GENETIC EFFECT OF ELECTRONIC-WASTE LEACHETE FROM ALABA INTERNATIONAL MARKET ON DROSOPHILA

Ogunkanmi, L.A.[1*], Atujor, A.O.[1], Fakorede, S.T.[1], Bolarinwa, K.A.,[2] Bamkefa Bukola Ayodeji.,[3] Adekoya, K.O.[1], Sifau, M.O.[1] and Oboh, B.O.[1]

[1]Department of Cell Biology and Genetics, University of Lagos, Nigeria,
[2]Department of Education Science (Biology-Unit), Distance Learning Institute, University of Lagos, Nigeria
[3]Department of Biological Sciences, Lead City University, Ibadan, Nigeria.
*Correspondence email: logunkanmi@unilag.edu.ng

ABSTRACT

Electronic industry is one of the most prominent and advanced in the world, producing significant complex waste known as electronic-waste (e-waste). One of the challenges of e-wastes is the concern raised about the toxic effect of their components. This study investigates the effect of e-waste from Alaba International market in Lagos, Nigeria on Drosophila melanogaster (fruit fly). Wild type drosophila were collected and fed with a prepared fruit fly medium. Different concentrations (0.1, 1, 2, 10, 25, 50% (v/v), positive control and negative control of the e-waste medium were prepared. The toxicity of e-waste was studied by varying the e-waste concentration in the media on which the fruit flies were bred for two successive generations. Physico-chemical analysis of the e-waste was carried out. Data were analyzed using Microsoft excel and SPSS statistical analyses. The results showed that as the concentration of e-waste increases from 0.1-100 v/v%, the time interval for development of the drosophila was prolonging. In the F_1 and F_2 generations, there was a significant change in the number of offspring observed. The morphology of the wings was found to be affected by 10-100 % (v/v) in F_2 generation with curved wings and wings hanging away from abdomen. The physicochemical analysis showed the presence of heavy metals, with lead and copper having the highest values 6.55 ppm and 4.38ppm respectively. This study ascertains that increased concentration of e-waste affect the reproductive cycle, growth and development, fecundity and morphology of the fruit fly.

Keywords: Drosophila, E-waste, Genetics, heavy metals

INTRODUCTION

Electronic waste has become an emerging threat given the volumes of e-waste being generated and the content of both toxic and valuable materials in them. These valuable components of e-waste accounts for 60%, while pollutants comprise 2.70%, these pollutants are hazardous especially when burned or

recycled in uncontrolled environments (Rolf *et al.,* 2005). With rapid advancement in economic, technological and industrial developments in recent years-in a bid to increase the standard of living, there is a significant increase in production of e-waste that has resulted from electronic equipment such as smart phones, personal computers (PCs), televisions, radio tabloids, etc. that became obsolete or damaged to users. This has been predicted to rise inevitably due largely to accelerated industrialization, urbanization and population growth (Bakare *et al.,* 2013). Over the next few years, one billion computers may have become obsolete. By 2022, the total waste electrical and electronic equipment (WEEE) is estimated to grow between 2.5% and 2.7% annually, reaching a total of approximately 12.3 million tons. The reason is that the number of appliances entering the market every year is increasing in developed and developing countries (Scluep, *et al.,* 2009).

There are 80,000 chemicals in commercial use today, and approximately 2,000 new chemicals introduced each year for which there is insufficient toxicological data (Matthew, 2010). Among these chemicals are the major components of electronic waste (e-waste) - also known as waste electrical and electronic equipment (WEEE)-such as copper, tin, lithium, cobalt, indium and most prominently, hazardous substances such as lead, polychlorinated biphenyls (PCBs), polybrominated biphenyls (PBBs), mercury, polybrominated diphenyls ethers (PBDEs), and brominated flame retardants (BFRs) (Chancerel, *et al.,* 2009). *Drosophila melanogaster* as a model organism has been a very useful tool in genotoxic study and DNA repair testing. This organism is a valuable model for research related to human health, including DNA damage response. Since its first application, it has been used to analyze the genotoxicity and action mechanisms of different chemicals, demonstrating good sensitivity and proving its usefulness (Isabel and Maria. 2014). The uses of this organism as an *in vivo* model is due largely to the simplicity of its biology, it's easy to maintain in laboratory conditions. It has a considerably short lifespan, and ease of genetic manipulability. This study investigated genotoxic effect of electronic waste on *Drosophila melanogaster* and ascertains the potential health implication.

