals, salts or metals dissolved in a given volume of water. TDS (1155 mg/l), TSS (200 mg/l) and TS (1355 mg/l) were higher in sewage water irrigated semi-urban area (MAAW) whereas comparatively lowest values of TDS were found to be in KNAW (720 mg/l), ETAW (824 mg/l), and KKAW (1120 mg/l). The results indicate that urban water contains more pollutants than rural water. That increase in the value of TDS indicated pollution by extraneous sources (Benit and Roslin, 2015). The total hardness values were high in KKAW (470 mg/l) and MAAW (461 mg/l) respectively. High nitrate content was observed in KNAW (41 mg/l). But in the case of phosphate and sulphate concentration was shown higher in KKAW 2.8 and 42 mg/l respectively. Phosphates are chemicals containing the element phosphorous, and they affect water quality by causing excessive growth of algae, but irrigation of this water may substitute the use of fertilizers to the crop. Sulphate content was comparatively higher than the FAO (FAO, 1985) suggested standard. High chloride and alkalinity content were found to be in the MAAW sample and that is within the permissible standard of FAO (FAO, 1985). COD is also registered with the same pattern and those are within the acceptable range of FAO. In general, the total concentrations of micronutrients were higher in domestic sewage water irrigated areas than that in the rural well-water irrigated areas.

### Heavy Metal Concentration in Irrigation Water Samples of *A. tricolor*

Concerning the heavy metals content of irrigation water, data show that sewage effluents contained Al, Cr, Fe, Ni, Cu, Zn, As, Se, Cd, Pb and Mn, in relatively higher values compared to well water. Al-though sewage effluents had elevated concentrations of such metals compared to well water, the concentrations of these metals in these two sources of irrigation water were within the permissible limits for their use as irrigation water as suggested by FAO (Al: 5, Cr: 0.1, Fe: 5, Ni: 0.01, Cu: 0.1, Zn: 2, As: 0.1, Cd: 0.01, Pb: 5, Mn: 0.2 mg/l). Domestic sewage water irrigated Madukkarai area showed higher heavy metal content mainly in the case of Al (5.1 mg/l), Fe (4.27 mg/l), Zn (2.38 mg/l) and Cd (0.095 mg mg/l). This may be due to various industrial activities. But in the case of well water, all the heavy metal content was comparatively lesser than the domestic sewage water irrigated areas. Nickel has been considered to be an essential trace element for human and animal health (Hassan *et al*., 2012). The maximum permissible limit for Ni in water is 0.01 mg/l, high nickel content was observed in ETAW (0.94 mg/l) well water irrigated sample. The maximum permissible limit for Cr in water is 0.1 mg/l. The present study shows the Chromium content ranges from 0.0045 (KNAW) to 0.1 mg/l (KKAW). The range was below the maximum permissible limits of FAO standards. It accumulates in the water reservoirs and agricultural soil as a result of the intensive use of Cd-contaminated phosphate fertilizers (Bandara *et al.,* 2010). The maximum permissible limit for Cd in water is 0.01 mg/l. Cadmium content ranges from 0-0.095 mg/l and it lies within the permissible limit. Lead is a naturally occurring heavy metal and it is, however, toxic to plants and humans. KKAW was the only sample that contains Lead that is 1.3 mg/l and it was found to be below the FAO standards. Higher concentrations of zinc can be toxic to the organism. The permissible limit of zinc in irrigation water according to WHO standards are 2 mg/l. The zinc content of domestic sewage water of MAAW was observed at 2.38 mg/l which was slightly higher when compared to the maximum permissible limits of FAO standards.

Related studies on sewage water irrigation in three cities in India have shown similar trends. The

**Table 1.** Physico chemical parameters of irrigation water samples of *A. tricolor*

| Sample | ETAW | KNAW | MAAW | KKAW | FAO (1992)/ WHO (1994) |
|---|---|---|---|---|---|
| pH | 7.5 | 7.5 | 8.1 | 7.9 | 8.5 |
| Conductivity (mS/cm) | 1.32 | 1.17 | 1.78 | 1.75 | 3 |
| TDS (mg/L) | 824 | 720 | 1155 | 1120 | 2000 |
| TSS (mg/L) | 130 | 134 | 200 | 225 | - |
| TS (mg/L) | 954 | 854 | 1355 | 1345 | - |
| Total Hardness (mg/L) | 345 | 335 | 461 | 470 | - |
| Ca hardness (mg/L) | 225 | 215 | 325 | 305 | 250 |
| Mg hardness (mg/L) | 37 | 34 | 49 | 51 | - |
| Nitrate (mg/L) | 30 | 41 | 32 | 35 | - |
| Phosphate (mg/L) | 0.9 | 1.14 | 2.6 | 2.8 | 2 |
| Sulphate (mg/L) | 26 | 29 | 38 | 42 | 1000 |
| Chloride (mg/L) | 176 | 154 | 276 | 260 | 1100 |
| Alkalinity (mg/L) | 154 | 168 | 242 | 236 | - |
| BOD (mg/L) | 10.4 | 13.6 | 18.2 | 17.4 | 100 |
| COD (mg/L) | 36 | 48 | 76 | 62 | 80-500 |
| DO (mg/L) | 3.09 | 3.14 | 3.18 | 4.02 | |
| Be (mg/L) | 0 | 0.012 | 0.002 | 0 | - |
| Na (mg/L) | 987.3 | 1547.9 | 1191.54 | 1945.1 | 900 |
| Mg (mg/L) | 318.8 | 679.9 | 248.04 | 464.79 | 60 |
| K (mg/L) | 460.7 | 354.1 | 268.27 | 431.12 | - |
| Mo (mg/L) | 0 | 0.0002 | 0.004 | 0 | - |
| Ca (mg/L) | 63.2 | 64.7 | 88.1 | 93.2 | 400 |
| OD (mg/L) | 3.09 | 3.14 | 3.18 | 4.02 | 5.0 |
| Oxidation Reduction Potential (Eh) (mV) | 408 | 409 | 324 | 354 | 300-500 |

ETAW: Ettimadai - *A. tricolor* irrigation water sample, KNAW: Kannamanaickanur - *A. tricolor* water sample (Rural areas). MAAW: Madukkarai - *A. tricolor* water sample, KKAW: Kinathukadavu - *A. tricolor* water sample (Domestic sewage water irrigated Semi urban areas).

study of Alghobar *et al.* (2004), effect of sewage water irrigation on soil properties and evaluation of the accumulation of elements in Grass crops in Mysore city, Karnataka, shows lower values of heavy metals in the wastewater. In Varanasi, Singh *et al.* (2010) reported equally low levels of heavy metals in wastewater. Continuous application of irrigation from wastewater could contribute to heavy metal accumulation in soils and crops, even if their levels are low in irrigation water. Similar results were obtained by Abu Nada (2009) who reported that heavy metals concentrations in the irrigation water UWW and GW were very low values and they also comply with the standards of reused wastewater in agriculture.

### Physico-chemical Parameters of Soil Samples of *A. tricolor* Cultivated in Different Study Areas

Soil texture is an important soil characteristic that drives crop production and field management. The textural class of soil is determined by the percentage of sand, silt, and clay (Berry *et al.,* 2007). The data on physicochemical parameters of the *A. tricolor* cultivation areas are given in Table 2. The results of lime status shown that rural areas soil samples that are irrigated with well water shows non-calcareous and the semi-urban areas samples that were irrigated with domestic sewage wastewater show calcareous. The soil texture of the Ettimadai soil sample shows clay loam, the Madukkarai soil sample of Amaranthus cultivating areas was observed with silty clay loam and the other two samples KNAS and KKAS resulted with sandy clay loam. In the case of soil type, ETAS and KNAS show brown, but the MAAS soil sample was a reddish-brown colour and the KKAS soil sample was red. Sandy soils have relatively high bulk density since the total pore space in sands is less than in silt and clay soils (Marshall and Holmes, 1999). The effect of sand content on soil bulk density is found to be higher than that of any other soil properties. Clayey soils have lower bulk densities and higher porosities than sandy soils (Chaudhari *et al.,* 2013).

Soil pH is affected by the duration of wastewater irrigation. Many types of research pointed out that there is inconsistency in wastewater irrigation's effect on soil pH (Rusan *et al*., 2007). The soil pH of *A. tricolor* cultivating areas ranged from 6.5 to 8.2. Among the soil samples, the highest pH was observed in the KKAS whereas the lowest value was observed in the MAAS. The soil pH decreased as a result of sewage irrigation which might be due to the higher input of organic matter was increased from 17% to 30% as a result of long-term wastewater irrigation (Al Omron *et al*., 2012).

The electrical conductivity (EC) of the analyzed soil samples was in the range of 0.04 to 0.29 dS/m. The highest electrical conductivity was found in

**Table 2.** Physico chemical parameters of soil samples of *A. tricolor* in different study areas

| Parameters | ETAS | KNAS | MAAS | KKAS | Indian standards 1983 (mg/kg) |
|---|---|---|---|---|---|
| Lime status | Non-calcareous | Non-calcareous | Calcareous | Calcareous | |
| Soil texture | Clay loam | Sandy clay loam | Silty clay loam | Sandy clay loam | |
| Soil Type | Brown | Brown | Reddish Brown | Red | |
| pH | 7.3 | 7.7 | 6.5 | 8.2 | 8.5 |
| EC (dS/m) | 0.04 | 0.25 | 0.29 | 0.14 | |
| TOC (%) | 2.2 | 2.7 | 1.1 | 0.92 | |
| N (Kg /Ha) | 175 | 187 | 235 | 208 | |
| P (Kg /Ha) | 47 | 53 | 64 | 60 | 0-20 |
| K (Kg /Ha) | 215 | 196 | 245 | 301 | 0-450 |
| Be(mg/kg) | 0.125 | 0.14 | 2.4 | 0.155 | |
| Na(mg/kg) | 179.45 | 251.06 | 453.79 | 539.19 | |
| Mg(mg/kg) | 139.76 | 158.34 | 159.06 | 182.83 | 0-500 |
| K(mg/kg) | 283.95 | 105.46 | 161.3 | 1626.06 | |
| Mo(mg/kg) | 0 | 0.01 | 0 | 0.0125 | |
| Ca (mg/kg)* | 74 | 68 | 79 | 83 | 0-3500 |

ETAW: Ettimadai - *A. tricolor* irrigation soil sample, KNAW: Kannamanaickanur - *A. tricolor* soil sample (Well water irrigated rural areas). MAAW: Madukkarai - *A. tricolor* soil sample, KKAW: Kinathukadavu - *A. tricolor* soil sample (Domestic sewage water irrigated semi urban areas). *Flame photometric method domestic sewage water irrigated semi-urban area of MAAS, and the lowest value was observed in ETAS, a rural area soil sample irrigated with well water. The electrical conductivity of the soil is connected to the magnesium and potassium ions of the soil (Ashraf *et al.*, 2012). In general, soils irrigated with treated or raw wastewater present higher salinity than soils irrigated with other water sources due to salt input with the irrigation water (Adrover *et al.*, 2017). Soil organic matter is considered to be an important indicator of soil quality as it covers the major reserves of essential nutrients for plants and microorganisms (Hamdi *et al.*, 2019). The total organic carbon content of the samples was found to range between 0.92 and 2.7 %. When compared to rural areas which were irrigated with well water, semi-urban areas have been shown to be high organic matter content. This indicates that sewage wastewater irrigation provides the essential nutrients including carbon sources to the crops along with as well as improves the fertility levels of soil (Singh *et al.*, 2012).

