An environmental challenge from leather processing arises from the nature and quality of the chemicals used along with the amount of wastes generated and discharged. Thus the effluent from tannery industry is considered as a serious environmental threat throughout the world (Gupta and Sinha, 2007). The tannery industries discharge large quantities of common salt during the process of tanning and deposition of this salt into the soil takes place when the effluent comes in contact with the soil. Besides chlorides, toxic substances like chromium, sodium sulphide, sodium carbonate and ammonium sulphate are present in the discharged effluent which manifolds the soil pollution (Rajan and Arias, 2007).

The continuous input of wastes containing toxic metals on the agricultural land causes imbalance in ecosystem. The plants growing under such habitats accumulate high amount of toxic metals, which in turn are being assimilated and transferred within food chain by the process of biomagnification and results in various health hazards to animal and human beings (Adesodun et al., 2009).

The untreated industrial effluents and agriculture wastes are often discharged into the water bodies. This contaminated water spreads a wide range of water borne diseases around water bodies (Morales et al., 2007).

Hence in the present study the effect of tannery effluent on water and soil samples in the target area were analysed. In the next phase the effect of diluted tannery effluent on selected plants were also studied. In the third phase the impact of effluent on the health status of tannery workers based on their years of exposure were studied. The experimental procedure adopted for the study is detailed as follows.
Results and Discussion

Effect of tannery effluent on water and soil profile, plant growth and human health

PHASE I

3.1 Collection and characterization of tannery effluent, target area water and soil samples

3.1.1 Selection of the tannery industry
3.1.2 Selection of the target area
3.1.3 Collection of the tannery industry effluent
3.1.4 Characterization of the tannery industry effluent
3.1.5 Collection of target area water samples
3.1.6 Biochemical profile of target area water samples
3.1.7 Collection of soil samples
3.1.8 Physicochemical characterization of soil samples

PHASE II

3.2 Growth studies of selected plants using diluted tannery effluent

3.2.1 Preparation of different dilutions of tannery effluent
3.2.2 Selection of two crop plants for the study
3.2.3 Preparation of soil samples for pot culture experiment
3.2.4 Seed collection
3.2.5 Design and layout of the experiment
3.2.6 Biometric observations of the plant
3.2.7 Biochemical analysis of the plant samples
3.2.8 Histochemical observation of the root samples
3.2.9 Measurement of yield parameters

PHASE III

3.3 Biomonitoring the clinical status of the tannery workers

3.3.1 Selection of the participants for the study
3.3.2 Grouping of the participants
3.3.3 Collection of blood and urine samples from the participants
3.3.4 Assessment of selected hematological parameters
3.3.5 Assessment of liver function
3.3.6 Assessment of renal function
3.3.7 Assessment of metal contents
PHASE I

3.1 Collection and characterization of the tannery effluent, target area water and soil samples

3.1.1 Selection of the tannery industry

Waste generated from tanning generally contains much higher concentrations of total dissolved solids, suspended solids, phenols, chromium, chlorides, ammonia, and heavy metals (Shukla et al., 2007). Besides these, chemicals like zinc chloride, mercuric chloride and formaldehyde used as disinfectants, sodium chloride in curing and as bleaching powder and sodium fluoride to prevent putrefaction, lime in liming, sodium sulphate, ammonium chloride, borax and hydrochloric acid in deliming, sodium for decreasing and basic or acidic dyes in leather finishing are used (Barrerea and Urbina, 2007).

More than 80 tannery industries are located in Dindigul. Many are situated in Begampur, Madurai bypass, Chinnalapatty and other areas in Dindigul. Waste water generated from these industries are released into the ground and in the pond named Thamaraikulam.

Tannery effluents being highly voluminous, when discharged, damage the normal life of the living and if allowed to percolate into the ground affect the water table and soil of that locality. An assessment of the ecological damage of the affected area is very essential taking into consideration the ground water damage, soil damage and natural habitat damage (plants) around the industrial site.

Hence in the present study, the tannery industry located in Dindigul bypass was selected to assess its physicochemical characteristics and effect on components of biosphere.