MATERIALS AND METHODS STUDY LOCATION

The study area is a major land fill dumpsite of about $300m^2$ in size located outside the market at the Alaba Ragon axis, Alaba International market (an electronics market) located in Ojo, Lagos State, Nigeria. E-waste collectors and recyclers work and indulge in burning and other crude recycling practices, to recover important components/scrap from e-waste.

SAMPLE COLLECTION

The soil sample was collected from four different locations at the dumpsite outside the market where E-wastes have been dumped and burnt over a long period of time. The sample was collected in a polyethylene bag, transported to the laboratory and air dried to give an accurate weight measurement of the soil. The wild-type *Drosophila melanogaster* used in this study were caught from the Biological Garden of the University of Lagos, using empty and clean bottles that served as traps with fruits inside that attracted the flies.

THE FRESH FRUIT FLY MEDIUMAND MEDIUM PREPARATION

One unit of Drosophila food prepared for this experiment contained 1000ml of distilled water, 250g of banana which provided the sugar on which the 5g yeast grew, 250g of garri, 10g of nutrient agar and 5ml propionic acid (Bakare *et al.* 2013). *Drosophila* culture medium was prepared by adding 10g of agar to 1000ml of boiled distilled water and stirred to dissolve. A 250g of finely crushed banana was added to the mixture and allowed to stay for 5 minutes. Thereafter, 250g of garri was added and mixed thoroughly for 5 minutes. Five milliliters (5ml) of propionic acid was added when the pot was removed and allowed to cool for 1 minute to avoid its evaporation. The warm medium was poured into vials (10cm x 2cm), using funnel. The medium was kept for 24h to solidify before transferring the wild type drosophila into the vials. The vials were labeled according to the concentrations with each concentration divided into two parts; A and B. Each of the vials was corked with sterile foam.

E-WASTE CONCENTRATION

The e-waste concentration consists of 1000ml of distilled water which was added to 1kg of soil sample for 24h and sieved to give 100% liquid concentration of the e-waste. Different concentration was prepared from the stock (0.1% (v/v), 1% (v/v), 2% (v/v), 10% (v/v), 25% (v/v), 50% (v/v)), and the positive control of 0% (v/v) (made with only distilled water) and negative control 100% (v/v) (stock with dilution).

ANESTHETISATION AND TRANFER

The wild-type *Drosophila melanogaster* were anesthetized using diethyl ether and cotton wool. Thereafter, one male and three females drosophila were crossed into each of the concentrations to raise F_1 generation. There were no replications and same method was repeated for the F_2 generation.

PHYSICO-CHEMICAL ANALYSIS OF THE E-WASTE

The heavy metal contents of the e-waste were analyzed at the Central Research Laboratory of the University of Lagos. Physico-chemical properties of the test samples were determined according to the procedure of American Public Health Association (APHA, 1998) and United States Environmental Protection Agency (USEPA, 1996).

STATISTICAL ANALYSIS

The data were analyzed using Microsoft Excel and SPSS Version 20 statistical analysis.

RESULTS

PHYSICO-CHEMICAL ANALYSIS

Table 1 shows the results of the physico-chemical analysis of the e-waste. Six (6) heavy metals were analyzed. The results showed the composition by mass in (ppm) of the presence of heavy metal contents of the e-waste.