Soil inorganic nitrogen content depends on the supply of this element not only from the irrigation water but also on soil organic matter mineralization and the nitrogen uptake of the plants and soil microorganisms (Adrover *et al.*, 2017). The results indicated the total nitrogen content varies from 175 (ETAS) to 235 Kg/Ha (MAAS). These results show that the domestic sewage water irrigated semi-urban area soil sample registered higher values than the well water irrigated rural area sample. The same trend was followed in the case of phosphorous (P) also, a higher value was noticed in the Semi-urban soil sample MAAS (64 kg/Ha) and the least concentration was found to be in ETAS (47 kg/Ha). Potassium concentration varied in all the samples and it was significant at the level of $P<0.05$. Soil phosphorous influences the plants to improve the quality of crops and it offsets the harmful effects of excess nitrogen. When applied to leguminous crops, it has tended and encouraged the development of nitrogen-fixing module bacteria. Be concentration varies in different samples and it was significant at $P<0.05$. The highest concentration was observed in MAAS (2.4 mg/l) and the least concentration was registered in ETAS (0.125 mg/l).

Sodium concentration was high in both the sewage water irrigated semi-urban areas compared with the well water irrigated rural areas. The values are KKAS-539.19; MAAS-453.79; KNAS- 251.06; ETAS; 179.45 mg/l. The Magnesium concentration of the

**Table 3.** Mineral profile and heavy metal composition of *A. tricolor*

| Minerals | ETA | | KNA | | MAA | | KKA | |
|---|---|---|---|---|---|---|---|---|
| | Raw | Boiled | Raw | Boiled | Raw | Boiled | Raw | Boiled |
| N (%) | 4.6 | 5.41 | 4.9 | 4.91 | 4.93 | 5.98 | 3.89 | 4.03 |
| Be (mg/kg) | 0.01 | 0.015 | 0 | 0 | 0 | 0 | 0.01 | 0.01 |
| Na (mg/kg) | 1352.95 | 1309.88 | 1358.45 | 1218.55 | 1869.08 | 1819.02 | 1699.5 | 1285.2 |
| Mg (mg/kg) | 739 | 686 | 748.45 | 734.8 | 827.2 | 834 | 760.5 | 724 |
| Al (mg/kg) | 26.67 | 27.54 | 23.41 | 21.34 | 43.755 | 39.345 | 40.6 | 38.6 |
| P[*] (mg/kg) | 794 | 711.2 | 641.45 | 599.64 | 1293.16 | 1023.8 | 1186.2 | 1038.5 |
| K (mg/kg) | 765.13 | 723.5 | 998.13 | 871.32 | 1312.1 | 1214.23 | 1175.93 | 1146.11 |
| Cr (mg/kg) | 1.04 | 0.79 | 1.06 | 0.96 | 1.675 | 1.35 | 1.15 | 1.01 |
| Mn (mg/kg) | 6.13 | 5.61 | 5.63 | 5.1 | 11.53 | 10.03 | 9.63 | 6.87 |
| Fe (mg/kg) | 29.55 | 28.09 | 27.25 | 27.67 | 41.36 | 38.9 | 36.21 | 35.2 |
| Ni (mg/kg) | 0.025 | 0.16 | 0.035 | 0.34 | 0.075 | 0.09 | 0.015 | 0 |
| Cu (mg/kg) | 0.165 | 0.035 | 0.14 | 0.06 | 1.415 | 0.25 | 0.9 | 0.85 |
| Zn (mg/kg) | 1.26 | 1.65 | 0.04 | 0.03 | 1.85 | 0.585 | 0.04 | 0.025 |
| As (mg/kg) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Se (mg/kg) | 0 | 0 | 0 | 0 | 0.01 | 0.005 | 0 | 0 |
| Mo (mg/kg) | 0.03 | 0.035 | 0.01 | 0.01 | 0.015 | 0.04 | 0 | 0.025 |
| Cd (mg/kg) | 0 | 0 | 0 | 0 | 0.01 | 0 | 0 | 0 |
| Pb (mg/kg) | 0 | 0.015 | 0 | 0 | 0.65 | 0.1 | 0.25 | 0.01 |
| Ca*(mg/kg) | 234 | 212.6 | 242 | 241.2 | 256.1 | 248.7 | 246.7 | 244.3 |

ETA: Ettimadai - *A. tricolor* Sample, KNA: Kannamanaickanur - *A. tricolor* sample (Well water irrigated rural areas), MAA: Madukkarai - *A. tricolor* Sample, KKA: Kinathukadavu - *A. tricolor* Sample (Sewage wastewater irrigated semi-urban areas). [a]Micro Kjeldahl method, [*]Spectrophotometric method, *Flame photometric method

Amaranthus cultivating soil samples of different areas varies significantly. The maximum concentration was noticed in KKAS (182.83 mg/l) and the lowest concentration was recorded in ETAS (139.76 mg/l). Generally, the Mg level decreases in small amounts and this may be due to the increase in sodium level in wastewater as the volume of Mg gets replaced by Na. Mg level in the present investigation was still in the acceptable range according to EPA (EPA, 2012) and FAO (FAO, 1985) guidelines as the maximum allowable level is 60 mg/l. In all the investigated soil samples, Ca concentration was found to be in the acceptable range according to the EPA (EPA, 2012) and FAO (FAO, 1985) guidelines where they recommend Ca values of 0 - 400 mg/l. The results show that the calcium content ranges from 68 (KNAS) to 83 mg/l (KKAS). High concentrations of Ca and Mg ions in irrigation water can increase soil pH, resulting in reducing the availability of phosphorous $PO_4$ (Al-Shammiri *et al.,* 2005), but they are also considered to be essential plant nutrients. Further, the present investigation is in good agreement with the previous results obtained by Kiziloglu *et al.* (2008) who described that wastewater irrigation increased soil salinity, exchangeable Ca and Mg ions. Singh (2007) proves that the significantly higher values of exchangeable cations have been reported in soils irrigated using paper mill effluent and also these results were similar to the values of Kumar and Chopra (2013); Kumar *et al.* (2014) who reported that irrigation using wastewater leads to a significant increase in Ca and Mg in the soil as compared to groundwater or well water.

### Heavy Metal Concentration of Soil Samples of *A. tricolor* Cultivated in Different Study Areas

The heavy metal concentration of all the heavy metals analyzed in the soil samples was not at elevated levels when compared with the FAO (FAO,1985) suggested pattern.

**Nickel:** The values of Ni in samples were ranging between 0.39 and 0.86 mg/kg. The lowest value was recorded in rural areas whereas the highest value was observed in urban areas.

**Cadmium:** Concentration of cadmium in all the collected samples was found to be negligible except the KKAS sample that contained 0.031 mg/kg of soil sample. Cd has a greater exchangeable capacity, thus easily becoming available and soluble in soils and becoming bio-available and accumulating in the edible parts of plants (Luo *et al.,* 2011). The concentration of copper ranged between (0.06 to 0.9605 mg/kg). The highest concentration is present in the urban areas (Nazir *et al*., 2015).

**Chromium:** Chromium concentration in all the experimented soil samples was found to range between 6.02 and 8.5 mg/kg. Nonetheless, the estimated concentration of chromium in both the Rural and Urban vicinity was well below the permissible limit established by WHO. However, the highest concentration of Cr was present in the urban area when compared to the value found in the rural areas. Hence the present investigation suggests the intake of samples devoid of chemical pollution as the samples collected from the rural vicinity satisfies the recommended dietary limit of intake of the essential minerals for a healthy balanced diet when compared with the samples collected from the urban vicinity. The results of the present investigation are by the findings of Kumar and Chopra (2013) who found that irrigation with wastewater significantly increased Cr in soil. These results are not consistent with Lu *et al.* (2016) who revealed that the wastewater did not increase the Cr content of the soil.

**Lead:** The concentration of lead in soil samples is a serious threat because of its toxicity, bioaccumulation and biomagnifications in the food chain (Khan *et al*., 2012). The concentration of Pb ranged between 0.126 and 0.378 mg/kg. In almost all the collected soil samples the concentration of lead was recorded above the permissible limit suggested by WHO. In general, the urban soil had a higher concentration of heavy metals compared with the rural soil samples and this may be due to the relative and moderate deposition of wind flow aerosol containing Pb in sewage water as well as in irrigation soil from nearby industrial activities.

**Iron:** Compared with rural soil samples, the chromium concentration was reported to be higher in a semi-urban area. Iron is an essential trace element for haemoglobin formation, normal functioning of the central nervous system and the oxidation of carbohydrates, proteins and fats and it also plays an important role in oxygen and electron transfer in the human body**.** The concentration of iron in all the collected soil samples was significantly higher, but not exceeding the maximum permissible limit (51.17 to 131.92 mg/kg). Dikinya and Areola (2010) observed that the Fe was not significantly higher in wastewater-irrigated soil in comparison to soils irrigated with groundwater.

**Zinc:** Zinc requirements are related to tissue turnover rate and growth. Zinc is one of the important trace elements that play a vital role in the physiological and metabolic processes of many organisms. Nevertheless, higher concentrations of zinc can be toxic to the organism. The concentration of zinc in the experimental soil samples ranged between 0.76 and 3.54 mg/kg. In all the soil samples concentration of zinc was recorded above the permissible limit described by WHO.

