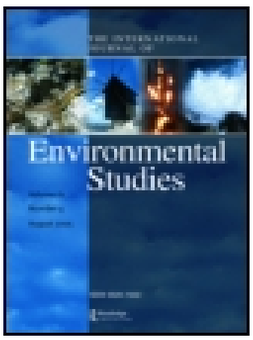

International Journal of Environmental Studies

ISSN: 0020-7233 (Print) 1029-0400 (Online) Journal homepage: http://www.tandfonline.com/loi/genv20

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To cite this article: Nadia Khlaif & Mazin B. Qumsiyeh (2016): Genotoxicity of recycling electronic waste in Idhna, Hebron District, Palestine, International Journal of Environmental Studies, DOI: 10.1080/00207233.2016.1236650

To link to this article: http://dx.doi.org/10.1080/00207233.2016.1236650

Published online: 27 Oct 2016.

INTERNATIONAL JOURNAL OF ENVIRONMENTAL STUDIES, 2016
http://dx.doi.org/10.1080/00207233.2016.1236650

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Genotoxicity of recycling electronic waste in Idhna, Hebron District, Palestine

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ABSTRACT

Most Electronic waste (e-waste) ends up in landfills while some is recycled. A major site for e-waste recycling in Palestine is the village of Idhna in the Hebron District and most of this waste originates from Israel. The objective of this study was to evaluate the effects of e-waste on human DNA damage and chromosome breaks. The test sample was 46 non-smoker individuals with direct exposure to e-waste, either employed in the workshops or resident in Idhna. Genotoxicity data were compared with a control sample of sixteen unexposed individuals from Bethlehem and Al-Aizariya (Bethany). DNA damage was evaluated using the Comet assay while chromosome aberrations were tested by using conventional cytogenetic techniques. We noted an average of 4.83 aberration/cell/subject in test samples while in controls the average was 0.75. Chromosome aberration frequency was statistically different between exposed and control samples for total aberrations, for chromatid and chromosome breaks, and for formation of rings but not for dicenterics and tetraploidy. The Comet assay likewise showed that there was significant difference between exposed and control samples for DNA damage ($p < 0.05$). We therefore recommend measures to mitigate the health impact of e-waste recycling.

KEYWORDS

Electronic waste; DNA damaged; chromosomal aberration

Introduction

The expanding use of electronic equipment coupled with proportionally shortening life cycle for these devices (obsolescence) has led to a significant increase of electronic waste (e-waste) that potentially impacts human health and environmental sustainability because of the toxic substances contained in these products [1–3]. At present, the annual global e-waste generation is estimated at 50 million tonnes but only 15–20% of e-waste is recycled; the rest is burned or dumped in landfills [4,5]. Dozens of chemical elements are integrated in electronic and electrical equipment which, when released into the environment, can enter the human body by inhalation, ingestion, and direct contact.

There are many methods to test genotoxocity including the Comet assay, chromosomal aberration test, micronucleus assay, and sister chromatid exchange [6]. The aim of this study was to examine the effect of e-waste recycling, especially burning of the computer parts, on chromosomal and DNA damage in Idhna town in the Hebron Governorate. This is an area

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Figure 1. Electronics recycling facility next to a school in Idhna, Hebron District, OPT.

which receives large quantities of e-waste. Most of it is transferred from Israel and much of it is extracted by environmentally irresponsible methods such as burning [1]. Crebelli and Caiola [7] noted that chronic exposure to air pollution can be assayed by studies of peripheral blood lymphocyte DNA damage. A previous study from Bethlehem University on genotoxicity of industrial waste served as a model to show the impact on human health in Palestine [8]. In the present study, we picked an area that handles a particular, but different, problem of e-waste. Idhna seemed ideal because most of the waste being recycled is e-waste and Idhna is isolated and not subject to other contaminants (no industrial settlement nearby and no other local industries).

