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Determination of Heavy Metals and Acute Toxicity Studies of Vat Dyes on Earthworm (*Lumbricusterrestris*) As Ecological Risk Indicators

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ABSTRACT: Objective: This study was carried out to determine the heavy metal contents and the toxicity of three vat dyes (blue, green, and red dyes and their additives NAOH (caustic soda) and $NAHSO_4$) commonly used by local dyers on earthworms (*lumbricusterrestris)*

Method: 24-hour filter paper contact test was used to make five different concentrations (0.1, 0.2, 0.3, 0.4, 0.5mg/l) using warm distilled water and the lethal concentration LC_{50} was determined using probit analysis software. Heavy metals were also detected using atomic absorption spectrophotometer.

Result: The heavy metals concentration using atomic spectrophotometer in each 0.1mg of the dye and their additives $NaHSO_4$ and NaOH and all the dyes shows a significant amount of concentration of the metals such as Zn (zinc), Cd (cadmium), Mn (manganese), Pb (lead), Cr (Chromium), Fe (iron), and Cu (copper). The results indicate that the red vat dye is the most toxic to the earthworms with LC_{50} of 0.12 mg/L and 0.12 mg/l respectively with additives at 24-h of exposure. The LC_{50} of the blue vat dye is 0.129mg/l and 0.21 mg/l with additives and green dye is the least toxic when compared to the red with LC_{50} of 0.3 mg/l and 0.137mg/l. NAOH and $NaHSO_4$ shows LC_{50} of 0.13 and 0.5 respectively which shows that the NaOH is most toxic between the two additives used.

Conclusion: The Seven heavy metals detected were found to be present at 0.1g of each of the vat dye with their absorption and concentrations. The study concludes that all the vat dyes commonly used by the local dyers in Kano metropolis are highly toxic and can cause potential damage to the organisms in the soil ecosystem as well as possible carcinogenic effect.

Key words*: lethal concentration, vat dyes, Atomic absorption spectroscopy (AAS), filter paper Contact test.*

I. INTRODUCTION

The use of textile dyes or textile industry is a flagship business of a number of youths in both urban and rural areas of the northern part of Nigeria. An average seven dye pits are found in a single ward in a local government area in Kano state and constitute one of the most outstanding small scale business of the state. Even the government introduces textiles dyeing training programs for rural settlers as a means of alleviating poverty and employment opportunities for the citizen of the state (personal interview with dyers). The textile industries are to satisfy the ever-growing demands in terms of quality, variety, fastness and other technical requirements. The large variety of dyes and chemicals used in an attempt to make more attractive popular shades of fabrics for a competitive market render them very complex [1].

The last decade has seen tremendous environmental issues associated with dyestuff production and application has risen sharply and is one among the major menace affecting the textile dye industry till today [2]. For the local dyers very little or no regulation is done to these industries and perhaps they neither even educated on the potential dangers of the business while for larger industries little implementation of the safety guidelines have been strictly adhered to as stipulated. Large amounts of dyes have been noticed in these textile wastewaters, due to their incomplete use and washing operations, which are mostly done inappropriately [3]. The dyes disposed off, can be found in dissolved state or in suspension in the wastewater. These dyestuffs are highly structured polymers and are very difficult to decompose biologically [4]. The most obvious impact of the discharge of dye colored effluent is the persisting nature of the color. It is stable and fast, difficult to degrade, toxic, rendering the water unfit for its intended use. Further, the color removal is also not adequate by the conventional chemical and biological treatment [5]. Such dyestuffs can reach the aquatic environment, primarily dissolved or suspended in water, since the conventional treatment of wastewaters from textile mills and dyestuff factories are unable to remove most of the vat and other dyes effectively.

Many reports indicate that heavy metals interfere with the biochemistry of diverse group of microorganisms isolated from their natural environments [6]. However, information relating to the sensitivity of whole soil bacterial communities to heavy metals is not common [7]. Microorganisms do not live in isolation but in complex biological communities within which exists complex interactions arising from biotic and abiotic influence [8]. It is difficult to relate single species behaviors to the overall ecosystem. Since traditional methods of microbial ecology require that organisms from an environment be cultured in the laboratory before they can be identified and studied [9], monitoring the effect of pollutants on the activities [10] of the overall communities poses a great challenge [11], because less than 10% of microorganisms from any environment can be cultured [12]. For eco-toxicological research to be capable of influencing policies and regulations pertaining to environmental sustainability and conservation it must be methodologically planned and ecosystem oriented [13] This is because the existence of bacterial species that are tolerant to a pollutant is not indicative of the effect of selective pressure due to that pollutant on other species [14]. Heavy metals come from a variety of sources but principally anthropogenic activities such as chemical manufacturing [15], electric power generation, coal and ore mining, melting and metal refining, metal plating and to some extent domestic sewage [16]. Some of the metals such as copper, nickel and zinc are essential in very low concentrations serving as components of enzymes [17], structural proteinsand pigments in maintaining the ionic balance of cells [18].