Table 1: Physico-chemical analysis of E-waste from Alaba international market

S/N	HEAVY METALS	CONCENTRATION (ppm)
1	Lead	6.55
2	Cadmium	0.51
3	Iron	0.79
4	Copper	4.38
5	Nickel	1.37
6	Chromium	1.86

EFFECT OF E-WASTE ON THE REPRODUCTIVE CYCLE OF DROSOPHILA MELANOGASTER

The presence of e-waste in the culture medium of *D. melanogaster* showed a significant effect on the life cycle of the fruit fly. In the first generation, there was little or no significant manifestation of the effect of e-waste at lower concentrations up to 10% v/v (Table 2a). However, at higher concentrations from 25% v/v to 100% v/v, the conversion rate decreased. The larval and pupal period did not show a dependent or uniform increment on the concentration of the e-waste across all the concentrations. There was increased metamorphosis (larva-pupa-adult) period from lower to higher concentrations in the first generation.

At the second generation (F_2) (Table 2b), there was a significant decrease in the percentage rate of transition across the concentration from the lower and to the higher concentrations of the test media, while the control medium remain unchanged. The results show that the presence of e-waste in the medium increased the larval and pupa period, but decreased the rate of larva transition into pupa, adult, and eggs hatching (Table 2b).

Table 2a: Effect of e-waste on the reproductive cycle of fly at first filial generation - F_1.

Conc.	Larva period (hours)	Pupa period (hours)	Larva emerged (days)	Pupa emerged (days)	Adult Emerged (days)
Control	72	24	5	8	9
0.10%	96	72	4	8	11
1%	96	48	4	9	10
2%	120	48	5	10	12
10%	72	48	4	9	10
25%	96	48	6	11	13
50%	120	96	5	10	13
100%	96	72	5	11	12

Table 2b: Effect of e-waste on the reproductive cycle of the fly at F_2.

Concentrations	Larva period (hours)	Pupa period (hours)	Larva emerged (days)	Pupa emerged (days)	Adult emerged (days)
Control	72	48	4	7	9
0.10%	96	48	4	8	11
1%	96	48	5	9	11
2%	96	72	6	9	12
10%	120	72	5	11	14
25%	144	72	7	13	16
50%	144	96	8	14	17
100%	192	96	8	15	18

FECUNDITY ASSESSMENT

At the first filial generation - F1 (Table 3a), there was little or no noticeable effect of the e-waste on the reproductions of *D. melanogaster* at lower concentrations up to 25%. The effect of e-waste on the average number of flies in the test media of 0.1%, 1%, 2%, 10%, and 25% was not concentration dependent. The result showed that even though there was a decrease in the number of offspring as seen from the control medium to the concentrations above, the decrease was in no particular order of the concentrations.

At the second filial generation- F2 (Table 3b), there was a significant decrease in the number of offspring in the test media from lower to higher concentrations. Comparing the control medium and the test media of lower concentrations of 0.1% and 1%, the e-waste showed no observable effect on the average number of offspring. At higher concentrations of 2%, 10%, 25%, 50%, and 100%, the result showed a significant decrease in the reproductions of the fly.

Table 3a: Number of offspring in the controls and each of the concentrations at F_1

	CONC	0.10%	1%	2%	10%	25%	50%	100%
A	27	24	23	18	27	25	15	13
B	33	23	23	14	21	25	15	12
MEAN	30	23.5	23	16	24	25	15	12.5
STD	4.24264	0.70711	0	2.82843	4.24264	0	0	0.70711

Table 3b: Number of offspring in the controls and each of the concentrations at F_2

	CONTROL	0.10%	1%	2%	10%	25%	50%	100%
A	33	29	25	13	13	10	7	2
B	25	23	29	15	15	9	9	3
C	37	19	31	11	11	0	0	0
MEAN	31.6667	23.6667	28.3333	13	13	9.5	8	2.5
STD	4.98888	4.10961	2.49444	1.63299	1.63299	4.76751	4.08248	1.31498