Materials and methods

Study area

Idhna is a town in the Hebron Governorate in the Occupied Palestinian Territories (OPT) (more than 140 countries recognize Palestine as an independent state). The OPT have rapidly become a centre for recycling for both local e-waste and material transported across the borders from Israel [1]. Idhna receives 200–500 tonnes of e-waste every day. In order to process this waste the people in Idhna established 55 main workshops in addition to many small workshops inside homes [9]. Workers extract useful or worthy materials such as copper, nickel and lead which is then sold and re-used. The extraction process includes dismantling and burning components of electronic material to separate the useful materials. Burning is a common method in Idhna both in workshops and nearby. There are poor legal frameworks and/or little enforcement of law relating to transfer and processing of e-waste in Idhna. Most of it enters through the Tarqumiya checkpoint/border crossing [10]. Workshops are distributed next to residential areas and even schools (e.g. Figure 1). Many workshop

operators also burn the material for recycling metals inside plastics. This occurs next to residential areas and near water wells, potentially contaminating the underground water.

Samples

Blood samples were collected in 9 ml sodium heparin tubes (Greiner Bioone, Germany, Cat #95,057–415) from 45 subjects from Idhna and 16 control subjects from Bethlehem and Bethany/Al-Aizarya. Subjects from target area (Idhna) had an age range of 17–58 years old (average 28) while in the control area 17–65 (average 24). Test subjects were chosen to meet the following criteria: non-smokers, residing in Idhna, and working in or living next to e-waste workshops. Consent forms and information and sample gathering was in compliance with Bethlehem University Institutional ethics guidelines/rules. The consent forms included some information about the aim of the study and a separate form without subject name collected information age, health status, and other relevant information. Control subjects were dealt with in a similar fashion. All samples and information forms were coded so that no patient names or the source of the sample was visible during laboratory work (i.e. double blind study). Coded blood samples were transported in dark insulated containers (room temperature) to the cytogenetic lab in Bethlehem University and processed as soon as possible to prevent damage of DNA from light. The chromosome aberration test was possible for all samples (n = 46) while one sample from Idhna produced no Comet data because of a technical error (i.e. n = 45 for Comet).

Chromosome aberration methods

We followed the same procedure as in Hammad and Qumsiyeh [8]. A quantity of 0.5 ml whole blood in 5 ml blood culture medium containing PHA (Biological Industries, Cat # 01-201-1A) was incubated at 37 °C for three days. Then 50 µl Colcemid solution (Biological Industries, 10 µg/ml in DPBS, cat.#12-004-1D) was added 45 min prior to harvest to arrest cells in their metaphase. The tubes were centrifuged at 1000 RPM for10 min. The supernatant was removed with a pipette and 10 ml hypotonic solution was added (0.75 M KCl), the cells suspended, and then incubated at 37 °C for 18 min. About 2 ml fresh Carnoy's fixative (1: 3 Glacial Acetic Acid: Absolute Methanol) was added to the top of each tube, then mixed and the tube centrifuged at 1000 RPM for 10 min. the supernatant was removed and the pallet was mixed gently in the remaining few drops (by tapping with fingertip or gently with pipette). The critical step of adding 10 ml of fix to the tube followed (slowly at first).

The tube was inverted gently to be mixed and centrifuged 1000 RPM for 10 min. The supernatant was aspirated and 8 ml of fresh fix was added. Fixation and centrifugation were repeated twice (total 3 times) until the pellet was white. The last step included suspending the pellet in a small volume of fixative (a few drops) and dropping it onto a clean wet microscope slide (frosted ends) held at 45° angle. The slides were put into a warm and humid environment (40 °C, 80% humidity) for spreading for about 30 s; then kept on a hot plate at 90 °C for one hour, or at room temperature overnight for chromosome hardening. Slides were stained using 3% stock Giemsa solution in pH 6.8 Gurr's Buffer for three minutes, then washed with water. 100 metaphases were scored per subject for chromosomal breaks using a light microscope (Leica ATC2000) with 100× magnification (immersion oil).