3.1.2 Selection of the target area

The target area selected for collection of soil and water samples is within one kilometer radius of effluent discharge site. Area 15 kms away from the
3.1.3 Collection of the tannery industry effluent

Fifteen litres of the effluent samples were collected from the tannery industry in clean plastic cans and stored at 4 °C for the analysis. The effluent was directly collected from the outlet of the industry.

3.1.4 Characterization of the tannery industry effluent

Tanning industries, due to the complexity of transformation of the animal hide or skin into leather, use a great number of chemical agents and produce an enormous volume of residual waters and solid residues (Wionczyk et al., 2006). Earlier studies by Babu et al. (2007) suggested that tannery waste water presented a high concentration of biological oxygen demand, chemical oxygen demand, total dissolved solids, ammonia, nitrate and sulphate when the samples were collected from the outlet of the industry. To characterize the tannery effluent, selected physiochemical characteristics were analyzed in the effluent samples. The procedures adopted for the study are given in Table 2.

TABLE 2

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Method of analysis</th>
<th>References</th>
<th>Appendix No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour</td>
<td>Visual</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Odour</td>
<td>Sense</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Turbidity</td>
<td>Visual</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>pH</td>
<td>Digital pH meter</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total hardness</td>
<td>EDTA titrimetric method</td>
<td>APHA (2005)</td>
<td>1</td>
</tr>
<tr>
<td>Electrical conductivity</td>
<td>Conductivity method</td>
<td>APHA (2005)</td>
<td>2</td>
</tr>
<tr>
<td>Total suspended solids</td>
<td>Filtration method</td>
<td>APHA (2005)</td>
<td>3</td>
</tr>
</tbody>
</table>
Results and Discussion

Effect of tannery effluent on water and soil profile, plant growth and human health

Characteristics | Method of analysis | References | Appendix No.
--- | --- | --- | ---
Total dissolved solids | Filtration method | APHA (2005) | 4
Chemical oxygen demand | Titrimetric method | APHA (2005) | 5
Biological oxygen demand | Winkler’s Iodometric method | APHA (2005) | 6
Carbonate and bicarbonate | Titrimetric method | Natarajan et al. (1988) | 7
Calcium | EDTA titrimetric Method | APHA (2005) | 8
Magnesium | Calculation method | APHA (2005) | 9
Chloride | Silver nitrate titricmetric method | Vogel (1978) | 10
Sodium and potassium | Flame photometric method | Natarajan et al. (1988) | 11
Fluoride | Ion selective electrode method | APHA (2005) | 12
Nitrite | Colorimetric method | APHA (2005) | 14
Sulphate | Turbidometric | APHA (2005) | 15
Chromium, nickel, zinc and cadmium | Atomic absorption spectrophotometric method | APHA (2005) | 16

3.1.5 Collection of target area water samples

Most of the hazards coming to human and ecosystem are due to water pollution. The untreated sewage, industrial effluents and agriculture wastes are often discharged into the water bodies. This contaminated water spread wide range of water borne diseases. The agricultural field around these water bodies are affected (Waziri and Ogugbauja, 2010). Indiscriminate discharge of effluents (either from industrial, municipal and agricultural activities) containing toxic substances into aquatic environment, create problems of water pollution rendering water no longer fit for drinking, agriculture and aquatic life (Bailey et al., 2005).
In this study, potable water sample near the target area (within one kilometer radius) was collected for the analysis and a sample of water from a distant area (15 kilometers away), free from industrial pollution, and was also collected for comparison.

