**Joint Biotechnology Master Program**

Palestine Polytechnic University

Deanship of Graduate Studies and Scientific Research

Bethlehem University

Faculty of Science

# The effect of electronic waste (e- waste) on DNA and chromosomes in Idhna, Hebron District, Occupied Palestine

By

Nadia Mahmoud Khlaif

In Partial Fulfillment of the Requirements for the Degree of

Master of Science in Biotechnology

**September 2015**

**“The effect of electronic waste (e- waste) on DNA and chromosomes in Idhna, Hebron District, Occupied Palestine”**

**By: Nadia Mahmoud Khlaif**

# Abstract

Electronic waste (e-waste) is solid waste which accumulates because of short life span of electronic equipments. The major problem of e-waste is recycling that use primitive techniques such as dumping in landfills or burning. In Palestine, a major site for work on e-waste is the village of Idhna in the Hebron Governorate. 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 with residency in Idhna town. 16 controls were used from Bethlehem and Al-Aizariya (Bethany).

The DNA damaged was evaluated using comet assay, while chromosome aberration was tested by using conventional cytogenetic. The average of the total number of chromosomes with aberration was 3.77 while in controls the average was 0.75. Chromosome aberration frequency was statistically different between exposed and control samples for chromatid and chromosome breaks, formation of rings, and total CA. No significant difference was observed between exposed and controls for incidents of dicenterics and tetraploidy (P-value >0.05). Comet assay showed that there was significant different between exposed and control for DNA damage (P-value <0.05). There no significant difference noted by age.

The results of this study demonstrate potentially grave health consequence for recycling e- waste in Idhna and we suggest some potential remedies.

Key word: Electronic waste, DNA damaged , Chromosomal aberration, comet assay , Genotoxocity.

**"تأثير النفايات الالكترونيه على الكرموسوم والصبغه الوراثيه في منطقه أذنه**

**ناديه محمود أحمد خليف**

# ملخص

النفايات الالكترونيه هي اسرع النفايات الصلبه تكونا نتيجه لتطورها المستمر وقصر فتره كفائتها وتعرف بانها نهايه عمر المعدات الاتكترونيه. من المشاكل الرئيسيه في النفايات الالكترونيه هي اعاده تدويرها والتي تعتمد على طرق بدائيه مثل الدفن و الحرق.الموقع الرئيسي في فلسطين لتدوير النفايات هو منطقه اذنه في الخليل.

الهدف من هّذه الدراسه هو دراسه تأثير النفايات الألكترونيه على الماده الوراثيه والكرموسومات في الأنسان.العينه احتوت على 46 شخص غير مدخن من منطقه اذنه تعرضوا مباشره للنفايات الأ لكترونيه ويسكنون اذنه.بالاضافه الى 16 عينه من من منطقه بيت لحم و العيزريه.

استخدم طريقتين للتحليل التحليل بالفصل الكهربائي للخليه الواحده (التحليل المذ نب) والتحليل الكروموسومي لتحديد كميه الكسر في الكرموسوم.

نتا ئج التحليل الكرموسومي تظهر معدل الكرموسوم التي كسر هو 3.77 بينما في العينات المرجعيه معدل كان 0.75 وهذا الفرق دال.وأيضا يوجد فرق دال من ناحيه تكسر الكرموسومات ,تكسر الكروماتيد, والكرموسوم الحلقي والاختلال الكلي في الكروموسوم (P value <0.05).

ولا يوجد فرق دال من ناحيه الكرموسومات ذات السنترومير ين ورباعيه المجموعه الكرموسوميه (P-value >0.05) .

الفحص الاخر للماده الوراثيه كان الفرق دال بين العينه المتأثره والعينات المرجعيه (P-value <0.05) .

لا يوجد فرق دال من ناحيه العمر والعمل في الورشات او السكن فقط (P-value >0.05).

# Declaration

I declare that the Master Thesis entitled " The effect of electronic waste (e- waste) on DNA and chromosomes in Idhna, Hebron District, Occupied Palestine " is my own original work, and hereby certify that unless stated, all work contained within this thesis is my own independent research and has not been submitted for the award of any other degree at any institution, except where due acknowledgment is made in the text.

Name and signature: Nadia Mahmoud Khlaif

Date: September 2015.