II. MATERIALS AND METHODS

2.1 Materials

Vat Dyes (Blue, green and red) were obtained in powder form. Matured earthworms with well developed clitella (*Lumbricusterrestris*) from Salantariver Kano State polytechnic along Buk road by digging.

2.2 Sample Collection (dyes)

Three Synthetic vat dyes whose chemical constituents were mentioned on the containers were obtained from two places kurmimarket and one mini market (Haurenwanki) along buk road in their powder forms. Sodium Hydrogen sulphide (NaH_2S) and caustic soda was also collected as they are used together with the dyes in the dyeing process as additives. Blue, red, and green were chosen among the various colors of the dye at random. Total 5 samples were used for this analysis.

2.3 Collection of earthworms

Matured earthworms (*Lubricusterrestris*) were collected from Salantariver Kano State polytechnic along Buk road by digging. This site is relatively free from industrial waste and other domestic waste. There after samples were taken to the biological science garden and kept in a pot of sand and sorted out before being transferred into the laboratory for test. Matured worms were sorted out with well developed clitella of about1.3- 1.5g were selected for the test

2.4 Methods

2.4.1 Digestion of the dye samples

0.100 g of powdered each dye sample was taken in a silica crucible (150 cm^3). Then 1M concentrated hydrochloric acid (9 cm^3) was added followed by 1 M concentrated nitric acid (3 cm^3). The content of the crucible was carefully heated in sand bath nearly to dryness in fume hood. After cooling the crucible at room temperature, deionized water was added to the sample and was filtered through a filter paper (Whatman No. 42). The filtrate was collected in a measuring flask and was preserved for the determination of Pb, Cd, Zn, Cu, Cr, Fe and Mn [19]. All regents used were Merck, Analytical Grade (AR) including standard stock solutions of known concentrations of different heavy metals [20].

2.4.2 Formulation of test concentration

Dye solutions were made by dissolving 0.1, 0.2, 0.3, 0.4, 0.5 g of dry powder of the dye each in 100 ml of warm, sterile, distilled water to form each five different concentrations. The test substance is dissolved (the five samples) in water to a concentration of 1000 mg/l) as appropriate, to give a range of known concentrations. Hence concentration of 0.1mg/l,0.2mg/l,0.3g/l,0.4g/l,0.5g/l was formed.

2.4.3 Earthworm Bioassay

(24 hour contact test (acute toxicity test))

This test was carried out according to the protocol obtained from Organisation for Economic Cooperation and Development (OECD) [21],[22].Flat-bottomed petridish approximately 8.5 cm in diameter was used and there sides are lined with filter paper cut to a suitable size so it does not overlap appreciably.5 ml of solution is pipette into each Petri dish with ventilation holes and evaporated to dryness by air, the petridish is being rotated horizontally as it dries. The control petri dish is treated with 5ml of deionized water. After drying, 1 ml of deionized water is added to each vial to moisten the filter paper [21]. For each treatment, two replicates, each consisting of five worms per vial, was used. In each test a range of treatment levels and five control vials were used. Worms were kept on moist filter paper for three hours before being placed in test petridish so they can void their gut contents [21]. They are then washed and dried before use. Tests was done in the dark and for a period of 24 hours kept in a cupboard with little air entrance for moisture. Worms are classified as dead when they do not respond to a gentle mechanical stimulus to the front end. All behavioral response was reported. Two parameters were used mortality and survival [11].

2.4.4 Mortality counts

Mortality is recorded by numerical and direct counting if they do not respond to a gentle mechanical stimulus to the front end. Those dead ones were separated from the live ones and discarded away in a bin to avoid contamination with the live ones. Also the live ones itself separated themselves away from the dead ones by moving to the other side of the petridish.