### Mineral Profile and Heavy Metal Composition of *A. tricolor*

Red amaranth is one of the most common useful leafy vegetables that are highly perishable food items and always needs special processing treatments to lengthen its storage time. The nutritional content may vary during this storage time and the refrigerator is usually used to preserve red amaranth (*A. tricolor*). The macro and micronutrients and heavy metals composition of diluted domestic sewage water irrigated raw and boiled *A. tricolor* are depicted in Table 3. Diluted domestic sewage water commonly contains high concentrations of nutrients in the form of Nitrogen (N), Phosphorus (P), Potassium (K), Magnesium (Mg) and Calcium (Ca) which are essential for plant growth. The present study revealed that both the raw and processed red amaranthus plant samples from respective treatments contain adequate minerals. All the samples analysed in the present study shows significant nitrogen content and it ranges from 3.89 to 5.98%. Domestic sewage water irrigation increases nitrogen concentration in the samples because of nutrient enrichment. Among the different samples, a significantly higher nitrogen value was observed in the Madukkarai Amaranthus sample (MAA) 5.98%.

Na and K maintain the ionic balance of the human body and maintain tissue excitability. Na plays an important role in the transport of metabolites and K is important for its diuretic nature. The ratio of K/Na in any food is an important factor associated with hypertension and arteriosclerosis. Na enhances and K depresses blood pressure (Datta *et al*., 2019). Na concentration of the *A. tricolor* was as follows MAA (Raw: 1869.08 mg/kg; Boiled: 1819.02 mg/kg) > KKA (Raw: 1699.5 mg/kg; Boiled: 1285.2 mg/kg) > KNA (Raw: 1358.45 mg/kg; Boiled: 1218.55 mg/kg) > ETA (Raw: 1352.95 mg/kg; Boiled: 1309.88 mg/kg). Comparatively, high sodium content was found to be in MAA sample. In the case of potassium, the higher concentration was obtained in the same MAA sample (Raw: 1293 mg/kg; Boiled: 1186.2 mg/kg). In the case of both sodium and potassium, the least concentration was recorded in the well water irrigated rural area samples. The content of phosphorous was also found to be higher in domestic sewage water irrigated MAA samples (Raw: 1312.1 mg/kg; Boiled: 1214.23 mg/kg).

High magnesium content was also found in the MAA sample (Raw: 827.2 mg/kg; Boiled: 834 mg/kg). Magnesium helps to prevent muscle degeneration, growth retardation, cardiomyopathy, immunologic dysfunction, impaired spermatogenesis and bleeding disorders (Chaturvedi *et al*., 2004). High calcium levels were observed in the entire experimental Amaranthus samples. There were no significant differences observed in the calcium content among the samples and, however, the high concentration was reported in the Madukkarai raw sample (256.1 mg/kg), and its boiled sample slightly reduces the calcium concentration, and it was found that 248.7 mg/kg. The results from this study were comparable to that of the wild leafy vegetables consumed in Bangladesh (Satter *et al*., 2016).

### Heavy Metal Composition of *A. tricolor*

The contamination of soil and vegetables by heavy metals is also a global environmental issue. However, the consumption of heavy metal contaminated food can seriously deplete some essential nutrients in the body that are further responsible for decreasing immunological defenses, such as intrauterine growth retardation, impaired psycho-social facilities, disabilities associated with malnutrition and a high prevalence of upper gastrointestinal cancer rates (Tasrina *et al*., 2015).

**Copper:** The copper concentration of *A. tricolor* was varied significantly and being an essential trace element, it is necessary for many enzymes. It is needed for normal growth and development. The high concentration of Cu present in the underground parts of the plants may be due to the absorption ability of the plants to get the trace heavy metals from the polluted soils. The maximum permissible limit of Cu in food is 30 mg/kg (Osakwe and Okolie, 2015). The highest copper concentration was found to be in MAA (Raw: 1.415 mg/kg; Boiled: 0.25 mg/kg) sample which was irrigated with domestic sewage water. The least concentration of the sample was observed in the well-water irrigated KNN sample (Raw: 0.14 mg/kg; Boiled: 0.06 mg/kg). While comparing the Cu concentration of the whole samples, it was found that there was a great reduction of copper concentration in processed samples and this might be occurred due to the leaching of minerals during the processing. The high concentration of Cu causes metal fumes fever, hair and skin discolourations, dermatitis, respiratory tract diseases, anaemia, liver disease and kidney damage and some other fatal diseases in human beings (Enemugwem *et al.,* 2016). According to an estimate, only 1.5-3 mg/day of copper has been determined safe for human consumption. The transfer factor of copper was exceeded 1, only in the Madukkarai raw sample (1.99), but processing reduced the transfer factor by less than one (0.92) (Table 4.).

**Iron:** Iron is an essential micronutrient for almost all living organisms because of its critical role in metabolic processes such as DNA synthesis, respiration, and photosynthesis (Rout and Sahoo, 2015). From the results, it was observed that the concentration of iron (Fe) in samples varied between raw and processed samples of *Amaranthus tricolor.* The maximum iron concentration was found to be in the Madukkarai area sample (MAA). Its raw sample possessed 41.36 mg/kg and the concentration was slightly decreased in a boiled sample with a value of 38.9 mg/kg. The maximum permissible limit of Fe in food is 48mg/kg. The most significant and common cause of anaemia is iron deficiency if iron intake is limited or inadequate due to poor dietary intake (Abbaspour *et al*., 2014). Since leafy vegetables are prescribed medicine for iron deficiency and anaemic patients due to their high iron content and mineral composition. In the present study, all the experimented red amaranthus samples were found to be a good source of Fe content and it could be advocated for wider consumption to eradicate such micronutrient deficiency among school children and pregnant women.

**Zinc:** Zinc is the least toxic and an essential element in the human diet as it is required to maintain the functioning of the immune system (Amin *et al.*, 2013). The value of zinc observed in this study was found to be higher than 0.3 mg/kg (FAO/WHO, 1993) which is suggested to be the safe limit for consumption. Particularly, Zn concentration was found to be slightly higher in the samples from both wells water irrigated and domestic sewage water irrigated areas. Madukkarai area sewage wastewater irrigated raw amaranthus leaf sample registered as 1.41 mg/kg of Zn and during the boiling process, the content of Zn was recorded as 0.58 mg/kg with 81% of reduction. It was slightly higher than the maximum permissible limits suggested by FAO/ WHO (1993). The transfer factor of zinc was also observed to be higher than one in the semi-urban area of the Madukkarai (Table 4) plant sample. *A. tricolor* tend to bioaccumulate this metal, the proper dilution of sewage water before irrigation and continuous monitoring and evaluation of the Zn content in such plant sample before wider consumption are essential.

**Lead:** In the present study plant samples, Pb concentration was ranged between 0 and 0.65 mg/kg. Interestingly, there was no lead content was observed in ETA and KNA samples were irrigated with well

**Table 4.** Transfer factor of heavy metals from soil to *A. tricolor*

| Heavy Metal | ETA | | KNA | | MAA | | KKA | |
|---|---|---|---|---|---|---|---|---|
| | Raw | Boiled | Raw | Boiled | Raw | Boiled | Raw | Boiled |
| Al | 0.10 | 0.08 | 0.13 | 0.13 | 0.14 | 0.11 | 0.13 | 0.10 |
| Cr | 0.12 | 0.04 | 0.18 | 0.10 | 0.44 | 0.13 | 0.16 | 0.10 |
| Fe | 0.58 | 0.53 | 0.33 | 0.28 | 0.31 | 0.29 | 0.45 | 0.44 |
| Ni | 0.05 | 0.20 | 0.04 | 0.04 | 0.19 | 0.15 | 0.02 | 0.16 |
| Cu | 0.20 | 0.41 | 0.11 | 0.14 | 1.99 | 0.92 | 0.07 | 0.31 |
| Zn | 1.65 | 1.94 | 0.04 | 0.04 | 3.43 | 5.19 | 0.03 | 1.43 |
| As | 0.00 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Se | 1.00 | 0 | 0 | 0 | 1.00 | 0 | 0 | 0 |
| Cd | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.32 |
| Pb | 0.08 | 0.02 | 0 | 0 | 0.34 | 0.31 | 0.09 | 0.02 |
| Mn | 0.16 | 0.03 | 0.16 | 0.04 | 0.31 | 0.09 | 0.13 | 0.04 |

ETA: Ettimadai - *A. tricolor* sample, KNA: Kannamanaickanur *A. tricolor* sample (Well water irrigated rural areas), MAA: Madukkarai - *A. tricolor* sample, KKA: Kinathukadavu - *A. tricolor* sample (Sewage wastewater irrigated semi urban areas).

water. The high concentration of lead was found to be in the MAA sample (raw: 0.6mg/kg; boiled: 0.1). Kinathukadavu area samples (KKA) were obtained with the lead concentration of raw sample 0.25mg/kg and the processed sample was observed with 0.1mg/kg. Though the content of Pb in MAA sample was found to be higher than the safe limit (0.3 mg/kg) recommended by FAO/WHO (1993), it had been significantly reduced during the hydrothermal processing. The high level of lead in this study could be attributed to acid-lead batteries as waste dumped or flung in the upstream sewage water flow region of the Residential area which is subsequently used to irrigate the farmlands in downstream areas (Amin *et al.,* 2013). Further, the possibilities for the contamination of Pb on the surface of sewage wastewater and water irrigating soil through particulate materials which were generated from industrial activities and subsequently flowing via air could also not be ruled out.

**Cadmium:** Cadmium is also another non-essential heavy metal. It is extremely toxic even at low concentrations. It causes learning disabilities and hyperactivity in children (Enemugwem *et al*., 2016). In our study, the concentration of Cd was noted only in the MAA raw sample (0.1mg/kg) which was the same as the critical level of 0.1 mg /kg as reported by WHO (1995). But it was found to be disappeared when processing the sample before consumption. Food substances containing excess Cd are known to result in bone fracture, cancer, diarrhoea, stomach pains, severe vomiting, reproductive failure and damage of CNS and DNA (Wilberforce, 2016).

**Chromium:** Chromium plays a vital role in the metabolism of cholesterol, fat, and glucose. Its deficiency causes hyperglycemia, elevated body fat, and decreased sperm count, while at high concentrations it is toxic and carcinogenic. Chromium is an essential trace metal but a high concentration in edible food materials could be disastrous to human health. WHO's permissible limit of chromium in the plant is 1.5 mg/kg, while its daily dietary intake is 0.2 mg/kg (Enemugwem et al., 2016). From the results, it was found that Cr concentration of all the analysed samples was below the maximum permissible limit prescribed by WHO except MAA raw sample where it was shown to be a concentration of 1.67mg/kg which was slightly higher than the permissible limit. However, the domestic and conventional processing (boiling) reduced it to 1.35 mg/kg which was found to be under the allowable limit. This may be due to the metal transformation and unavailability for estimation or leaching out during processing.