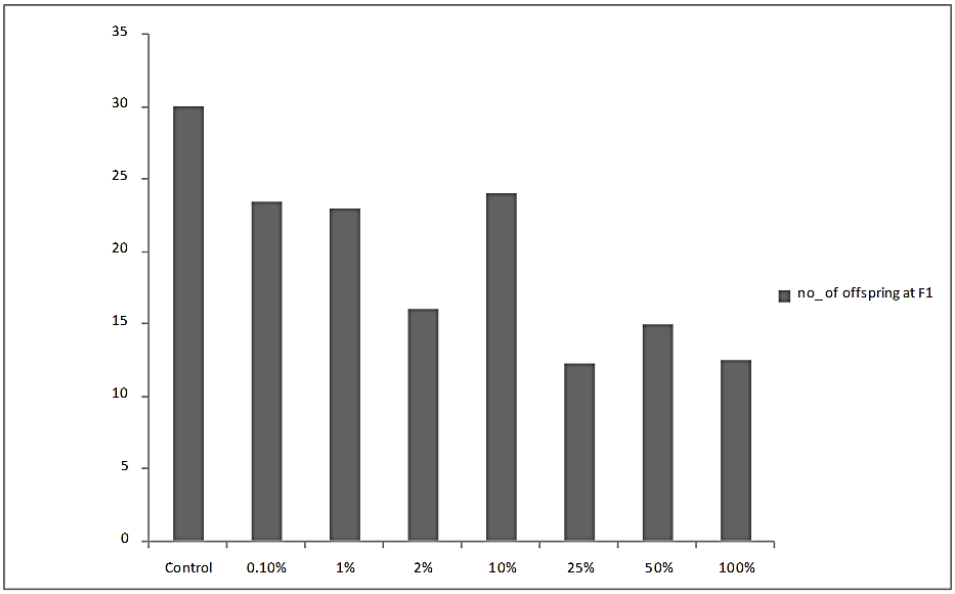


Fig 1: Number of offspring at F1 in response to concentrations gradients of e-waste

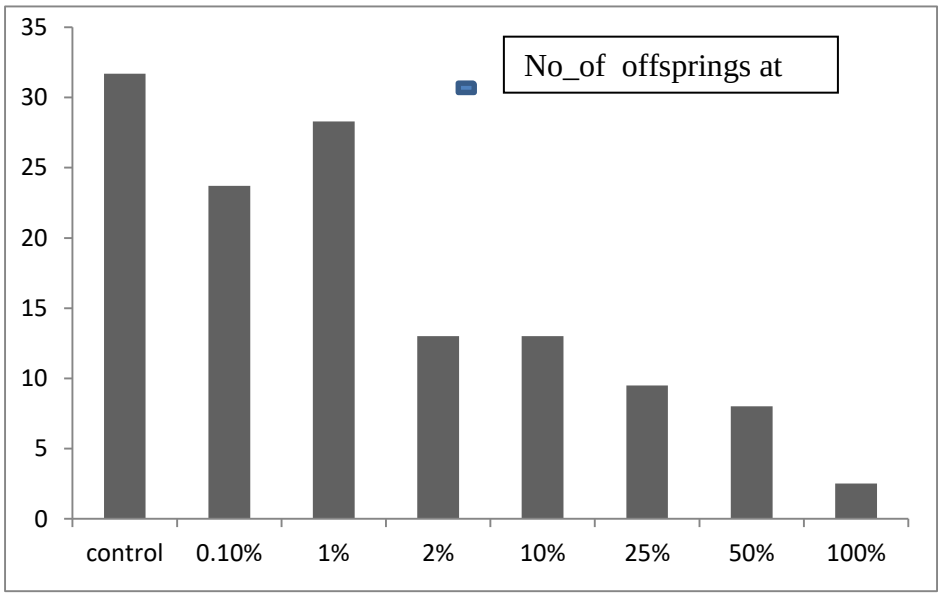


Fig. 2: Number of offspring at F2 in response to concentrations gradients of e-waste

COMPARING THE PERIOD OF LARVAL EMERGENCE AT F_1 AND F_2

The time taken for the larva to emerge at F_1 and F_2 was studied. It was observed that the time taken for the larva to emerge across the different concentrations

increased as concentration increases. The time interval for larva emergence was compared between the first and second generation (Fig.1 and Fig. 2). This effect was not seen at F_1, the average day of the emergence of larva at F_1 was at day four (4) across all concentrations. This showed that the emergence of larval at F_1 was not concentration-dependent when compared to F_2. At F_2, the time interval for the emergence of larva increased across the concentration from lower to higher concentrations from day 4-8 in an increasing order.

