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Modified Continuous Flow Respirometer To Measure the Effect of Tannery Effluents on the Respiration of Earthworms

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Chromium that is extensively discharged into the environment by the tanning industry (Rajamani and Madhavkrishna, 1982) is several times higher than the background levels of the element (Elwood et al., 1980; Rajendra Babu and Nandkumar, 1982; Somashekhar et al., 1982). Discharged through "dug out" culverts these effluents adversely affect soil organisms, especially earthworm populations (Shivas, 1980). The earthworm is an ubiquitous component of soil ecology. Since earthworms are easily available and easy to maintain in the laboratory they provide an economical and useful organism for detection of environmental toxicants as well as may serve as indicators of various types and levels of pollutants. One way to determine the effect of the pollutant is to measure the oxygen uptake by the earthworms, in an aquatic medium, under conditions of environmental stress caused by the pollutant (Bharathi and Subba Rao, 1984).

MATERIALS AND METHODS

A simple continuous flow system was used by Sharief (1986) to determine oxygen consumption by the hemichordate *Ptychodera flava* based on the respirometer described by Fry and Hart (1948) and later modified by Job (1955) and Azariah (1969). This apparatus has been further modified by us to investigate the effect of tannery effluent (chrome tanning) on earthworms.

Lampito mauritii Kinberg (Annelida : Oligochaeta) has been selected as the test organism because of its dominance in the sandy loam soils of Madras (Ismail and Murthy, 1985; Ismail et al, 1990). Although earthworms normally live in soil they are able to survive in water for prolonged periods of time. This adpatation allows them to be used in an aquatic medium for purposes of relating toxicity to oxygen consumption (Saroja, 1959).

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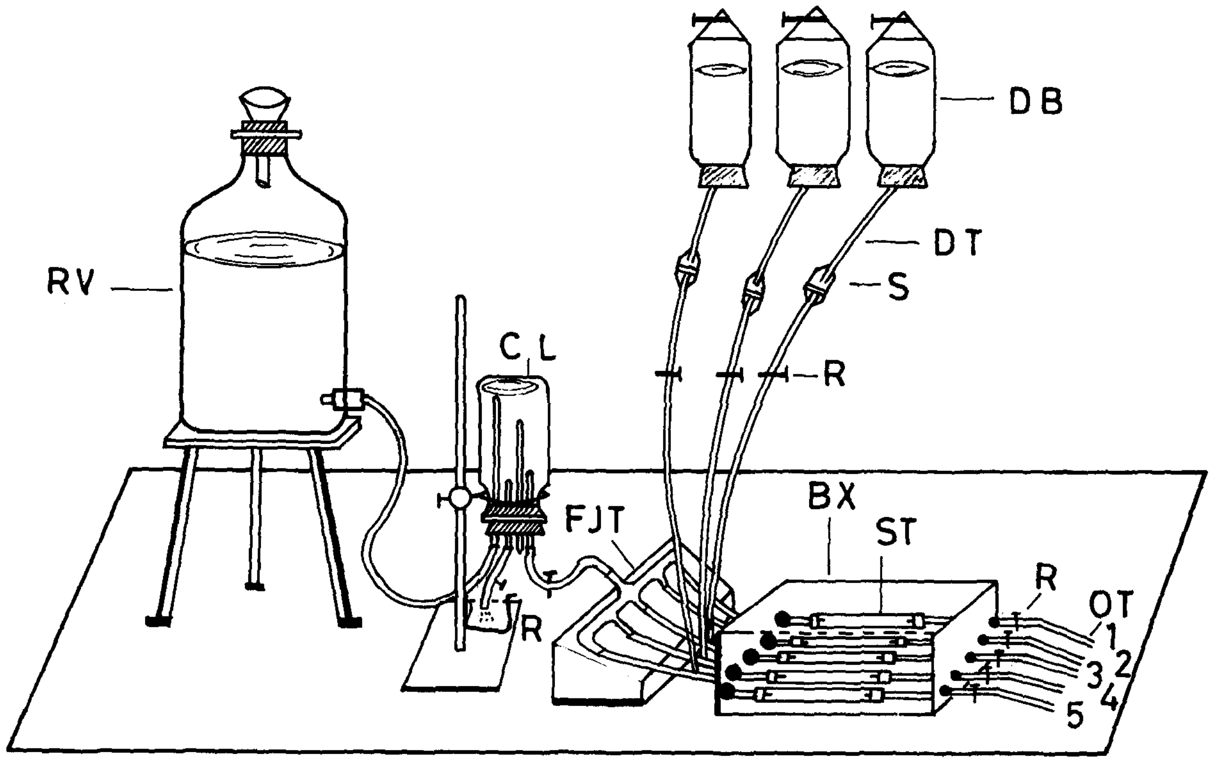