# STATEMENT OF PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for the joint master degree in biotechnology at Palestine Polytechnic University and Bethlehem University, I agree that the library shall make it available to borrowers under the rules of the library. Brief quotations from this thesis are allowable without special permission, provided that accurate acknowledgement of the source is made.

Permission for extensive quotation from, reproduction, or publication of this thesis may be granted by my main supervisor, or in his absence, by the Dean of Higher Studies when, in the opinion of either, the proposed use of the material is for scholarly purposes. Any copying or use of the material in this thesis for financial gain shall not be allowed without my written permission.

Signature: Nadia Mahmoud Khlaif

Date: September 2015.

# Dedication

I dedicate this thesis to my dear mother, husband Ghassan, sister Niveen, my children (Ramiz and Rand) and friends for their patience, support and encouragement, with love and respect.

# Acknowledgment

I extend my great thanks to my supervisor Prof. Dr Mazin Qumseiyh, PhD, Professor of genetics and cytogenetic at Bethlehem University for help, encouragement and for time and effort he put to have their thesis completed.

I am thankful to my mother and husband for their love, encouragement and support for being with me on each step of my life.

I also would like to thanks the lecture in Bethlehem University and Palestine Polytechnic University for their help and guidline.

I would like to thanks the employees in the cytogenetic lab at Bethlehem University, Reem Helal and Regine Handal .

I would also like to thanks Mr. Mohamed Mahesneha and Mrs. Uraib Sharaha for help in blood collection .

Great thanks to Mr. Mohamed Zawahra, Palestine Ahliya University for help in statistical analysis.

Final thanks to subject in Idhna that participated in this work.

# Abbreviations

| | |
|---|---|
| BER | Base excision repair |
| CA | Chromosomal aberration |
| Cd | Cadmium |
| ChrB | Chromosome break |
| CtB | Chromtid break |
| Cu | Copper |
| E-Waste | electronic waste |
| GaAs | Gallium Arsenides |
| Hg | Mercury |
| HR | Homologous recombination |
| MMR | Mismatch repair |
| NER | Nucleotide excision repair |
| Ni | Nickel |
| NL | Nucleus Length |
| PAHs | polycyclic aromatic hydrocarbon |
| Pb | Lead |
| PBDEs | polybrominateddipheny ether |
| PCBs | polychlorinated biphenyle |
| PCS | Premature chromaticd separation |
| Pt | Platinum |
| PVC | Polyvinylchlorides |
| TL | Tail Length |

# List of Figures

# List of tables

# Table of Contents

# CHAPTER ONE

## Introduction or General Introduction

The growth of electronic equipment and easy availability has impacted nearly every aspect of modern life. Although there are numerous benefits of the electronic equipment in modern society, there is a high increase of electronic waste (e-waste) generated from it. At present, the annual global e-waste generation is estimated at 50 million tones but only 15-20 % of e-waste are recycled, the rest are burned or dumped in landfills (UNEP, 2013). E-waste has become an issue of public health concern because it consists of dangerous substances and toxic chemicals which have potential to pollute the environment and risk human health when processed, recycled or disposed of (ARIJ, 2012; Bakare et al., 2013).

Dozens of chemical elements are integrated in electronic and electrical equipment. These inter human body by inhalation, ingestion , direct contact in addition to informal and formal sector of recycling. In this study genotoxicity study want to study the effect of chemical relase from e-waste in chromosome and DNA. Genotoxicity consider a primary step in initiate of cancer when they result in inactivation of tumor suppressor gene or activation of oncogene (Kang et al,2013).The damaged DNA subjects to different fate: repaired by efficient repaired mechanism or arrested by apoptosis or lead to mutation leading to cancer (Fox et al,2012).

DNA repair mechanism can be divided into five categories: base excision repair (BER), nucleotide excision repair (NER), mismatch repair (MMR) and double-strand break repair, which includes both homologous recombination (HR) and non-homologous end joining( Dexheimer,2013)

There are many methods to test genotoxocity including comet assay, chromosomal aberration test , micronucleus (MN) and sister chromatid exchange(Kang et al., 2013).