3.1.6 Biochemical profile of target area water samples

The parameters analyzed were pH, electrical Conductivity, turbidity, total alkalinity, total solids, total dissolved solids, total suspended solids, total hardness, chromium, nickel, zinc and cadmium. The method of analysis and the corresponding appendix numbers for detailed procedures are given in Table 3.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method of analysis</th>
<th>References</th>
<th>Appendix No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>Digital pH meter</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Electrical conductivity</td>
<td>Conductivity method</td>
<td>APHA, (2005)</td>
<td>2</td>
</tr>
<tr>
<td>Turbidity</td>
<td>Visual</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total alkalinity</td>
<td>Titrimetric method</td>
<td>Natrajan et al. (1988)</td>
<td>7</td>
</tr>
<tr>
<td>Total suspended solids</td>
<td>Filtration method</td>
<td>APHA, (2005)</td>
<td>3</td>
</tr>
<tr>
<td>Total dissolved solids</td>
<td>Filtration method</td>
<td>APHA (2005)</td>
<td>4</td>
</tr>
<tr>
<td>Total hardness (calcium and magnesium hardness)</td>
<td>EDTA titrimetric method</td>
<td>APHA (2005)</td>
<td>1</td>
</tr>
<tr>
<td>Fluoride</td>
<td>Ion selective electrode method</td>
<td>APHA (2005)</td>
<td>12</td>
</tr>
<tr>
<td>Chloride</td>
<td>Silver nitrate titrimetric method</td>
<td>Vogel (1978)</td>
<td>10</td>
</tr>
<tr>
<td>Chromium, nickel, zinc and cadmium</td>
<td>Atomic absorption spectrophotometric method</td>
<td>APHA (2005)</td>
<td>16</td>
</tr>
</tbody>
</table>
3.1.7 Collection of soil samples

Dindigul area is rich in red soil and black soil. People cultivate seasonal crops in these soil types. In the present study, samples of both the soils were selected separately and analysed for the effect of tannery effluent on these soil types. Their influence on the plant growth was also studied.

The soil samples were collected from a depth of 15 cm with a wooden spade, dried and crushed. It was sieved using a steel sieve and filled in plastic cans. Sampling was done from areas within one kilometer radius of effluent discharge site and another from a distant area (15 kilometers away), which was free from industrial pollution and compared.

3.1.8 Characterization of soil samples

The soil samples were analyzed for pH, electrical conductivity, sodium, phosphorus, nitrogen, potassium, calcium, magnesium, chromium, zinc, iron, copper, nickel and cadmium. The details are given in Table 4.

TABLE 4

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method of analysis</th>
<th>References</th>
<th>Appendix No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>Digital pH meter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Electrical</td>
<td>Conductivity method</td>
<td>APHA (2005)</td>
<td>2</td>
</tr>
<tr>
<td>conductivity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium</td>
<td>Flame photometric Method</td>
<td>Natarajan et al. (1988)</td>
<td>11</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>Titrimetric method</td>
<td>Humphrie (1956)</td>
<td>17</td>
</tr>
<tr>
<td>Phosphorous</td>
<td>Spectrophotometry</td>
<td>Jackson (1973)</td>
<td>18</td>
</tr>
<tr>
<td>Potassium</td>
<td>Flame photometric method</td>
<td>Natarajan et al. (1988)</td>
<td>11</td>
</tr>
</tbody>
</table>
### Results and Discussion

#### Effect of tannery effluent on water and soil profile, plant growth and human health

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method of analysis</th>
<th>References</th>
<th>Appendix No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>Titrimetric method</td>
<td>Raghuramulu et al. (2003)</td>
<td>8</td>
</tr>
<tr>
<td>Magnesium</td>
<td>Titrimetric method</td>
<td>Raghuramulu et al.(2003)</td>
<td>9</td>
</tr>
<tr>
<td>Copper</td>
<td>Colorimetric method</td>
<td>Raghuramulu et al.(2003)</td>
<td>19</td>
</tr>
<tr>
<td>Iron</td>
<td>DTPA method</td>
<td>Shanmugam et al. (1994)</td>
<td>20</td>
</tr>
<tr>
<td>Chromium, nickel, zinc and cadmium</td>
<td>Atomic absorbtion spectrophotometric method</td>
<td>APHA (2005)</td>
<td>16</td>
</tr>
</tbody>
</table>

**PHASE II**

#### 3.2 Growth studies of selected plants using diluted tannery effluent

With the high generation of tannery effluent from the tannery industry, the possibility for using the effluent as an irrigant for crops were studied (Thirunavukarasu and Lourdraj, 2005). With the possibility of water scarcity increasing it has become the need of the hour to investigate on alternative irrigation sources. The effluent from industries can reduce the pressure on water scarcity for irrigation. Hence in the present study various dilutions of the selected tannery effluent were used for plant growth.

#### 3.2.1 Preparation of different dilutions of tannery effluent

The tannery effluent collected from the discharge site was diluted as follows and used for the pot study.