Figure 1. BX, box containing specimen tubes; CL, constant level bottle; DB, drip bottle; DT, discharge tube; FJT, finger joint tube; OT, outlet tubes (1- 5); R, regulators; RV, reservoir; S, satchet; ST, specimen tubes.

The apparatus is illustrated in Figure 1. It consists of a glass reservoir (RV) which is filled with fresh water. The outlet from the RV leads to an inverted Kilner bottle used as a Constant Level bottle (CL) and is fitted with a four-holed cork - one for the tube from the RV, one for the outlet from the CL to the Finger Joint Tube (FJT), one for the thermometer to record temperature of water, and one for regulating the constant level and enabling flow. The all glass FJT has five outlets and the rubber tubing from each of these outlets is connected to a narrow glass tube (60mm long, 2mm dia) which passes through the wall of the Plastic Box (BX) and is held in position by Rubber Seals (RS) (Fig. 2). Short rubber tubings act as suspensions on either side of each Specimen Tube (ST) through narrow glass tubes entering the ST through one-holed Rubber Corks (RC). Each ST is a glass tube 18mm long and 20mm diameter. The suspension on the farther side of the tube is connected to a short glass tube which passes out through the opposite wall of the BX and then through a rubber tubing opens into an Outlet Tube (OT) (Figure 2).

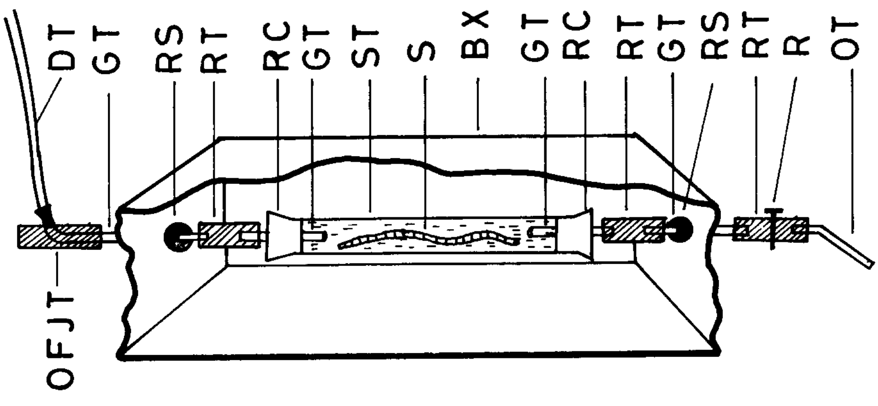

Figure 2. Position of specimen tube inside the box. BX, box; DT, discharge tube; GT, glass tube; OFJT, outlet from finger joint tube; OT, outlet tube; R, regulator; RC, rubber cork; RS, rubber seal; RT, rubber tube; S, specimen (earthworm); ST, specimen tube.

Tannery effluent of known chromium concentration is taken in glass Drip Bottles (DB) each fitted with a rubber stopper with provision for a Discharge Tube (DT) enabling flow of the effluent. The three DB are suspended at a higher level and the DT of each is led through a hypodermic needle (No.20) into the 3rd, 4th & 5th rubber outlet tube from the FJT (OFJT) close to the respective ST (Fig. 2). Regulators (R) are placed at various points to regulate the flow of water and effluent (Figs.1 & 2).