In my research, I used both alkaline comet assay and conventional cytogentic methods (chromosome aberration) because comet simple , very sensitive low level of DNA damaged ,not time consuming ,small number of cell needed, low cost and can apply to different cell type as lymphocyte ,spermatocyte, epithelial cell and fibroblast cell (Tice et al., 2000; Wong. et al, 2005)

The chromosomal aberration assay is a useful tool for study for study the effect of chemical agent and can be used as validation test for comet (Silva.et al, 2000).

The main objective of this study is to examine the effect of e-waste on chromosomal and DNA damage in Idhna town in the Hebron Governorate which receives large quantities of e-waste, most of which is transferred from Israel (ARIJ, 2012).

The main objective of this study is to examine the effect of e-waste on chromosomal and DNA damage in Idhna town in the Hebron Governorate which receives large quantities of e-waste, most of which is transferred from Israel (ARIJ,2012).

# Chapter Two

# Literature Review

## 2.1 E-waste overview

The electronic revolution in computing and e-communication grew in the second half of the 20th century and has even accelerated in the 21stcentury. The electronic growth and easy availability of computer technology has affected nearly every aspect of modern life and entered nearly all households around the world. At this time nearly every family owns an electronic device of some sort or another: a mobile phone, a microwave, a television, a refrigerator, or a personal computer. The benefit of increased use electronic tools in modern society was accompanied by a voluminous increase of electronic waste (e-waste). E-waste broadly, covers waste from all electronic and electrical devices such as mobile phone, computer, television , washing machine, refrigerators and other household equipment (Akinseye, 2013).

Robinson (2009) explained that separating electrical appliances such as refrigerators and ovens from e-waste is not quite accurate anymore. Today, E-waste is defined as any end of life goods which is dependent on electrical or electromagnetic field, in order to do their function perfectly. This definition includes small and large household items such as TVs, refrigerators, information and communication tools, electrical and electronic lighting tools, some sports equipment, medical equipment, and monitoring and control equipment (Massey et al., 2013).

E-waste became a significant source of income in some developing areas, even though it can and does have noticeable health impact on the community (Widmera et al., 2005). E-waste is one of the fastest growing waste in the world(UNEP, 2007). In 2013 the annual global e-waste generation was estimated at 50 million tons of e-waste and only 15-20% of e-wastes were recycled while the 75-80% ended up in either landfills or

burned or both (UNEP, 2013). Most of e-wastes are produced in the developed world: Europe, United States and Australia (Robinson, 2009). 50-80% of e-waste of these developed countries are exported to Asia and Africa where they cause damage (Liu et al., 2009).

E-waste may represent 1-3% of global municipal waste (Bakare et al.,2013).However, E-waste is physically and chemically different from other forms of municipal and industrial waste because it contains both beneficial and hazardous materials that need special recycling methods to get away from a negative effect on human and environment (Robinson, 2009).

When e-waste is either land filled or burnt, harmful substances can go into the air or pollute underground water (Figure 1). Harmful chemical substances may enter the human body through air, water and food (Akinseye, 2013; Bakare et al., 2013; Liu et al., 2009). Recycling methods are expensive due to labor costs and many rich country export e-waste to poor countries to recycle it by using primitive technique in recycling mostly burning. In addition in poor countries there is no legislation to prevent illegal exported of e-waste and recycling it (Robinson, 2009).

According to the way of recycling, electronic devices are classified into three major categories: a) White goods include household equipment such as refrigerators, washing machines, and air conditioners, b) Brown goods such as cameras and TVs, and c) Gray goods such as computers, fax machines, printers, and scanners. The gray good are considered more complex to recycle this because the toxic component (Pinto, 2008).