**Nickel:** The daily dietary intake of nickel in food is suggested to be 3-7mg/day. The negligible concentration of nickel was detected from all the samples of the present investigation and it ranged from 0 to 0.75mg/kg. As this study shows high concentration was found to be in MAA raw sample. In the entire sample, processing reduces the Ni concentration. Nonetheless, Selenium concentration was also found to be negligible in all the samples analysed. The symptoms of Ni toxicity in plants include chlorosis, stunted root growth, and sometimes brown interveinal chlorosis and symptoms specific to plant species (Gupta and Gupta, 1998).

**Manganese:** The level of Mn varied significantly in all the samples**.** The advocated dose of daily dietary intake of Mn in food is 11mg/day. The Mn content obtained for the studied Amaranthus samples were high. MAA sample contains significantly high value (Raw: 11.53 mg/kg; Boiled: 10.03 mg/kg). The factors responsible for the high content of manganese in vegetables were proposed to be the soil type, composition and quality of sewage wastewater, application of agricultural pesticides and fertilizer (Amin *et al.,* 2013). Excessive Mn concentrations in plant tissues can alter various processes, such as enzyme activity, absorption, translocation and utilization of other mineral elements (Ca, Mg, Fe and P), causing oxidative stress (Millaleo *et al.,* 2010). Arsenic (As) is ubiquitous in many environments and highly toxic to all forms of life. In this study, it was not found in any of the samples. Interestingly, the transfer factors of all the heavy metals except Zn were not exceeding the suggested limit.

### Acute Toxicity and Dose Fixation Study

Animals showed good tolerance to the testing doses. The acute toxicity study was performed as per OECD 423 guidelines. Both the doses of 2000 mg/kg and 3000 mg/kg of *A. tricolor* L acetone extract were found to be safe and also non-toxic because rats did not show any signs of writhing, gasping, palpitation, decreased respiratory rate, or mortality until the end of the study period.

### Subchronic Toxicity Study in Rats: Observational Study

No death was observed in any of the groups of rats during 28 days of treatment. The subchronic oral administration of extract of *A. tricolor* had no noticeable effect on the body weight or general behaviour of the treated rats. Weight gain was normal over the treatment period. There was no significant difference in the relative organ weights of the liver and kidney between the control and all treatment groups (Table 5). Both the control and treated rats appeared uniformly healthy at the end of the experiment as well as throughout the 28-day treatment period. Evaluation of oral toxicity via a repeated dose 28-day experiment has been advocated as a fundamental requirement for assessing safety (Hor *et al.,* 2012). Food and water consumption for all the animals were found to be normal and similar between the control and treated groups. The animals did not show any signs of neurotoxicity as there were no changes in sensory and motor signs. The findings of the mental alertness study also showed a normal mental activity pattern before and at the end of the study. Further, no significant changes were found in the locomotor activity for the control and treated groups.

The acute and sub-chronic toxicity studies did not show any adverse clinical symptoms or mortality at all tested doses. Bodyweight changes are often found to be the first sign of toxicity and are an important parameter for the objective evaluation of the effects of a test substance in experimental animals. Both acute and sub-chronic toxicity studies revealed no drastic changes in organ weight measurement of treated rats compared to the control group. Conforming to that, no variation in food and water consumption was recorded during the acute and subacute toxicity studies between control and treated groups.

### Haematological and Biochemical Analysis

The biochemical evaluations are important to verify any toxic effects on hepatic and renal function. The liver is the main organ responsible for drug metabolism. Serum enzymes AST and ALT are considered to be sensitive markers of hepatocellular toxicity and increased activity indicates damage in this organ (de Lima *et al.,* 2018). The analysed haematological parameters, which included RBC, WBC, PLT, Hb and ESR of rats treated with raw and processed *A. tricolor* extracts (250, 500, 1000 mg/kg) did not show any significant difference between the control and solvent extracts treated groups at all doses (Table 6).

**Table 5.** Effect of *A. tricolor* on body weight changes in control and experimental rats

| Groups | Body weight of animals | | |
|---|---|---|---|
| | 1st day (g) | 28th day (g) | Weight difference (g) |
| Normal | 210.8±6.4 | 229.2±4.5 | 18.4 ns |
| ACR 250 mg/kg | 237.2±8.2 | 255.1±6.6 | 17.9 ns |
| ACR 500 mg/kg | 242.3±7.6 | 260.4±5.8 | 18.1 ns |
| ACR 1000 mg/kg | 224.8±6.8 | 241.9±4.9 | 17.6 ns |
| ACB 250 mg/kg | 218.7±5.7 | 237.4±4.2 | 18.7 ns |
| ACB 500 mg/kg | 231.2±7.1 | 249.8±6.9 | 18.6 ns |
| ACB 1000 mg/kg | 222.5±6.4 | 240.9±5.2 | 18.4 ns |

Values were mean ± SEM; n = 6. P > 0.05 when compared to control (one-way ANOVA followed by Dunnett's test); ACR – *A. tricolor* raw, ACB – *A. tricolor* boiled.

**Table 6.** Hematological parameters in control and experimental groups

| Group | RBC($\times10^6$/µl) | WBC($\times10^3$/µl) | PLT($\times10^5$/µl) | Hb (mg/dl) | ESR(mm/hr) |
|---|---|---|---|---|---|
| Normal | 6.26±0.2 | 12.08±0.6 | 8.29±0.1 | 12.18±0.3 | 5.28±0.1 ns |
| ACR 250 mg/kg | 6.12±0.1 ns | 11.86±0.4 ns | 8.02±0.2 ns | 11.98±0.1 ns | 5.16±0.2 ns |
| ACR 500 mg/kg | 6.16±0.1 ns | 11.90±0.2 ns | 7.92±0.4 ns | 11.82±0.4 ns | 5.12±0.2 ns |
| ACR 1000 mg/kg | 6.18±0.3 ns | 11.92±0.8 ns | 7.99±0.3 ns | 11.90±0.2 ns | 5.11±0.1 ns |
| ACB 250 mg/kg | 6.42±0.2 ns | 12.42±0.2 ns | 8.27±0.2 ns | 12.22±0.3 ns | 5.30±0.2 ns |
| ACB 500 mg/kg | 6.32±0.1 ns | 12.54±0.4 ns | 8.24±0.3 ns | 12.12±0.1 ns | 5.26±0.3 ns |
| ACB 1000 mg/kg | 6.52±0.1 ns | 12.62±0.1 ns | 8.39±0.2 ns | 12.41±0.2 ns | 5.38±0.1 ns |

Values were mean ± SEM; n=6. nsP> 0.05 when compared to control (one-way ANOVA followed by Dunnett's test); ACR – *A. tricolor* raw, ACB – *A. tricolor* boiled. RBC-Red Blood Cell Count; WBC-White Blood Cell Count; PLT-Platelet Count Test; HB-Hemoglobin Concentration; ESR-Erythrocyte Sedimentation Rate.

These parameters remained within normal limits throughout the experimental period.

Considering the haematological parameters which evaluated showed an increase in erythrocyte count (RBC). In the normal control group, it was reported $6.26 \times 10^6/\mu l$. Rats received 250 mg/kg raw *A. tricolor* extract showed the slightly decreased RBC rate ($6.12 \times 10^6/\mu l$) while its processed sample of 250mg/kg was observed with an increased RBC rate ($6.42 \times 10^6/\mu l$). There were no significant differences ($p < 0.05$) between the raw and processed sample treated rat's RBC Count. Rats obtained 500 mg/kg *A. tricolor* extract raw and processed sample exhibited $6.16 \times 10^6/\mu l$ and $6.32 \times 10^6/\mu l$ respectively. Rats received 1000 mg/kg of raw and processed *A. tricolor* solvent extract samples were reported with 6.18 and $6.52 \times 10^6/\mu l$ respectively. The haematopoietic system is one of the most sensitive targets of toxic compounds and is an important index of physiological and pathological status in men and animals (Das *et al.,* 2015). In the case of WBC, the normal control group was observed with $12.08 \times 10^3/\mu l$. WBC concentration of all the other groups was ranged between 11.86 (250 mg/kg of *A. tricolor*) to $12.62 \times 10^3/\mu l$ (1000 mg/kg of boiled *A. tricolor)*. The WBC concentration of raw samples was as follows; rats received 500 mg/kg (ACR) observed with WBC count of $11.90 \times 10^3/\mu l$, while in the case of 1000 mg/kg *A. tricolor* sample was reported to be $11.92 \times 10^3/\mu l$ of WBC count. Boiled *A. tricolor* treated samples exhibited slightly increased WBC count (ACB 250mg/kg: $12.42 \times 10^3/\mu l$ and ACB 500 mg/kg $12.54 \times 10^3/\mu l$) but did not show any significant deviation ($P > 0.05$) concerning the normal control group. In the case of platelet (PLT) count of the normal or control sample was observed with $8.29 \times 10^5/\mu l$. Raw *A. tricolor* 250 mg/kg received rats were observed with a platelet count of $8.02 \times 10^5/\mu l$, 500 mg/kg received rats were observed with a platelet count of $7.92 \times 10^5/\mu l$ and 1000 mg/kg received rats were shown with a platelet count of 7.99 $10^5/\mu l$. Though the boiled *A. tricolor* sample showed slight increase values, no significant deviations were observed among the samples with dose increases (ACB 250mg/kg: $8.27 \times 10^5/\mu l$, ACB 500 mg/kg: $8.24 \times 10^5/\mu l$ and ACB 1000 mg/kg: $8.39 \times 10^5/\mu l$).

The hematopoietic system is one of the most sensitive targets for toxic compounds and an important index of physiological and pathological status in man and animals (Wang *et al*., 2014). Platelets counts of the control rats were reported to be $8.29 \times 10^5/\mu l$. There was no significant difference ($p < 0.05$) was observed between all dosages treated rats and with control. The maximum platelets count was observed in boiled *A. tricolor* treated rats ($8.39 \times 10^5/\mu l$ in 1000 mg/kg) and the least concentration was characterized in raw *A. tricolor* (500 mg/kg) treated rats ($7.92 \times 10^5/\mu l$). Furthermore, the PLT count of all the above said experimented rats were well comparable and corroborated with that of normal or control experiment rat values.