2.4.5 Time of death

The experiment was carried out and recorded in 24 hours because the preliminary experiments carried out record mortality in the control petridish above 10% due to environmental factors such as temperature. Mortality recorded in less than 4 -6 hours was also noted. Final reading is taken in 24 hours contact [21].

2.4.6 Conditions for the validity of the test

The mortality in the controls should not exceed 10 per cent at the end of either test [21],[22].

2.4.7 Data Analysis

Result obtained for the LC_{50} was calculated using probit analysis comparing with the Finney's table to determine the lethal concentration of each test samples prepared. Probit software was used to confirm the figures obtained from calculated values. Mortality for the test organisms is tabulated against the concentration causing the mortality after taking the average from the replicates of each sample prepared.Result of absorption from the spectrophotometer is used to calculate the concentration using the values obtained from slope of the graph obtained. The concentration of the heavy metals placed in a tabular form parallel to the vat dye color.

III. RESULTS AND DISCUSSION

Since eco-toxicology focuses upon the adverse effects of chemicals in the environment, acute toxicity in this discipline is more commonly described by the LC50 or EC50. LD50 and ED50 values are more commonly used when evaluating toxicity from a human health perspective [23, 24]. Clearly, the LC50 value is not indicative of an acceptable level of the chemical in the environment. Allowing an environmental concentration of chemical that is predicted to kill 50% of the exposed organisms is hardly an example of good environmental stewardship. Rather the LC50 is used as an indicator of relative acute toxicity. The LC50 is used to this end rather than a more relevant descriptor of an environmentally suitable concentration because the LC50 value has the greatest level of confidence associated with it due to its central location on the concentration response line [24]. LC50 and LD50 values are often interpreted as given in by [23, 24]. Acute toxicity of vat dyes to earthworm was summarized in Table 1 and 2. In the present study, toxicity was evaluated from concentrations of 0.10 mg/L for blue, red, green vat dyes, caustic soda and sodiumhydrosulphate which determined the LC50 ranges. Also, control solutions (concentration of 0.0 mg/L) were conductedto confirm the accuracy of the test. Table 1 and 2 shows the number of dead earthworm after 24-h contact tests. The results indicate that the LC50 of red vat dye on earthworms is about 0.12 mg/L and 0.12 mg/l with mixtures at 24-h of exposure, the LC50 of the blue vat dye is 0.129mg/l and 0.21 mg/l with mixtures and green dye also with LC50 of 0.3 mg/l and 0.137mg/l. NAOH and NaH_2SO_4 shows LC50 of 0.13 and 0.5 respectively. No mortality or lethal effects were observed in the green dye at 0.10 mg/l without mixture and 100%mortality was observed at 24 -h in 0.5 mg/L highest concentration tested in all the dyes used except in NaH_2SO_4. This means that 50% of the earthworms were dead at concentration between 0.3 down to 0.5 mg/L after 24-h contact test. Table 1 and 2 also shows that all the dyes used can be expressed as been extremely toxic which corresponds to the work of [24].

Table 3 shows the result of the heavy metals concentration using spectrophotometer in each 0.1mg of the dye and their additives NaH_2SO_4 and NaOH and all the dyes shows a significant amount of concentration of the metals such as Zn (zinc), Cd (cadmium), Mn (manganese), Pb (lead), Cr (Chromium), Fe (iron), and Cu (copper). When the result obtained is compared to the amount of the dyes used to dye locally 5 yards of the fabric where 120g 0f the dyes and 60g of the additives is used as practiced by the local dyers, then it can be classified that they are above the save limits when disposed indiscriminately in the soil . If all the values obtained were converted into grams and then multiplied by 120g, then this result confirms the literature submitted by [25] where he described vat dyes as the most toxic of all the dye types and described it further to be corrosive and ignitable. The concentration of Zinc was about 0.64 , chromium 0.5, and lead 0.53 in the red dye in every 0.1g of the stock sample, in the blue dye zinc was 0.77, lead was 0.73 and chromium was 0.33, in the green dye zinc was 0.5, lead was 0.4 and chromium was 0.25. Comparing these values it appears the concentration of chromium is responsible for the increased mortality in the red dye then followed by the blue dye and then the green dye.