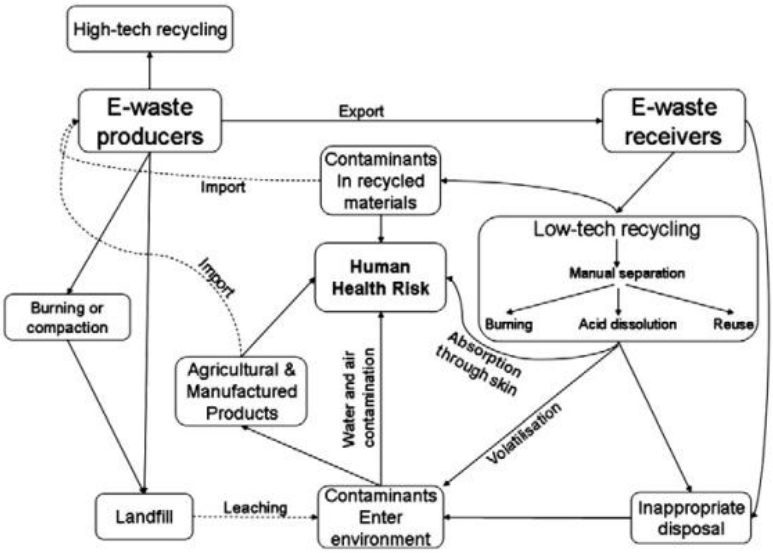


Figure 1: E-waste fate (from Robinson,2009)

Dozens of chemical elements/substances are integrated in electrical and electronic equipments (see Table 1).

e-waste contain both valuable metals such as Cu, Pt and also hazardous substances that make contaminate to the environment such as Pb, Hg, Cd, Ni, polybrominateddiphenyl ether (PBDEs) and polychlorinated biphenyle (PCBs). Burning of e-waste may produce dioxin, furans, polycyclic aromatic hydrocarbon (PAHs)(Robinson, 2009).

E-waste can be classified into substances found in small amount as Mercury, Hallium and Cadmium As well as substances found in trace amounts, including Gold , Nickel , Silver, Americium, Antimony, Arsenic, Barium, Bismuth,Boron, Cobalt, Europium, Gallium, Germanium, Indium, Lithium, Manganese, Niobium, Palladium, Platinum, Rhodium, Ruthenium, Selenium, Tantalum, Terbium, Thorium,Titanium and Vanadium. Other elements exist in large amount such as Carbon, Copper, Lead, Iron , Aluminium,Epoxy Resins, Fiberglass, PCBs (Polychlorinated biphenyls), PBDEs (Polybrominateddiphenyls ethers), PVCs (polyvinylchlorides), Thermosetting plastics, Tin, Silicon and Beryllium (Akinseye, 2013).

Also the components of e-waste can be classified according to their effect into hazardous substances and non-hazardous substances. The hazardous elements include Mercury, Lead, Americium, Sulphur, Polychlorinated Biphenyls (PCB), PBDEs, Cadmium, Beryllium Chloride, And Polyvinyl Chloride. Non-hazardous substances include Gold, Iron, Tin, Copper , Zinc, Aluminum, Germanium, Silicon, and Nickel (Akinseye, 2013). Hazardous chemicals produced from burning of plastics found in the covers and printed circuit board include Furans (Fs) and Poly Brominated Dibenzo Dioxin (PBDD) (Allsopp, 2006).

Dioxin and furans are considered highly toxic, for that exposure to furan and dioxin increase the risk of cancer, in addition the people who are exposed to dioxin and furan may change in hormone level and high dose of exposure caused skin disease called Chloracne. Also furan and dioxin can effect the immune system and reproductive system (Allsope et al., 2006). Dioxins enter the body through inhalation of contaminated air or through contaminated water and food. A bout 90% of exposure to dioxin and furan from contaminated food, dioxin and furan can participate in fatty tissue in animal ((WHO), 2014)

Table 1:Components of e-waste and the route of exposure (from Grant et al, 2013)