Haemoglobin is the predominant protein in the red blood cell and is responsible for transporting oxygen, carbon dioxide, and protons between the lungs and tissues. In the present study, all the groups were observed with good haemoglobin content. The Control group was observed with 12.18 mg/dl. The raw *A. tricolor* treated rats haemoglobin was range between 11.82 mg/dl (ACR 500 mg/kg) to 11.98 mg/dl (ACR 250 mg/kg). There was no significant difference observed between the control and treated groups. In the case of boiled *A. tricolor* re-

**Table 7.** Effect of *A. tricolor* on biochemical parameters in control and experimental rats

| GROUP | AST/ SGOT | ALT/SGPT | ALP | TP | UREA | CREATININE |
|---|---|---|---|---|---|---|
| Normal | 58.4±3.6 | 44.6±1.8 | 163.7±6.4 | 7.54±0.09 | 16.02±0.1 | 0.71±0.01 |
| ACR 250 mg/kg | 55.2±2.8$^{ns}$ | 43.5±2.2$^{ns}$ | 159.5±7.2$^{ns}$ | 7.62±0.08$^{ns}$ | 15.82±0.2$^{ns}$ | 0.69±0.03$^{ns}$ |
| ACR 500 mg/kg | 56.8±1.6$^{ns}$ | 55.3±3.1$^{ns}$ | 160.2±4.2$^{ns}$ | 7.38±0.04$^{ns}$ | 15.92±0.1$^{ns}$ | 0.72±0.05$^{ns}$ |
| ACR 1000 mg/kg | 60.6±5.4$^{ns}$ | 46.9±3.4$^{ns}$ | 164.8±8.9$^{ns}$ | 7.36±0.06$^{ns}$ | 16.22±0.2$^{ns}$ | 0.74±0.01$^{ns}$ |
| ACB 250 mg/kg | 57.1±4.3$^{ns}$ | 43.6±4.1$^{ns}$ | 161.6±7.4$^{ns}$ | 7.58±0.07$^{ns}$ | 15.91±0.1$^{ns}$ | 0.70±0.04$^{ns}$ |
| ACB 500 mg/kg | 58.5±3.2$^{ns}$ | 43.2±2.2$^{ns}$ | 161.2±5.6$^{ns}$ | 7.42±0.08$^{ns}$ | 15.86±0.2$^{ns}$ | 0.71±0.02$^{ns}$ |
| ACB 1000 mg/kg | 59.2±5.4$^{ns}$ | 43.4±3.8$^{ns}$ | 162.3±8.8$^{ns}$ | 7.44±0.05$^{ns}$ | 16.06±0.1$^{ns}$ | 0.72±0.02$^{ns}$ |

Values were mean± SEM; n=6. $^{ns}P > 0.05$ when compared to control (one way ANOVA followed by Dunnett's test) aspartate aminotransferase (AST)/ serum glutamic-oxaloacetic transaminase (SGOT) = U/L, Alanine Aminotransferase (ALT)/Serum glutamic-pyruvic transaminase (SGPT)= U/L, Alkaline Phosphatase (ALP) = U/L, Total Protein= g/dl, Urea= mg/dl, and Creatinine=mg/dl, ACR – *A. tricolor* raw, ACB – *A. tricolor* boiled.

ceived rats shows slightly increased haemoglobin content as follows ACB 250 mg/kg: 12.22 mg/dl, ACB 500 mg/kg: 12.12 mg/dl, ACB 1000 mg/kg: 12.41 mg/dl. Since *A. tricolor* raw and processed sample showed high mineral Iron (Fe) concentration which was reflected in the haemoglobin concentration. Erythrocyte Sedimentation Rate (ESR) was also showed to be without any significant difference (P>0.05) between the control and other groups. The Control group was found to have an ESR of 5.28 mm/hr**.** Among all the tested groups, a high ESR value was reported in ACB 1000 mg/kg treated rats (5.38 mm/hr) and the minimum value was registered in ACR 1000 mg/kg treated rats (5.11 mm/hr). Changes in blood parameters are considered to be one of the most reliable pieces of evidence for toxicity studies. Measurement of levels of blood components is relevant to assess the risk of toxicity in animal studies that can be translated into human values (Hor *et al.*, 2012; Christopher *et al*., 2017). The Hb and RBC levels were not reduced and also confirming that there was no development of anaemia or other haematological disorders. Likewise, there was no significant change observed in the biochemical parameters between the control and extract treated groups also. The above said results indicated that the consumption of red amaranthus leafy vegetables irrigated with domestic sewage wastewater was found to be a good, cheap and nature-based solution for the eradication of micronutrient deficiency and supply of nutraceutical bioactive substances not only among the children but also among the pregnant women and adults without any toxicity/health issues.

**Biochemical Parameters**

The biochemical parameters analysed were serum glutamic pyruvic transaminase (SGPT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein (TP), Urea, Creatinine and the results are given in Table 7. The biochemical parameters that evaluate hepatocellular injury are liver transaminases, such as ALT, AST and SGOT. It is known that several toxic compounds accumulate in the liver where the detoxification occurs (Burci *et al.*, 2018). Liver damage is usually assessed by the determination of serum transaminases (ALT and AST) and also by the measurement of total proteins and was not observed any significant alterations in serum levels of these three markers in liver function after acute and subchronic administration of *A. tricolor* extracts and also confirming that the administration of these extracts did not cause liver damage. AST level of the control group was observed with 58.4 U/L. *A. tricolor* raw extract in different dose received rats did not show any significant difference in the AST concentration (ACR 250 mg/kg: 55.2 U/L, ACR 500 mg/kg: 56.8 U/L, ACR 1000 mg/kg: 60.6 U/L). Moreover, the AST values of both raw and processed *A. tricolor* extract-treated rats were found to be similar to that of the values of normal/control-treated rats. However, among the processed samples of *A. tricolor*, the maximum AST value was reported in ACB 1000mg/kg dose received in rats (59.2 U/L) and the minimal concentration (57.1U/L) was characterized in the ACB 250 mg/kg treated rats. A serum glutamic pyruvic transaminase (SGPT) value of all the *A. tricolor* samples that treated rats were found to be similar to that of normal control rat experiments. Serum glutamic pyruvic transaminase, an enzyme that is normally present in liver and heart cells. SGPT is released into the bloodstream when the liver or heart is damaged and thus the blood SGPT levels are elevated and it is also known as alanine aminotransferase (ALT). The normal or control group rats showed the SGPT value of 44.6 U/L and this value was found to be on par with those values obtained for the raw *A. tricolor* treated rats such ACR 250mg/kg (43.5 U/L), ACR 1000 mg/kg (46.9 U/L). However, the value (55.3 U/L) observed in the ACR 500 mg/kg treated rats was found to be relatively higher when compared to control rats value but not up to the elevated level to cause toxicity. Whereas in the processed sample, the SGPT values were shown as 43.6 U/L, 43.2 U/L, 43.4 U/L in ACB 250 mg/kg, ACB 500 mg/kg and ACB 1000 mg/kg *A. tricolor* extract-treated rats, respectively and these values were well compared to control value. Further, when compared to control, the SGPT value of all the above said raw and boiled samples treated rats showed without any significant differences (P>0.05).

Alkaline phosphatase (ALP) levels were found to be normal in all the treated rat groups and also comparable to that of value (163.7 U/L) was observed in the control sample. The high ALP concentration was reported in 1000 mg/kg treated ACR sample (164.8 U/L) and the lowest ALP value was observed in 250 mg/kg received rats of ACR sample (159.5 U/L). Interestingly, there was no significant difference observed in between the treated samples and with the control rat groups. The serum total protein of the control group of rats was reported as 7.54 g/dl and the value of all other samples treated rats were ranged between 7.36g/dl (ACR 1000 mg/kg) and 7.62 g/dl (ACR 250 mg/kg). Serum proteins are mainly synthesized in the liver, so the levels of these proteins are considered as the indicator of normal and synthetic functions of hepatocytes (Christapher *et al.,* 2017). In the present study, there was no significant difference (P>0.05) observed in serum total protein between the control and, *A. tricolor* treated raw and processed extract received rat groups.

Serum urea elevation is an indicator for various tissue injuries such as cardiac, renal parenchymal and renal calculi (Christapher *et al.,* 2017). In our present study, both parameters were not significantly different from control values, indicating that the extract has no deleterious effect on kidney function. Urea level in the control rat groups was observed as 16.02 mg/dl. The level of urea in raw and processed *A. tricolor* leaf sample extracts treated rat groups were as follows: In raw sample extracts such as ACR 250 mg/kg and ACR 500 mg/kg, ACR 1000 mg/kg treated rat groups recorded 15.82 mg/dl, 15.92 mg/dl and 16.22 mg/dl serum urea respectively. The boiled *A. tricolor* leaf sample extracts treated rat groups such as ACB 250 mg/kg, ACB 500 mg/kg and ACB 1000 mg/kg have also the same pattern of urea concentration such as 15.91 mg/dl, 15.86 mg/dl, and 16.06 mg/dl respectively. Kidney function is also an important aspect to indicate the potential toxicity of a compound. At this juncture, the level of creatinine is found to be an indicator of kidney function. An elevation in its level indicates the impaired glomerular filtration and kidney failure (Rhiouani *et al.*, 2008). Control rats were noticed with a creatinine level of 0.71 mg/dl. Creatinine level of *A. tricolor* treated sample was ranged from 0.69 mg/dl (ACR 250 mg/kg) to 0.74 mg/dl (ACR 1000 mg/kg). Renal functions are evaluated by urea and creatinine dosages and an increase in these markers is an indicator of a negative impact on renal function (Ezeja *et al.*, 2014). An increased level of protein, urea and creatinine in both plasma and urine are considered as significant markers of renal dysfunction. In the present study, there were no significant changes observed in the levels of protein, urea and creatinine in both raw and boiled *A. tricolor* given rats in all doses. The haematopoietic system is one of the most sensitive targets of toxic compounds and is an important index of physiological and pathological status in men and animals.

The lack of significant alterations in the levels of aspartate aminotransferase, alkaline phosphatase, and creatinine, which are good indicators of liver and kidney functions, suggests that sub-chronic administration of extract neither altered hepatocytes and kidneys of rats nor the normal Metabolism of the animals (Das *et al*., 2015). Serum biochemical parameters are used to predict adverse kidney and liver events. Creatinine level is an important parameter that can be used to estimate the glomerular function of the kidney (Abdalla *et al*., 2018).