- 25% - 25ml tannery effluent + 75 ml water
- 50% - 50ml tannery effluent + 50 ml water
- 75% - 75ml tannery effluent + 25 ml water
- 100% - 100ml tannery effluent
3.2.2 Selection of two crop plants for the study

Plants used for the study should be tolerant to metal contamination, should have fast growth and should translocate the metals to the non edible parts. *Vigna radiata* and *Vigna mungo* are very tolerant crops and grows healthy in contaminated soil and accumulates toxic metal ions in plant parts (Samantary, 2002). They are widely grown as mixed, inter crop or in rotation to improve nitrogen status of soil in most parts of the country. For their high nutritive value they are important agricultural crops as a food source crops in the poorer countries of Asia (Sinha *et al.*, 2008). Seedlings of this plant could be used as accumulators of common toxic metals such as arsenic, lead, nickel and chromium (Rout *et al.*, 2001).

Hence in the present study the plants *Vigna radiata* and *Vigna mungo* were selected for the growth studies with diluted tannery effluent.

3.2.3. Preparation of soil samples for pot culture experiment

Pot culture experiment was conducted for a period of 100 days. Red soil and black soil free from pebbles and stones were filled in pots separately. 10 kg of soil and sand in the ratio of 3:1 were filled before sowing.

3.2.4 Seed collection

Seeds of *Vigna radiata* and *Vigna mungo* were collected from Tamilnadu Agricultural Universitry, Coimbatore.

3.2.5 Design of the experiment

The seeds of the plant *Vigna radiata* and *Vigna mungo* were grown with red and black soil. There was no germination observed when seeds were grown with 100% effluent while with 75% effluent the leaf tips of the seedlings were burned, loops were formed in the young emerging leafs, leaves failed to expand and root tips turned brown and necrotic. Hence the study was continued with 25% and 50% effluent. Plants grown with water served as control (in both red and black soil). Plate 2 and 3 shows the growth pattern of the plants *Vigna radiata* and *Vigna mungo* respectively.
PLATE 2
GROUPING OF *Vigna radiata*

C1R - Control (Red soil + water)  T1R - Red Soil + 25% effluent
T2R - Red Soil + 50% effluent  C1B - Control (Black soil + water)
T1B - Black Soil + 25% effluent  T1B - Black Soil + 50% effluent
PLATE 3
GROUPING OF *Vigna mungo*

C1R - Control (Red soil + water)  
T1R - Red Soil + 25 % effluent  
T2R - Red Soil + 50 % effluent  
C1B - Control (Black soil + water)  
T1B - Black Soil + 25 % effluent  
T2B - Black Soil + 50 % effluent
Plants were grouped as follows:

- **C1R** - Control (Red soil + water)
- **T1R** - Red Soil + 25 % effluent
- **T2R** - Red Soil + 50 % effluent
- **C1B** - Control (Black soil + water)
- **T1B** - Black Soil + 25 % effluent
- **T1B** - Black Soil + 50 % effluent

Twenty seeds were sown in each pot and after germination. Fifteen healthy plants were maintained for establishment.

### 3.2.6 Biometric observations of the plant samples

Plant samples were uprooted carefully after 30, 60 and 90 days after sowing for recording the following biometric observations.

#### 3.2.6.1 Germination percentage

The number of seeds which germinated after sowing was counted and the percentage was calculated.

#### 3.2.6.2 Vigour index

Vigour index was calculated by the formula

\[
V.I. = \frac{(\text{shoot length} + \text{root length}) \times \text{germination percentage}}{100}
\]

#### 3.2.6.3 Root length and shoot length

The root length was measured from ground level to the tip of the root and shoot length was measured from ground level to the tip of the plant.

#### 3.2.6.4 Fresh weight and dry weight

The whole plant was weighed for fresh weight and it was dried at 70°C for 24 hrs and weighed for dry weight.
3.2.6.5 Number of leaves and number of roots

Number of leaves was counted and number of lateral roots was also counted.

3.2.7. Biochemical analysis of the plant samples

The selected biochemical constituents namely chlorophyll, flavanoid, carotenoid, carbohydrate, proteins, ascorbic acid, riboflavin, tocopherol, catalase, peroxidase, superoxide dismutase, chromium, nickel, cadmium and zinc were analysed in the plant samples of *Vigna radiata* and *Vigna mungo* collected at 30, 60 and 90 days after sowing (DAS).