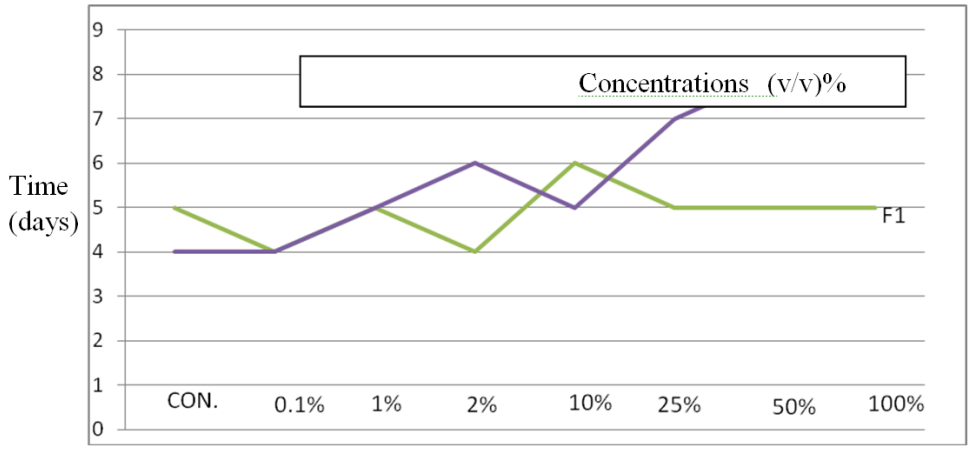
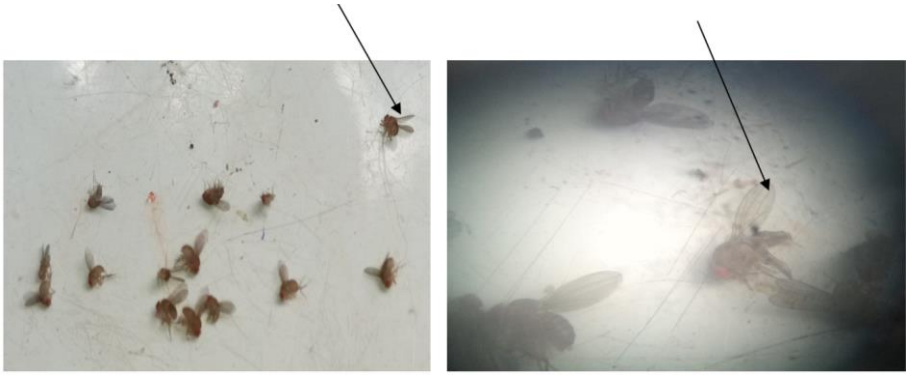


Fig.3: Comparing the time taken for the emergence of larva between F1 and F2

PHENOTYPIC MANIFESTATION

At the second filial generation, some morphological changes were observed in the test media from higher concentrations of 10% v/v to 100% v/v. The wings of some of the flies were malformed. (Fig. 4A-B) while some of the wings were curved inward others were left hanging away from the abdomen.

Other phenotypic changes that were observed include a change in the colour of some flies at 25% to 100% of the test media at the second filial generation. The colour of the entire body of those flies changed from brown to grey colour. While the eye colour of the same flies changed from red to black.



A Wing hangs away from abdomen B Wing curved inward

Fig.4: Malformed wings of *D. melanogaster* across different concentration of e-waste.
(A)-(B) showed varieties of malformed wings.