### Organ Weight- Liver and Kidney

Relative organ weight measurement provides valuable information about the toxicities of the compounds towards targeted organs. The alteration in organ weight could be observed if the tested compound is potentially toxic to them. In the present study, the physical conditions of the organs were normal and there was no sign of swelling, atrophy or hypertrophy. Normal / control group rat had a liver weight of 3.48 g/100 g of body weight and its kidney was 0.688 g/100 g of body weight (Figure 1). Group I was observed with 3.56 g/100 g and 0.692 g/100 g liver and kidney weight, Group II was observed with 3.32 g and 0.686 g liver and kidney per 100 g of body weight, respectively), Group III was observed with 3.27 g and 0.698 g liver and kidney per 100 g of the body weight respectively, Group IV was observed with 3.59 g and 0.681 g of liver and kidney per 100 g body weight, Group V was observed with 3.52 g and 0.67 g liver and kidney per 100 g of body weight and Group VI were observed with 3.41 g and 0.678 g of liver and kidney per 100 g of body weight. There was no difference in the

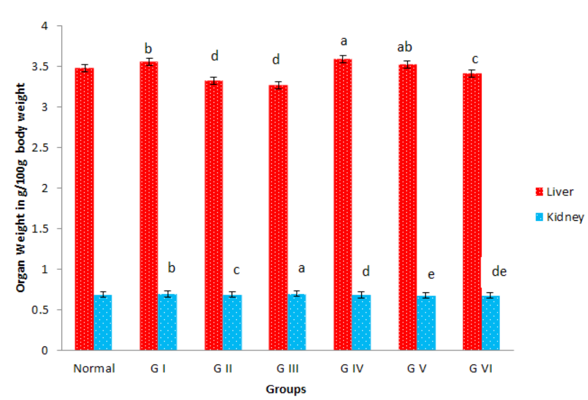



**Fig. 1.** Organ Weights - Liver and Kidney
Values were mean ± SEM; n=6. P >0.05 when compared to control (one-way ANOVA followed by Dunnett's test)

above mentioned relative organ weight between the control animals with the extract-treated animals.

**Histopathology Evaluation**

Histopathological findings following single and repeated doses of the extract provided clear evidence of organ toxicity, similar to those associated with known hepatotoxins and nephrotoxins Husain *et al*., 2018; Olayode *et al*., 2019)·. The relative organ weights of the extract-treated groups were found to be no different from that of respective control group organs. The histopathological evaluation conducted on vital organs such as the brain, liver, lungs, kidney and heart showed normal architecture and no abnormality in the control and extract treated groups. When the histological slides were examined, some minor changes were observed but the changes were found to be minimal and similar observations were marked in the control group as well (Figure 2 and 3).

The kidneys receive about 25% of the cardiac blood flow and any substance that reaches the systemic circulation will reach this organ. Therefore, they are considered as frequent targets of toxicity (Burci *et al.,* 2018). Renal function was evaluated by serum levels of urea and creatinine and by histological analysis. Histopathological evaluation of the vital organs such as liver and kidney displayed no relevant macroscopic or histological changes in animals that received *A. tricolor* extracts. The liver showed normal architecture and also observed no evidence of lesion. The kidney showed adequate glomeruli and normal tubules with normal orientation. The histopathology of liver from normal/control group rats and *A. tricolor* treated group rats are presented in Figure 2. In the liver of the control group (Group I), the cross-section (CS) showed the normal appearance of the liver, central vein, sinusoids, and hepatocytes in a conserved form with regular orientation. *A. tricolor* (250 mg/kg) treated rats showed liver with normal portal tract with hepatocytes. Group III, 500 mg/kg treated rats showed normal hepatocytes and central vein. Normal lobular architecture and central vein were shown in ACR 1000 mg/kg treated rat liver sample. In the case of processed *A. tricolor* sample under both ACB 500 mg/kg and 1000 mg/kg treated rats were observed with normal portal tract with hepatocytes. There was no liver toxicity induced in high doses treated samples also observed.

Assessment of the effect of the test substances on the cellular architecture of the kidney revealed that

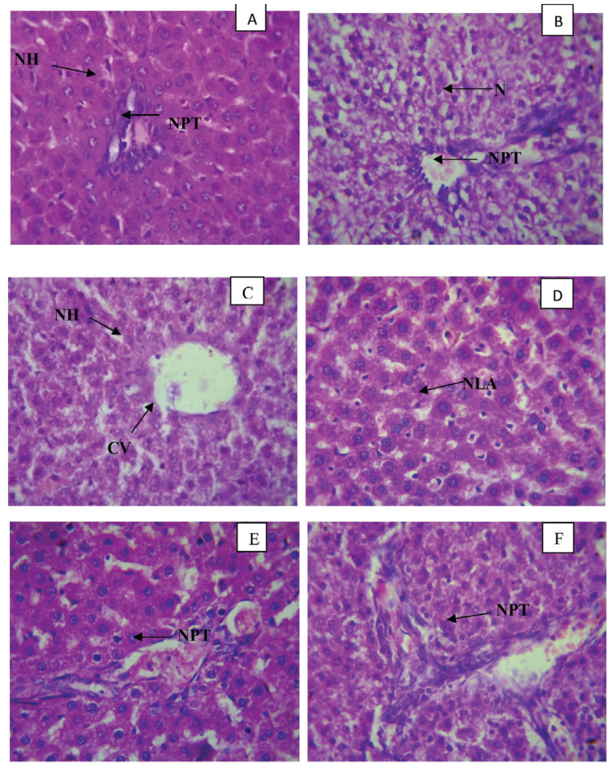



**Fig. 2.** Photomicrographs of Hematoxylin and Eosin Stained Histological Sections of Normal and Different Doses of *A. tricolor* Treated Rat's Liver A) Normal Control (NC); B) ACR 250mg/kg; C) ACR 500 mg/kg; D) ACR 1000 mg/kg; E) ACB 500 mg/kg; F) ACB 1000 mg/kg, NPT-Normal Portal Tract with Hepatocytes, N- Nucleus, NH-Normal Hepatocytes, CV-Central Vein (NLA)-Normal Lobular Architecture.

the control experiment indicates good histoarchitecture, clear distinct proximal and distal tubules; and distinct vascular; urinary poles and normal glomeruli (Figure 3). In the case of raw *A. tricolor* treated (ACR 250 mg/kg), rats showed kidneys with normal glomeruli. There was no significant difference observed between normal/control and processed sample. ACR 500 mg/kg extract-treated rats histopathology reveals the normal tubular cells and ACR 1000 mg/kg treated rats revealed the normal glomeruli cells. On the other hand, boiled *A. tricolor* treated rats did not show any toxic effect on the kidneys and renal functions. ACB 500 mg/kg treated rats were observed with normal tubular cells and the ACB 1000 mg/kg dose received rats were noticed with normal glomeruli and tubular cells in the kidneys. From the histopathological study, we found that in domestic sewage water irri-

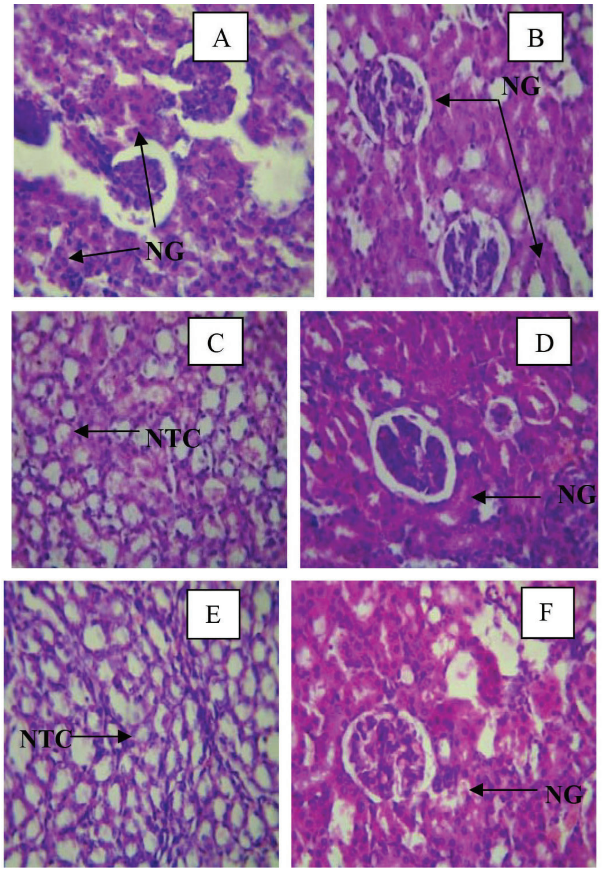
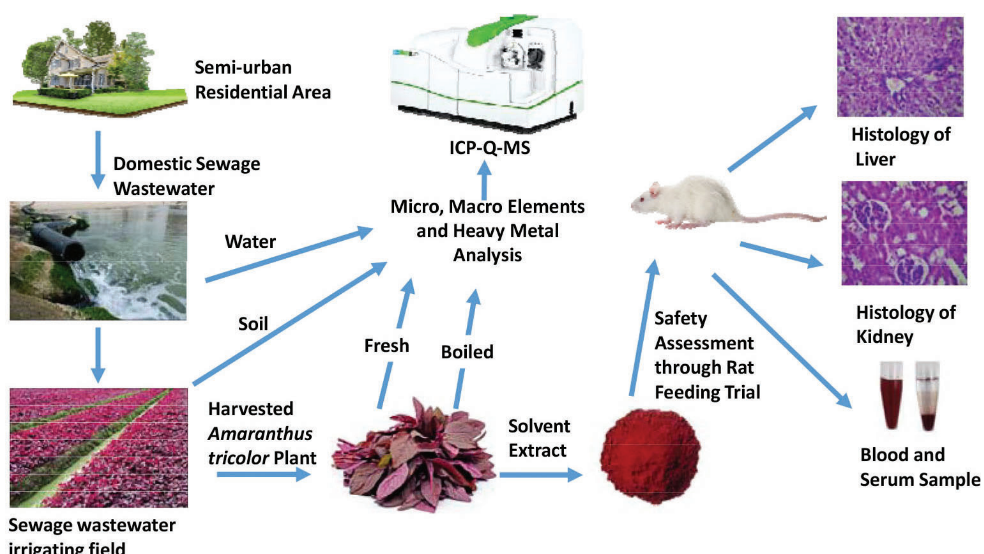



**Fig. 3.** Photomicrographs of Hematoxylin and Eosin Stained Histological Sections of Normal and Different Doses of *A. tricolor* Treated Rats Kidney A) Normal Control (NC); B) ACR 250 mg/kg; C) ACR 500 mg/kg; D) ACR 1000 mg/kg; E) ACB 500 mg/kg; F) ACB 1000 mg/kg. NG- Normal Glomeruli, NTC- Normal Tubular Cells

gated raw and boiled *A. tricolor* treated rat groups after 28 days, the organs showed no significant changes at the cellular level in comparison to control. The histopathological images also confirmed that the treated groups up to the dose level of 1000 mg/kg showed no toxicity in 28 days.