Table 1. Descriptive statistics for chromosome abnormalities scored (average of all cases in the two groups/cell/subject).

Parameter	GROUP	Mean	Standard deviation
Chrb	Exposed	2.15	1.58
	Control	0.44	0.89
Ctb	Exposed	0.7	0.96
	Control	0.00	0.00
Dicentric	Exposed	0.48	0.72
	Control	0.13	0.42
Ring	Exposed	0.54	0.72
	Control	0.06	0.2500
Tetraploidy	Exposed	0.35	0.850
	Control	0.13	0.342
PCS	Exposed	0.61	1.02
	Control	0.00	0.06
Total CA	Exposed	4.83	2.9
	Control	0.75	0.931

Comet assay or single cell gel electrophoresis (SCGE)

The comet assay was done according to Tice & Vasquez (1999) with modifications depending on trial and error in the cytogenetics lab at Bethlehem University lab as in Hammad and Qumsiyeh [8]. Observations and scoring were made from stained slides using a 10 X objective on fluorescent microscope (Olympus BX 41). Pictures were taken and analysed and measured using an Infinity camera and software (Lunenera Corporation, Ottawa, Canada). Tail and head lengths were measured on 100 randomly selected cells per sample.

Statistical analysis

Statistical analysis was done using SPSS 15. The null hypothesis was that there was no difference in genotoxic effect for the test and control sites tested. The Shapiro–Wilkes test was used for normality because the sample size was less than 50 and when normality was rejected, we used the Mann–Whitney 2-independent sample test to check for significant differences between the control and test sites [11].

Results

Chromosome aberrations (CA)

For each subject in the exposed group (n = 46) and control group (n = 16), the following variables were examined on 100 metaphases: chromosome breaks (ChrB), chromatid breaks (CtB), dicentrics, rings, tetraploidy, Premature Centromere Separation (PCS), and the total chromosome aberrations (CA). The total CA in the 46 test subjects varied from zero aberrations (1 case 2.2%) to having 11 aberrations (2 cases). In the control population 8 subjects had zero aberrations, 5 had two, 2 had two aberrations, and 1 had three aberrations. No subject in the control sample had four or more aberrations. Descriptive information of exposed and control data for ChrB, CtB, dicentrics, rings tetrapliody, PCS and total CA per 100 metaphases /person were calculated showing an increase in values for the test group (Table 1).

Table 2. The result of Mann–Whitney test for exposed and control group for chromosome aberrations.

	ChrB	CTB	Dicentrics	Rings	Tetraploidy	PCS	CA
Mann–Whitney *U*	118.500	200.000	274.000	236.000	338.000	248.000	53.000
Wilcoxon *W*	254.500	336.000	410.000	372.000	474.000	384.000	189.000
Z	−4.114	−3.232	−1.872	−2.581	−.726	−2.574	−5.101
Asymp. Sig. (2-tailed)	0.000	0.001	0.061	0.010	0.468	0.010	0.000

Table 3. Age effect on chromosome abnormalities was not significant for all variables.

	ChrB	Ct B	Dicentrics	Rings	Tetraploidy	PCS	CA
Mann–Whitney *U*	236.500	241.500	207.000	233.500	238.000	208.000	221.500
Wilcoxon *W*	467.500	566.500	532.000	558.500	469.000	533.000	546.500
Z	−.586	−.514	−1.436	−.727	−.781	−1.447	−.910
Asymp. Sig. (2-tailed)	0.558	0.607	0.151	0.467	0.435	0.148	0.363

To evaluate whether these differences are significant, we first did a normality test to determine which statistical test to use. The null hypothesis is that data follow the normal distribution of chromosome data (ChrB, CtB, dicentrics, rings, tetraploidy, PCS, and total CA). The result showed p values of Kolmogorov-Smirnov(a) and Shapiro–Wilk normality tests for the exposed group below 0.05 for both control and test sites for all variables (data not shown). Since normality was rejected, the 2-independent sample test (Mann–Whitney test) was used for comparison between exposed and control. There were significant differences between exposed and control samples for ChrB, CtB, rings, PCS, and total CA with p-value < 0.05 (Table 2). The p-value for dicentrics and tetraploidy was >0.05, however, and so there are no significant differences between exposed and control for these two variables (Table 2).