DISCUSSION AND CONCLUSION

This study demonstrated the genotoxic effect of e-waste on *D. melanogaster.* The results showed that with increased concentration of e-waste, the time taken for the larva to transform to pupa and pupa to adult increased in a nearly direct correlation to the concentration of e-waste in the medium. Other effects manifested such as the time taken for the larva to emerge increased with increase concentration of the e-waste. However, some of these effects were not profound at F_1 generation and at lower concentration (0.1%, 1%, and 2%) of the F_2 generation, probably due to the presence of protein, metallothionein (MT), which detoxify the e-waste at F_1 and at lower concentrations of F_2. But in higher concentration of the e-waste, probably the MT protein is incapable of detoxification. Also, high concentrations of e-waste (due to the presence of heavy-metals such as Lead) can lead to impaired expression of MTs, resulting in the accumulation of large metal structures in the body and inducing deleterious effects on the fly (Al-Momani and Massadeh, 2005; Chancered *et al*., 2009). Possible causes of increased life cycle duration, that is, the transformation of larvae to pupae, and pupae to adults can be noted in which high concentrations of heavy-metal contents of the e-waste can interfere with the function of the essential enzymes needed for the production of hormones involved in metamorphosis (Al-Momani and Massadeh, 2005). In this study, the increased concentrations of e-waste delay the developmental period of *D. melanogaster* egg to adult which increases both the larval and pupal periods.

The toxic effects of heavy-metal contents of the e-waste can be induced in the body of larva when swallowed and decreases its growth, delaying the development of the insect (Ding and Wang, 2006). The possible reason for late

emergence of larvae can be attributed to the possibility of metals entering into the egg coat or microphil and thereby affecting the developmental stages of embryo and delaying its growth. Moreover, the higher the concentration of the metal, the more this delay is observed and the later the larva emerges (Safaee *et al*., 2014).

A number of metal ions such as As, Cr, Cu, Cd, Fe, Mn, and Ni, are known to be potent inducers of DNA damage (Desoize, 2003). Metals like Cd, Cr, Cu, and Ni are strong oxidants, generating reactive oxygen species (ROS) (Valko *et al*., 2006). It has also been demonstrated that a number of organic carcinogens/mutagens induce oxidative DNA damage through metal-catalyzed ROS generation (Kawanishi *et al*., 2002). ROS induces a broad spectrum of DNA lesions, including DNA single-strand and double- strand breaks, apurinic/pyrimidinic (AP) sites, DNA base modifications, and bulky adducts (Halliwell and Aruoma, 1991; Scharer, 2003). In addition to the metals, it is possible that other unidentified organic chemicals and nonconventional pollutants (NCPs) (though not analyzed) may have contributed to the genotoxic responses produced by the effluent (National Research Council, 1991). Thus, the ROS generating potential of metals in e-waste is one possible source of the genotoxicity observed in the *D. melanogaster* that were fed with the medium containing the e-waste at higher concentrations. The change in eyes and body colour observed, are some of the morphological manifestations of this possible DNA damage induced by the e-waste.

CONCLUSION

This study demonstrated the genotoxic effect of e-waste from Alaba International market on *D. melanogaster*. The results indicated that an increased concentration of e-waste affects the reproductive cycle, growth and development, fecundity and morphology of the fruit fly. It can be inferred that the toxicity of e- waste would have a negative effect on the viability and development of the stages of the organism. However, these negative effects were not manifested at lower concentrations of the e-waste. This may be due to the ability of the fly to detoxify the e-waste using their MTs protein or other ways. The results also showed that these effects become more profound as the flies were transferred from one generation to another. It can be inferred that long term exposure to e-waste and its possible accumulation in the body can have negative health implications on other organisms as well as humans. This present study indicates the usefulness of *Drosophila* as a model organism for genotoxic and environmental assessment studies of e-waste in the places where they are generated and poorly managed.

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