3.2.7.1. Chlorophyll

Chlorophyll content in the leaves were estimated by the method of Yoshida *et al.* (1971). The detailed procedure of which is given in Appendix 21.

3.2.7.2. Carotenoid

Carotenoid contents in the leaves and seeds were estimated by the method of Zakaria *et al.* (1979). The procedure is detailed in Appendix 22.

3.2.7.3. Carbohydrate

Carbohydrate contents in the leaves and seeds were estimated by the method of Dubois (1956). Detailed procedure is given in Appendix 23.

3.2.7.4. Protein

Protein contents in the leaves and seeds were estimated by the method of Lowry *et al.* (1951). The procedure in detail is given in Appendix 24.

3.2.7.5. Total phenol

Total phenol content in the seeds of the plants was estimated by the method of Malick and Singh (1980). Detailed procedure is given in Appendix 25.
3.2.7.6. Protein profile of the seeds

The protein profile and the molecular weight of the seed protein was determined by Sodium dodecyl sulphide – polyacrylamide gel electrophoresis by Sambrook et al. (1989). The detailed procedure is given in Appendix 26.

3.2.7.7. Catalase

Catalase contents in leaves and seeds were estimated by the method of Luck (1974). Appendix 27 gives the detailed procedure.

3.2.7.8. Peroxidase

Peroxidase contents in the leaves and seeds were estimated by the method of (Reddy et al., 1995). Detailed procedure is given in Appendix 28.

3.2.7.9. Superoxide dismutase

Superoxide dismutase contents in the leaves and seeds were estimated by the method of Kakkar et al.(1984). The procedure is detailed in Appendix 29.

3.2.7.10. Glutathione reductase

Glutathione reductase contents in the leaves and seeds were estimated by the method of Glatzle et al.(1970). Detailed procedure is given in Appendix 30.

3.2.7.11 Ascorbic acid

Ascorbic acid contents in the leaves and seeds were estimated by the method of Roe and Kuether (1953). Detailed procedure is given in Appendix 31.

3.2.7.12. Riboflavin

Riboflavin contents in the leaves and seeds were estimated by fluoimetric method (Raghuramulu et al., 2003). Detailed procedure is given in Appendix 32.
3.2.7.13. Flavanoid

Flavonoid content in the leaves were estimated by the method of Cameron et al. (1943). Detailed procedure is given in Appendix 33.

3.2.7.14. Tocopherol

Tocopherol contents in the leaves and seeds were estimated by the method of Varley et al. (1991). Detailed procedure is given in Appendix 34.

3.2.7.15. Chromium, nickel, zinc and cadmium

Chromium, nickel, zinc and cadmium contents in the roots, shoots, leaves and seeds were estimated by atomic absorption spectroscopy, (APHA, 2005). The detailed procedure is given in Appendix 16.

3.2.8. Histochemical chemical observations of root samples

Sections of root samples were processed and observed for metal accumulation. The procedure followed was silver sulphide method of heavy metals by Timm (1958), given in Appendix 35.

3.2.9. Measurement of yield parameters

3.2.9.1 Number of nodules

The number of nodules in the roots of the plants after 30, 60 and 90 days were recorded.

3.2.9.2. Flowering time

The day on which plants of Vigna radiata and Vigna mungo flowered were noted.

3.2.9.3. Pod length, number of pods/plant, number of seeds/pod, number of seeds/plant, hundred seed weight, and total seed weight/plant

All these parameters were recorded in both the plants grown in both the soils after 90 days after sowing.
The enormous pollution load along with the toxic nature of wastewater makes the tanneries a potential threat to the areas in the vicinity of their location. The tannery wastewater is being contaminated with high levels of metals causes serious health hazards (Sinha et al., 2006). An alarming number of chemicals are used in leather making which are proved to be potentially toxic.

Since tannery industry has found its market and existence in Dindigul for more than three decades, it has become the prime source of employment for the people around that locality. Hence assessment of the clinical status of the workers was done to investigate the effects of pollutants handled, on their health conditions.

3.3.1. Selection of the participants for the study

Forty male and female workers in the age group 20 – 50 years involved in various stages of tanning processes in various tannery industries located in Dindigul were selected for the study. They were selected based on the following criteria.