## Conclusion

From the *in vitro* study and mineral analysis, it was found that trace of heavy metals was reported in *Amaranthus tricolor* L. Animal models were commonly used to assess the preliminary toxicity because the early identification of undesirable effects is usually predictive of the toxicity in humans and can also save time. Several parameters were evaluated after conducting the *in vivo* acute and subchronic administration of *Amaranthus tricolor* L. such as haematological and biochemical parameters, liver and kidney weight and histopathological study. Both acute and sub-chronic toxicity studies revealed that there were no drastic changes in organ weight measurement of treated rats compared to the control group. Because of conforming to that, no variation in food and water consumption were recorded and in addition, no variation in haematological, biochemical and histopathological analysis during the acute and sub-acute toxicity studies between control and treated groups were observed. Further, it also confirmed that there was no toxicity or mortality observed between domestic sewage water irrigated to *A. tricolor* including raw and boiled samples. It could be concluded that in domestic sewage water




**Graphical Abstract**

grown crops, uptake of metals may increase nutritional value and improves soil properties, plant growth, and yield without any contamination in soil and toxicity in the crop. However, the continuous monitoring on quality of diluted domestic sewage wastewater irrigation, soil characters with practicing of crop rotation are the more viable and alternative option for the sustainable development of crop production, maintaining of soil fertility and healthy environment consortium including water conservation, reutilization and recycling, food security and zero waste generating agroecosystem.

**Credit Authorship Contribution statement**

**Siddhuraju P**: Conceptualization, Methodology, Supervision, Writing – original draft and Editing**;** **Dhanya V**: Investigation, Methodology, Writing, Data Analysis; **Haritha T Nair:** Writing and Editing: **Roopika S:** Writing and Editing

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgement

The authors (DV and HTN) gratefully acknowledge the financial support provided by the Department of Science and Technology (DST), New Delhi under the INSPIRE Fellowship Program (Grant No: IF150462, Grant No: IF180788) and author PS is also much thankful to the PURSE, FIST under DST, New Delhi and RUSA Programmes under MHRD, New Delhi for the financial assistance and AvH Foundation, Bonn, Germany for the award of Equipment Grant along with a Research Project sanctioned for long term cooperation (Grant No. 3.4 INI/1051260). The authors are also grateful to Bharathiar University, Coimbatore, TN, India for providing the Necessary Research Laboratory Facilities and author, RS is much thankful for University Fellowship.

## References

Abbaspour, N., Hurrell, R. and Kelishadi, R. 2014. Review on iron and its importance for human health. *Journal of Research in Medical Sciences.* 19(2): 164–174.

Abdalla, Y.O.A., Nyamathulla, S., Shamsuddin, N., Arshad, N.M., Mun, K.S., Awang, K. and Nagoor, N.H. 2018. Acute and 2 day sub-acute intravenous toxicity studies of 1'S-1'-acetoxychavicol acetate in rats. *Toxicology and Applied Pharmacology.* 6: 204-213.

Abu Nada, Z.M. 2009. Long Term Impact of Wastewater Irrigation on Soil and Crop Quality Parameters in Gaza Strip. *Long Term Impact of Wastewater Irrigation on Soil and Crop Quality Parameters in Gaza Strip*. The Islamic University. 1-123**.**

Adrover, M., Moyà, G. and Vadell, J. 2017. Seasonal and depth variation of soil chemical and biological properties in alfalfa crops irrigated with treated wastewater and saline ground water. *Geoderma.* 286: 54-63.

Al Omron, A.M., El-Maghraby, S.E., Nadeem, M.E.A., El-Eter, A.M. and Al-Mohani, H. 2012. Long term effect of irrigation with the treated sewage effluent on some soil properties of Al Hassa Governorate, Saudi Arabia. *Journal of the Saudi Society* of *Agricultural Sciences.* 11(1): 15-18.

Alghobar, M.A., Ramachandra, L. and Suresha, S. 2004. Effect of sewage water irrigation on soil properties and evaluation of the accumulation of elements in Grass crop in Mysore city, Karnataka, India. *American Journal of Environmental Protection*. 3(5): 283-291.

Al-Shammiri, M., Al-Saffar, A., Bohamad, S. and Ahmed, M. 2005. Waste water quality and reuse in irrigation in Kuwait using microfiltration technology in treatment. *Desalination.* 185(1-3) : 213-225.

Amin, N.U., Hussain, A., Alamzeba, S. and Begum, S. 2013. Accumulation of heavy metals in edible parts of vegetables irrigated with waste water and their daily intake to adults and children, District Mardan, Pakistan. *Food Chemistry.* 136 : 1515–1523.

APHA, 2005. *Standard Methods For Examination Of Water And Waste Water*. 21st edition. American Public Health Association 800 I street Washington DC 20001-3710.

Araujo, M.C.D.P.M., Barcellos, N.M.S., de Abreu Vieira, P.M., Gouveia, T.M., Guerra, M.O., Peters, V.M. and Saude-Guimaraes, D.A. 2017. Acute and Sub chronic toxicity study of aqueous extract from the leaves and branches of *Campomanesia velutina* (Cambess) O. Berg. *Journal of Ethnopharmacology.* 201: 17-25.

Ashraf, M., Bhat, G.A., Dar, I.Y. and Ali, M. 2012. Physico-Chemical Characteristics of the Grassland Soils of Yusmarg Hill Resort (Kashmir, India). *Ecologia Balkanica.* 4(1): 31-38.

Balkhair, K.S. and Ashraf, M.A. 2016. Field accumulation risks of heavy metals in soil and vegetable crop irrigated with sewage water in western region of Saudi Arabia. *Saudi Journal of Biological Sciences*. 23(1): S32-S44

Bandara, J.M.R.S., Wijewardena, H.V.P and Seneviratne, H.M.M.S. 2010. Remediation of cadmium contaminated irrigation and drinking water: a large scale approach. *Toxicology Letters.* 198(1): 89-92.

Barman, S.C., Sahu, R.K., Bhargava, S.K. and Chaterjee, C.

2000. Distribution of heavymetals in wheat, mustard, and weed grown in field irrigated with industrial effluents. *Bulletin of Environmental Contamination and Toxicology*. 64 (4): 489-496.

Benit, N. and Roslin, A.S. 2015. Physico chemical properties of wastewater collected from different sewage sources. *International Journal* of *Innovative Science Engineering and Technology*. 2(11): 691-696.

Berry, W., Ketterings, Q., Antes, S., Page, S., Russell Anelli, J., Rao, R. and DeGloria, S. 2007. Soil texture. Agronomy fact sheet series, fact sheet 29. Cornell University Cooperative Extension, Ithaca, USA.

Biswas, G.C., Sarkar, A., Rashid, M.H., Shohan, M.H., Islam, M. and Wang, Q. 2015. Assessment of the irrigation feasibility of low-cost filtered municipal wastewater for red amaranth (*Amaranthus cruentus* L cv. Surma). *International Soil* and *Water Conservation Research.* 3(3): 239-252.

Burci, L.M., da Silva, C.B., Rondon, J.N., da Silva, L.M., de Andrade, S.F., Miguel, O.G., Dias, J.D.F.G. and Miguel, M.D. 2018. Acute and subacute (28 days) toxicity, hemolytic and cytotoxic effect of *Artocarpus heterophyllus* seed extracts. *Toxicology Reports.* 23(6): 1304-1308.

Chaturvedi, U.C., Shrivastava, R. and Upreti, R.K. 2004. Viral infections and trace elements: a complex interaction. *Current Science.* 1536-1554.

Chaudhari, P.R., Ahire, D.V., Ahire, V.D., Chkravarty, M. and Maity, S. 2013. Soil bulk density as related to soil texture, organic matter content and available total nutrients of Coimbatore soil. *International Journal* of *Scientific* and *Research Publication*. 3(2): 1-8.

Christapher, P.V., Parasuraman, S., Asmawi, M.Z. and Murugaiyah, V. 2017. Acute and subchronic toxicity studies of methanol extract of *Polygonum minus* leaves in Sprague Dawley rats. *Regulatory Toxicology and Pharmacology.* 86: 33-41.

Cui, Y.J., Zhu, Y.G., Zhai, R.H., Chen, D.Y., Huang, Y.Z., Qui, Y. and Liang, J.Z. 2004. Transfer of metals from near a smelter in Nanning, China. *Environment International.* 30 : 785-791.

Das, N., Goshwami, D., Hasan, M.S. and Raihan, S.Z. 2015. Evaluation of acute and subacute toxicity induced by methanol extract of *Terminalia citrina* leaves in Sprague Dawley rats. *Journal of Acute Disease.* 4(4): 316-321.

Datta, S., Sinha, B.K., Bhattacharjee, S. and Seal, T. 2019. Nutritional composition, mineral content, antioxidant activity and quantitative estimation of water soluble vitamins and phenolics by RP-HPLC in some lesser used wild edible plants. *Heliyon.* 5(3): e01431.

de Lima, R., Guex, C.G., da Silva, A.R.H., Lhamas, C.L., dos Santos Moreira, K.L., Casoti, R. and Manfron, M.P. 2018. Acute and Sub-acute toxicity and chemical constituents of the hydroethanolic extract of *Verbena litoralis* Kunth. *Journal of Ethnopharmacology.* 224: 76-84.

Dhanya, V., Roopika, S. and Siddhuraju, P. 2019. Effect of domestic sewage wastewater irrigation on nutritional and nutraceutical perspectives of *Eleusine coracana* and *Zea mays* (raw and processed) from selected semi-urban and rural areas of Coimbatore, Tamil Nadu. *Human and Ecological Risk Assessment.* 26 (8): 2203-2224.

Dikinya, O. and Areola, O. 2010. Comparative analysis of heavy metal concentration in secondary treated wastewater irrigated soils cultivated by different crops. *International Journal of Environmental Science and Technology.* 7(2): 337-346.

Elgallal, M., Fletcher, L. and Evans, B. 2016. Assessment of potential risks associated with chemicals in wastewater used for irrigation in arid and semiarid zones: A review. *Agricultural Water Management*. 177: 419-431.

Enemugwem, R.I., Baridagara, S.C., Mikue-Yobe, T.F., Ekejiuba, C., Nwileba, A. and Elechi, P. 2016. Effect of heavy metals on cassava tubers (*Manihot esculenta*) and pumpkin leaves (*Telfairia occidentalis*) from prison farm in Elele, Ikwerre LGA of Rivers State, Nigeria. *International Journal* of *Scientific Engineering and Research.* 7(12): 1454-1463.

EPA, 2012.U.S. Agency for International Development. Guidelines for water Reuse; U.S. Environmental Protection Agency, Washington DC, USA.