To show if there was any effect of age in the chromosome aberration we initially did a descriptive information (mean rank test) for two groups in the test site: <25 year old (n = 25) and 26 and older (n = 21). 2-independent sample test (Mann–Whitney test) for age showed no significance for any of the tested variables (Table 3). We did similar analyses within the test group for those actually working in the recycling workshops (n = 33) vs. those only living nearby (n = 13). The results were also not significant (data not shown).

The comet assay

Data were available from 45 individuals from the Idhna exposed group and 16 control group. For each group we measured the nucleus and tail length and the ratio of (tail length/total length) (TL/(TL + NL). The mean (M) and standard deviation (SD) ratio (TL/TL + NL) for exposed and control group subjects were respectively M 0.7088 SD 0.5595 (n = 45) and M 0.520 SD 0.0498 (n = 16). To determine whether the difference was significant, the data were first tested for each group for normality (the null hypothesis: the data are normal). The result showed p values of Kolmogorov–Smirnov and Shapiro–Wilkes normality tests for the exposed group with p values 0.006 and 0.016. The corresponding p values for the control group were 0.000 and 0.009. Since all p values were below 0.05, normality was rejected. The 2-independent sample test (Mann–Whitney test) was thus used for comparison between exposed and control data. Table 4 shows that the p-value < 0.05, so there was significant difference between exposed and control data in DNA damage (tail length relative

Table 4. Result of 2-independent sample test (Mann–Whitney test) comparing exposed and control for the Comet data ratio of TL/TL + NL.

	DNA
Mann–Whitney *U*	10.000
Wilcoxon *W*	146.000
Z	−5.748
Asymp. Sig. (2-tailed)	0.000

Table 5. Result of 2-independent sample test (Mann–Whitney test) on test group comparing 33 subjects who worked in the recycling workshops vs. 13 subjects not working there but living nearby.

	Group
Mann–Whitney *U*	208.000
Wilcoxon W	299.000
Z	0.000
Asymp. Sig. (2-tailed)	1.000

to tail plus nucleus' length). There was no age difference via the 2-independent sample test (Mann–Whitney test) in COMET results on two aged group first group <25 ($n = 24$) and second >26 ($n = 21$) ($p < 0.01$; data not shown).

To show if there was any excessive effect for the people who work in e-waste workshops we divided the population in Idhna into work in work shop ($n = 32$) or only living near the recycling workshops ($n = 13$). No significant difference was noted (Table 5).

Discussion

The total number of CA ranged from 0 to 11 (average 4.84 aberrations per cell per patient) for the e-waste exposed group and 0–3 (average 0.75) for the control sample. The differences for overall aberrations were significant but also for individual types of aberrations except for tetraploidy and dicentrics (Tables 1 and 2). This range of CA in our control subjects was similar to earlier data for control populations [12] and the significantly increased levels in Idhna thus portend a true effect of e-waste. The most frequent type of aberration, and with significant difference between test and control group in our study, was chromosome and chromatid breaks. In both our control and test data, the frequency of chromatid breaks was lower than chromosome breaks. Kopjar et al. [12] studies showed that the most frequent type of aberration in normal control subjects were chromatid breaks. These aberrations are induced by genotoxic agents mostly during S phase [12,13]. Chromosome type aberration caused by double strand breaks can be induced in G1 phase and these aberrations can be maintained during S phase so both sister chromatids carry aberrations in the same position and produce chromosome breaks [14]. Liu et al. [15], showed the effect of e-waste on chromosome in Jinghai County of Tianjin in China with total CA slightly higher but in line with our data.