When it is also compared to the value or concentration of chromium between the caustic soda and NaH_2SO_4 the value of chromium tends to be higher in the caustic soda than the later with a value of 0.58 similar to that of the red dye while that of the later which is NaH_2SO_4 gave a concentration of 0.23 from Table 4. This generally confirms that the reason for the increased mortality in the dyes and there additives is due to the presence of the heavy metals which are easily accumulated by the earthworms particularly chromium. [26] Reported this similarly in the journal on the bioabsorbtion of heavy metals by plants. The wastewater from vat dye synthesis will contain unreacted raw materials and by products, which are, soluble in addition to inorganic salts formed by neutralization. The heavy metal catalysts and reagents used in key intermediate steps, such as mercury, arsenic, copper and chromium, are primarily found in the wastewater as soluble salts, and can contaminate soil and groundwater if improperly treated or disposed off.

Table 1: Mortality profile of earthworm (*L. terrestris*) to acute exposure of vat dyes at different concentrations.

Dyes	Exposure Period	Initial No. of Worms	Concentration (mg/dl) 0.1	0.2	0.3	0.4	0.5	LC_{50} (mg/l)
Green	24	5	0	1	2	3	5	0.3
Blue	24	5	1	2	3	4	5	0.21
Red	24	5	2	3	4	5	5	0.137
NaH_2SO_4	24	5	0	0	0	1	2	0.5
NaOH	24	5	2	3	5	5	5	0.13

Table 2: Mortality profile of earthworm (*L. terrestris*) to acute exposure of Vat Dyes mix with other dyeing additives (NaH_2SO_4 andNaOH)

Dyes	Exposure Period	Initial No. of Worms	Concentration (mg/dl) 0.1	0.2	0.3	0.4	0.5	LC_{50} (mg/l)
Green	24	5	2	3	4	5	5	0.137
Blue	24	5	2	3	5	5	5	0.129
Red	24	5	2	3	5	5	5	0.12

Table 3: Concentration of heavy metals of the vat dyes and their additives obtained from markets in Kano. Using atomic spectrophotometer the heavy metals present in 0.1g of the samples obtained.

Dyes	Concentration (mg/l)						
	Cd	Pb	Cr	Fe	Mn	Cu	Zn
Red	0.25	0.53	0.58	0.2	0.32	0.19	0.64
Blue	0.14	0.73	0.33	0.30	0.11	0.14	0.77
Green	0.04	0.40	0.17	0.26	0.27	0.10	0.5
NaH_2SO_4	0.75	0.27	0.25	0.22	0.03	0.05	0.41
NaOH	0.07	0.23	0.58	0.09	0.16	0.01	0.14

IV. CONCLUSION

The results using *Lumbricusterrestris* have been used only as a model to determine the toxicological implications that may result from vat dyes in terrestrial environment, but these results are not sufficient to assess the holistic health risk for receptor soil ecosystem. However, the toxicity to the earthworms is enough to suggest potential damage to every receptor terrestrial ecosystem and emphasizes the need for toxicological study of local dyes. The results indicate that allthe dyes were very toxic to earthworms, within a 24 hr exposure with LC50 between 0.13-0.5mg/l. The research also indicates the presence of heavy metals in the dyes and their proportion in every 0.1mg of the dye and their additives as shown in the research. It also stipulates the high presence of zinc, cadmium and copper. The increase in chromium correlating with the increase in the mortality shows that chromium can be very detrimental. This has a lot of carcinogenic implications owing to large amount of heavy metals present in the dyes which has direct consequences on the aquatic habitat such as fishes.

From this research it is quite interesting to know the toxicity of the vat dyes and it's additives as used by the local dyers in Kano which can also form bases for further research. So also the aim of this research which was to investigate the effect of vat dyes using earthworms as bio indicators of environmental pollution was fully investigated.

IV. RECOMMENDATIONS

1. The use of synthetic vat dyes must be discouraged and replaced by the natural vegetables dyes as these measures has been employed in other countries due to its high toxic effects.
2. Improved varieties of the natural and vegetable dyes should be studied and produced to meet the high demands of the local dyers.
3. Indiscriminate disposal of the vat dyes sludge should be banned by the Government by initiating policies that are environmentally friendly.
4. Treatment of this ludges should be done before proper disposal because they contain high proportion of heavy metals such as chromium, zinc etc. that are not biodegrable.
5. Local dyers must be educated of the toxic effects of these dyes and there detrimental impacts to the environment.
6. Further studies should be carried out on the toxic effects of these dyes in both aquatic and the possible health implication of these dyes such as the carcinogenicity and mutagenicity of the vat dyes.

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VI. CONFLICT OF INTEREST

Declared None

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