Water is first made to flow continuously and the regulators (R) at the OT are regulated to permit a uniform flow of water. This is verified by placing 50ml measuring cylinders at the nozzles of the five OT and regulating the flow to achieve uniform flow at a given time. Care is taken to avoid air bubbles in the entire set-up, which is achieved without much difficulty.

Earthworms of known weights (W,W',W") are introduced, one each, in the second, fourth and fifth specimen tubes (ST2, ST4, ST5) by releasing the rubber corks (RC) and restoppering. Air bubbles, if any, in the ST are eliminated. After an hour of acclimitization in the ST with water flowing through it, the regulators on the DT from the DB are gradually released in such a way that the flow is uniform in all the three DT as visible in the satchet (S) present in each DT.

Water flowing out from the OT (1 to 5) is collected in separate microwinkler bottles (7.5ml capacity) and the dissolved oxygen content estimated using Winkler's method (Welch, 1948).

Calculation

Sample from OT1	indicates the dissolved oxygen content of water.
Sample from OT2	indicates the dissolved oxygen content of water after consumption of oxygen by the earthworm.
OT1 - OT2	indicates oxygen consumption by the earthworm per unit time.
Sample from OT3	indicates the dissolved oxygen content of the water contaminated by the effluent.
OT1 - OT3	indicates the amount of oxygen deplenished due to contamination by the effluent.
Sample from OT4	indicates the dissolved oxygen content of water after consumption of oxygen by the earthworm in the polluted water.
OT3 - OT4	indicates the amount of oxygen

	consumed by the earthworm in the polluted water per unit time.
OT5	is a duplicate of OT4. W, W', W" weights of earthworms (in g).
(OT1-OT2)/W	Rate of oxygen consumption by the earthworm in fresh water (Control).
(OT3-OT4)/W'	Rate of oxygen consumption by the earthworm in polluted water.
(OT3-OT5)/W"	Rate of oxygen consumption by the earthworm in polluted water (Duplicate).

Samples from OT3 to OT5 can be collected periodically and checked for chromium which will quantify the dilution of the effluent as it passes through the ST. The worms may also be oven dried (≈60°C) and chromium content estimated to identify bioaccumulation of the element.

Experiments are presently under progress using this apparatus and the results are encouraging. Oxygen consumption values for earthworms (control) under the condition of the test are 0.035 ± 0.012 ml/g/hr. There is a reduction by 48 percent in the oxygen consumption of L. mauritii in the first 30 mins of its exposure to the polluted water. The reduction is only by 10 percent in the next 30 mins (60 mins of exposure) and by 33 percent in the following 30 mins (90 mins of exposure). These results were obtained when the overall flow from the OT is 10 ml/min and the flow of effluent through the DT is 0.5 ml/min. The chromium (III) content of the effluent in the DB is 3840 ppm and that in the sample collected from OT3 is 1.5 ppm indicating a dilution of 0.04 percent. The chromium content of the samples from OT4 & OT5 is 0.3 & 0.4 ppm respectively indicating accumulation of chromium by the earthworms, which on analysis show a chromium accumulation of 2.674 & 1.643 percent of the dry weight of the respective earthworms.

The length of time earthworms may be used under the conditions of the test depends on the nature and concentration of the effluent. In the present experiment mortality of the earthworms recorded is 5% during the first 30 minutes, 14% during the next 30 minutes and 29% during the last 30 minutes (or 90 minutes of exposure) under the conditions of the test. Surviving worms from the test can be transferred to pots containing silt loam and monitored for further survival.

The apparatus is highly versatile for the following reasons:

1) The reservoir can be made to contain fresh, marine or brackish water depending on the experiment.

2) The rate of flow of water can be regulated.

3) Any type of pollutant (effluent, pesticide, etc.,) can be used at similar or dissimilar flow rates to test its effect on the rate of respiration of organisms.

4) The dimensions of the box (BX) and the specimen tubes (ST) can be modified to suit the desired test organisms.

5) The BX can be wrapped in dark cloth or painted black to study respiration in nocturnal animals, or can be illuminated to study either diurnal animals or photosynthetic activity of selected aquatic plants.

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