Ezeja, M.I., Anaga, A.O. and Asuzu, I.U. 2014. Acute and sub-chronic toxicity profile of methanol leaf extract of *Gouania longipetala* in rats. *Journal of Ethnopharmacology.* 151(3): 1155-1164.

FAO. 1992. Wastewater treatment and use in agriculture-FAO irrigation and drainage paper 47. Food and Agricultural Organisation of United states, 92-5-103135-5.

FAO, 1985. Guidelines: land evaluation for irrigated agriculture. Soils Bulletin 55. Food and Agriculture Organization (FAO) of the United Nations (UN), Rome, Italy.

FAO/WHO, 1993. Codex Alimentarius Commission, & Joint FAO/WHO Food Standards Programme. *Codex alimentarius: Residues of veterinary drugs in foods*. Food and Agriculture Organization (FAO) of the United Nations (UN), Rome Italy.

Gupta, U.C. and Gupta, S.C. 1998. Trace element toxicity relationships to crop production and livestock and human health: implications for management. *Communications in Soil Science and Plant Analysis.* 29(11-14): 1491-1522.

Hamdi, H., Hechmi, S., Khelil, M.N., Zoghlami, I.R., Benzarti, S., Mokni-Tlili, S., Hassen, A. and Jedidi, N. 2019. Repetitive land application of urban sewage sludge: Effect of amendment rates and soil texture on fertility and degradation parameters. *Catena.*

172 : 11-20.

Hassan, Z., Anwar, Z., Khattak, K.U., Islam, M., Khan, R.U. and Khattak, J.Z.K. 2012. Civic pollution and its effect on water quality of river Toi at district Kohat, NWFP. *Research Journal* of *Environmental* and *Earth Sciences.* 4(3): 334-339.

Hor, S.Y., Ahmad, M., Farsi, E., Lim, C.P., Asmawi, M.Z. and Yam, M.F. 2011. Acute and subchronic oral toxicity of *Coriolus versicolor* standardized water extract in Sprague–Dawley rats. *Journal of Ethnopharmacology.* 137 : 1067–1076.

Hor, S.Y., Ahmad, M., Farsi, E., Yam, M.F., Hashim, A.M., Lim, C.P., Sadikun, A. and Asmawi, M.Z. 2012. Safety assessment of methanol extract of red dragon fruit (*Hylocereus polyrhizus*): Acute and subchronic toxicity studies. *Regulatory Toxicology and Pharmacology.* 63: 106–114.

Husain, H., Latief, U. and Ahmad, R. 2018. Pomegranate action in curbing the incidence of liver injury triggered by Diethylnitrosamine by declining oxidative stress via Nrf2 and NFêB regulation. *Scientific Reports.* 8(1): 1-17.

Khan, M.J., Azeem, M.T., Jan, M.T. and Perveen, S. 2012. Effect of amendments on chemical immobilization of heavy metals in sugar mill contaminated soils. *Soil and Environment.* 31(1): 55-66.

Khan, S., Farooq, R., Shahbaz, S., Khan, M.A. and Sadique, M. 2009. Health risk assessment of heavy metals for population via consumption of vegetables. *World Applied Sciences Journal.* 6(12): 1602-1606.

Kiziloglu, F.M., Turan, M., Sahin, U., Kuslu, Y. and Dursun, A. 2008. Effects of untreated and treated wastewater irrigation on some chemical properties of cauliflower (*Brassica olerecea* L. var. botrytis) and red cabbage (*Brassica olerecea* L. var. rubra) grown on calcareous soil in Turkey. *Agricultural Water Management.* 95(6) : 716-724.

Kumar, V., Chopra, A.K. and Srivastava, S. 2014. Distribution, enrichment and accumulation of heavy metals in soil and *Vigna mungo* L. Hepper (Black Gram) after irrigation with distillery Wastewater. *Journal* of *Environment* and *Health Science.* 1(1): 1-8.

Kumar, V. and Chopra, A.K. 2013, Distribution, enrichment and accumulation of heavy metals in soil and *Trigonella foenum-graecum* L. (Fenugreek) after fertigation with paper mill effluent. *Open Journal of Metal.* 3(2A)L : 8-20.

Lu, S., Wang, J. and Pei, L. 2016. Study on the Effects of Irrigation with Reclaimed Water on the Content and Distribution of Heavy Metals in Soil. *International Journal of Environmental Research and Public Health.* 13(3) : 298.

Luo, C., Liu, C., Wang, Y., Liu, X., Li, F., Zhang, G. and Li, X. 2011. Heavy metal contamination in soils and vegetables near an e-waste processing site, south China. *Journal of Hazardous Materials.* 186(1): 481-490.

Marshall, T.J. and Holmes, J.W. 1999. *Soil Physics.* Great Britain: Cambridge University Press. UK, p- 374**.**

Mastro, A.M.D., Londonio, A., Rebagliati, R.J., Pereyra, M., Dawidowski, L. and Gómez, D. 2015. Plasma-based techniques applied to the determination of 17 elements in partitioned top soils. *Microchemical Journal.* 123: 224-229.

Millaleo, R., Reyes-Díaz, M., Ivanov, A. G., Mora, M. L. and Alberdi, M. 2010. Manganese as essential and toxic element for plants: transport, accumulation and resistance mechanisms. *Journal of Soil Science and Plant Nutrition.* 10(4) : 470-481.

Motsara, M.R. and Roy, R.N. 2008. Guide to Laboratory Establishment for plant Nutrient Analysis; FAO Fertilizer and Plant Nutrient Bulletin 19, Rome, Italy,

Munir, A.H., John, B., Gemma, C., Fenghua, Z. and Tamara, M.J. 2012. Wastewater irrigation and environmental health: Implications for water governance and public policy. *International Journal of Hygiene and Environmental Health.* 215 : 255-269.

Nazir, R., Khan, M., Masab, M., Rehman, H. U., Rauf, N. U., Shahab, S., Ameer, N., Sajed, M., Ullah, M., Rafeeq, M. and Shaheen, Z. 2015. Accumulation of heavy metals (Ni, Cu, Cd, Cr, Pb, Zn, Fe) in the soil, water and plants and analysis of physico-chemical parameters of soil and water collected from Tanda Dam Kohat. *Journal of Pharmaceutical Sciences and Research. 7*(3) : 89-97.

OECD, 2002. Acute oral toxicity. Acute oral toxic class method guideline 423 adopted 23.03.1996. In: Eleventh Addendum to the, OECD, Guidelines for the Testing of Chemicals. Organization for Economic Co-operation and Development, Paris, France**.**

OECD, 2008. Test No. 407: Repeated Dose 28-day Oral Toxicity Study in Rodents. OECD Guidelines for the Testing of Chemicals. Organization for Economic Cooperation and Development, Paris, France.

Olayode, O.A., Daniyan, M.O. and Olayiwola, G. 2019. Biochemical, hematological and histopathological evaluation of the toxicity potential of the leaf extract of *Stachytarpheta cayennensis* in rats. *Journal of Traditional and Complementary Medicine*. 10(6) : 544-554.

Olowoyo, J.O., van Heerden, E., Fischer, J.L. and Baker, C. 2010. Trace metals in soil and leaves of *Jacaranda mimosifolia* in Tshwane area, South Africa. *Atmospheric Environment.* 44 : 1826-1830.

Osakwe, S.A. and Okolie, L.P. 2015. Physico chemical characteristics and heavy metals contents in soils and cassava plants from farmlands along a major highway in Delta State, Nigeria. *Journal of Applied Sciences and Environmental Management.* 19(4): 695-704.

Rhiouani, H., El-Hilaly, J., Israili, Z.H. and Lyoussi, B. 2008. Acute and sub-chronic toxicity of an aqueous extract of the leaves of *Herniaria glabra* in rodents. *Journal of Ethnopharmacology.* 118(3): 378-386.

Rout, G.R. and Sahoo, S. 2015. Role of iron in plant growth and metabolism. *Reviews in Agricultural Science.* 3: 1-24.

Rusan, M.J.M., Hinnawi, S. and Rousan, L. 2007. Long term effect of wastewater irrigation of forage crops on soil and plant quality parameters. *Desalination.* 215(1-3): 143-152.

Satter, M.M.A., Khan, M.M.R.L., Jabin, S.A., Abedin, N., Islam, M.F. and Shaha, B. 2016. Nutritional quality and safety aspects of wild vegetables consume in Bangladesh. *Asian Pacific Journal of Tropical Biomedicine.* 6(2): 125-131.

Singh, A., Sharma, R.K., Agrawal, M. and Marshall, F.M. 2010. Risk assessment of heavy metal toxicity through contaminated vegetables from waste water irrigated area of Varanasi, India. *Tropical Ecology.* 51(2): 375-387.

Singh, P.K., Deshbhratar, P.B. and Ramteke, D.S. 2012. Effect of sewage wastewater irrigation on soil properties, crop yield and environment. *Agricultural Water Management.* 103 : 100-104.

Singh, S.K. 2007. Effect of irrigation with paper mill effluent on the nutrient status of soil. *International Journal of Soil Science.* 2(1) *:* 74-77.

Tasrina, R.C., Rowshon, A., Mustafizur, A.M.R., Rafiqul, I. and Ali, M.P. 2015. Heavy metals contamination in vegetables and its growing soil. *Journal of Environmental Analytical Chemistry.* 142(2): 2380-2391.

Wang, L., Li, Z., Li, L., Li, Y., Yu, M., Zhou, Y., Lv, X., Arai, H. and Xu, Y. 2014. Acute and sub-chronic oral toxicity profiles of the aqueous extract of *Cortex dictamni* in mice and rats. *Journal of Ethnopharmacology.* 158: 207-215.

Wasfi, I.A. 1994. Some Pharmacological Studies on *Citrullus colocynthis*. *Journal* of *Herbs*, *Spices and Medicinal Plants.* 2(2): 65-79.

WHO, 1994. Health guidelines for the use of wastewater in agriculture and aquaculture, Technical report series, 778, Geneva, Switzerland.

WHO, 1995. International Programme on Chemical Safety (IPCS), Environmental Health Criteria 165-Inorganic Lead, World Health Organisation, Geneva, Switzerland.

WHO, 2006. Guidelines for the safe use of wastewater, excreta and grey water. Vol. 2. Wastewater Use in Agriculture, Geneva, Switzerland.

Wilberforce, J.O.O. 2016. Heavy Metals in Tapioca Locally Processed by Sun-Drying Method along Enugu-Abakaliki Highway in Ezilo, Ebonyi State, Nigeria. *Journal of Environmental Science*, *Toxicology* and *Food Technology.* 10(7) : 85-87.