The Comet assay was the second technique used in this study because it is sensitive enough to detect DNA damage. Comet results TL/(TL + NL) showed that there were significant differences between control and exposed groups with p-value < 0.05 (Table 4). These results are in line with the chromosome aberration results.

There was no effect for age in terms of our chromosome data or COMET data. Some authors did find some effect for age (e.g. Musthapa et al. [16]), but others did not [17–19]. The conflicting data may be explained by types and duration of exposure or technical issues (sample size, methodology) as well as sample size. We chose to divide the data into those two age groups: less than or equal to 25 years and more than 26 years to obtain roughly equal sample sizes. If we had had enough sample size it would have been instructive to see the difference between those at a much older cut off value (like 45 or 50 years old).

Studies by Hammed and Qumsiyeh [8] similarly showed that Burqeen villagers in Palestine subjected to industrial waste had total aberrations ranging from 1 to 12% (average 3.86) for test and 0.5–4% (average 1.91) for control subjects. The chromosomal aberration test in the present study did not show significant differences for all parameters. The comet assay detects all DNA lesions; chromosome aberration tests detect changes that pass through to the metaphase stage. The comet assay thus reflects short term genetic damage while the chromosome aberration test gives information about genetic damage over a longer period [12]. The comet assay also includes all nucleated peripheral blood cells; the data in the chromosomal aberration test (conventional cytogenetics) focus mostly on T-lymphocytes [12].

In unstimulated peripheral blood cells, almost all nucleated cells are in the G0 phase. The frequency of CA may only lead to mutagenic effects many years after exposure to chemical mutagens and cells have to pass through the cell cycle to replicate a genomic change [19]. Carrano and Natarajan [20] noted that there are short lived and long lived lymphocytes and their response may also be different to mutagenic agents. DNA lesions or alkaline labile sites could also be repaired in the G0 phase of cell cycles and if repair were incomplete then these DNA lesions would become chromosome breaks [19]. Taken together, the data point to increased DNA damage and CA in the population of Idhna compared to our test site and thus suggest a potential significant health impact.

The type and frequency of CA are dependent on the time of genotoxin exposure and time of exposure in the cell cycle [21]. Some of the genetic damage may be reversible after time away from further chemical mutagen exposure. Studies of subjects away from pesticide exposure for 8 months showed that CA decreased significantly [19]. These results may give hope that some genetic damage may be reversed if the environment is improved. Thus, we need to mitigate the effects and we recommend governmental (including municipal actions) and non-governmental civil society action to create alternative healthy circumstances. Developed countries export 50–80% of e-waste to Asia and Africa where they cause damage [15] and most recycled e-waste in Idhna arrives across internationally recognized borders (Israel and Palestine) [1]. Such challenges are also noted in other developing countries [22–24].

Although it may be economically and politically infeasible at this time to dismantle the e-waste industry in Idhna, it is possible to carry out a number of intermediate steps. For example, one can prevent open air burning and the Palestinian Authority could invest in creating an alternative system of separating beneficial e-waste components. Another possibility is to move e-waste recycling to an area away from the populated areas and insist that workers wear appropriate protective gear, especially masks. Education and working with schools in the area (young children can help in behavioural change also). These remedies can have a significant health impact. Ultimately, increased cancer rates, congenital birth defects, and infertility will have much higher socioeconomic impact than changing the status quo.

Acknowledgements

We are grateful to Mohammad Mahasneh of the Environmental Quality Authority who is also a resident of Idhna for logistical support. We are also grateful to the people of Idhna for their cooperation. We thank Dr Sahar Qumsiyeh for statistical support and to an anonymous reviewer for editing.

Disclosure statement

No potential conflict of interest was reported by the authors.

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