1. They should have been working in tanning industry for at least 5 years.

2. They should not have been exposed to any other forms of pollutants.

3. They should not suffer from any serious illness or should not have undergone any treatment procedures.

Twenty age and sex matched control group were selected from Muthanampatty, a village 15 km away from Dindigul city, as control group.

3.3.2 Grouping of participants

Group I – Control group of 20 participants in the age group of 20 – 50 years.
Results and Discussion

Effect of tannery effluent on water and soil profile, plant growth and human health

Based on the number of years of exposure in tannery industry the participants were categorized into two groups

Group II – Twenty workers with one to five years of exposure.
Group III – Twenty workers with five to ten years of exposure.

3.3.3 Collection of blood and urine samples

a. Blood

6.0 ml of venous blood was collected from each participant. 4.0 ml of the collected blood was allowed to clot and the serum was separated (Oser, 1976). 2.0 ml of the blood was transferred to a tube containing EDTA for whole blood sample. Plate 4 shows the blood sample collection process by the investigator.

b. Urine

Twenty four hour urine samples were collected from the participants.

PLATE 4

BLOOD SAMPLE COLLECTION
3.3.4 Assessment of selected hematological parameters

Haematological parameters namely hemoglobin, total leucocyte count and immunoglobulin E were determined in the blood samples. The method of analysis of hematological parameters, are given in Table 5.

TABLE 5
HAEMATOLOGICAL PARAMETERS ANALYSED IN HUMAN PARTICIPANTS

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method of analysis</th>
<th>Reference</th>
<th>Appendix no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin</td>
<td>Cyanmethemoglobin</td>
<td>Wintrobe et al., (1965)</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>method</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total leucocyte</td>
<td></td>
<td>Raguramulu et al.,(2003)</td>
<td>37</td>
</tr>
<tr>
<td>count</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immunoglobulin E</td>
<td>Quorum EIA ferritin</td>
<td>Nakamura et al., (1991)</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>method</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3.3.5 Assessment of liver function

A chronic dose of zinc, cadmium, nickel and chromium increases the risk of developing anemia, damage to pancreas, lowers down HDL cholesterol levels and raises LDL cholesterol levels and causes renal damage (Sharma and Agrawal, 2005). To find out the adverse effects of these metals on the health status of the workers, the following parameters were done. The serum of the participants was used for the estimation of alanine transaminase, aspartate transaminase, alkaline phosphatase, acid phosphatase, lactate dehydrogenase, urea, uric acid, creatinine, chromium, nickel, zinc and cadmium. Urine samples were analysed for urea, uric acid, creatinine, chromium, nickel, zinc and cadmium.

3.3.5.1 Alanine transaminase

Activities of alanine transaminase were determined by the method of Reitman and Frankel (1957). Detailed procedure is given in Appendix 39.

3.3.5.2 Aspartate transaminase

Activities of aspartate transaminase were determined by the method of Reitman and Frankel (1957). Detailed procedure is given in Appendix 40.
3.3.5.3. Alkaline phosphatase

Activities of alkaline phosphatase were determined by the method of King and Armstrong (Varley et al., 1991). Detailed procedure is given in Appendix 41.

3.3.5.4 Acid phosphatase

Activities of acid phosphatase were determined by the method of Moog (1946). Detailed procedure is given in Appendix 42.

3.3.5.5 Lactate dehydrogenase

Activities of lactate dehydrogenase were determined by the King’s colorimetric method (Varley et al., 1991). Detailed procedure is given in Appendix 43.

3.3.6 Assessment of renal function

Urea, uric acid and creatinine contents in the blood and urine samples were determined by Diacetylmonoxime method (NIN manual 2003), Caraway method (Varley et al., 1991) and Alkaline picrate method (Varley et al., 1991). The detailed procedures are given in Appendix 44, 45 and 46.

3.3.7. Assessment of metal contents

Chromium, nickel, zinc and cadmium contents were determined in the blood and urine samples by atomic absorption spectroscopy (APHA, 2005). Detailed procedure is given in Appendix 16.

3.4. Statistical analysis

The results obtained were reported as mean ± SD. One way and three way Analysis of Variance (ANOVA) were performed to analyze statistical significance of the data at p<0.05.