| | Component of electrical and electronic equipment | Ecological source of exposure | Route of exposure |
|---|---|---|---|
| **Persistent organic pollutants** | | | |
| Brominated flame retardants<br>Polybrominated diphenyl ethers | Fire retardants for electronic equipment | Air, dust, food, water, and soil | Ingestion, inhalation, and transplacental |
| Polychlorinated biphenyls | Dielectric fluids, lubricants and coolants in generators, capacitors and transformers, fluorescent lighting, ceiling fans, dishwashers, and electric motors | Air, dust, soil, and food (bio-accumulative in fish and seafood) | Ingestion, inhalation or dermal contact, and transplacental |
| **Dioxins** | | | |
| Polychlorinated dibenzodioxins and dibenzofurans | Released as combustion byproduct | Air, dust, soil, food, water, and vapour | Ingestion, inhalation, dermal contact, and transplacental |
| Dioxin-like polychlorinated biphenyls | Released as a combustion byproduct but also found in dielectric fluids, lubricants and coolants in generators, capacitors and transformers, fluorescent lighting, ceiling fans, dishwashers, and electric motors | Released as combustion byproduct, air, dust, soil, and food (bioaccumulative in fish and seafood) | Ingestion, inhalation, and dermal absorption |
| Perfluroalkyls | Fluoropolymers in electronics | Water, food, soil, dust, and air | Ingestion, dermal contact, inhalation, and transplacental |
| **Polyaromatic hydrocarbons** | | | |
| Acenaphthene, acenaphthylene, anthracene, benz[a] anthracene, benzo[a]pyrene, benzo[e]pyrene, benzo[b]fluoranthene, benzo[g,h,i]perylene, benzo[j] fluoranthene, benzo[k]fluoranthene, chrysene, dibenz[a,h]anthracene, fluoranthene, fluorene, indeno(1,2,3-c,d]pyrene, phenanthrene, and pyrene | Released as combustion byproduct | Released as combustion byproduct, air, dust, soil, and food | Ingestion, inhalation, and dermal contact |
| **Elements** | | | |
| Lead | Printed circuit boards, cathode ray tubes, light bulbs, televisions (1·5–2·0 kg per monitor), and batteries | Air, dust, water, and soil | Inhalation, ingestion, and dermal contact |
| Chromium or hexavalent chromium | Anticorrosion coatings, data tapes, and floppy disks | Air, dust, water, and soil | Inhalation and ingestion |
| Cadmium | Switches, springs, connectors, printed circuit boards, batteries, infrared detectors, semi-conductor chips, ink or toner photocopying machines, cathode ray tubes, and mobile phones | Air, dust, soil, water, and food (especially rice and vegetables) | Ingestion and inhalation |
| Mercury | Thermostats, sensors, monitors, cells, printed circuit boards, and cold cathode fluorescent lamps (1–2 g per device) | Air, vapour, water, soil, and food (bioaccumulative in fish) | Inhalation, ingestion, and dermal contact |
| Zinc | Cathode ray tubes, and metal coatings | Air, water, and soil | Ingestion and inhalation |
| Nickel | Batteries | Air, soil, water, and food (plants) | Inhalation, ingestion, dermal contact, and transplacental |
| Lithium | Batteries | Air, soil, water, and food (plants) | Inhalation, ingestion, and dermal contact |
| Barium | Cathode ray tubes, and fluorescent lamps | Air, water, soil, and food | Ingestion, inhalation and dermal contact |
| Beryllium | Power supply boxes, computers, x-ray machines, ceramic components of electronics | Air, food, and water | Inhalation, ingestion, and transplacental |

### 2.1.1 The effect of hazardous substances found in e-waste on human health and the environment

Lead: Lead exists in many electrical and electronic equipment such as circuit boards and liquid crystal displays (LCD), in TV and computer screen monitors (Messay et al., 2013). It corresponds to 6% of weight of computer monitors (Mussom et al., 2000). Human activities like burning of fossil fuel, mining and manufacturing are major lead sources (Truta et al., 2011). Humans can be exposed to these metals through inhalation of contaminated air and through contaminate foods (Akinseye, 2013).The reason for the toxicity of lead is due to the ability to mimic calcium and substitute it in many cellular processes that dependent on calcium. In addition, lead can pass through cell membranes in mechanism not fully understood until now.

The molecular mechanism of lead poisoning is not clear but some studies show indirect mechanism of action of lead such as inhibition of DNA repairor production of free radical (Garcia-Leston et al., 2010). Lead binds to DNA and RNA, disrupts DNA synthesis and can alter transcriptional processes and mitotic activity affecting genome integrity (Truta et al., 2011).

Mercury: Mercury is volatile and easily become free by breaking or burning. Mercury is found on environment in two forms: metal form and organic compound as methyl mercury ,which accumulates in living organism such as fish and reach the human body by eating contaminated fish. Both these forms are very harmful to human health. Mercury is volatile and easily become free by breaking or burning the electronic equipment that contain mercury. Mercury is found in high amount in LCD screen, fluorescent light, batteries and computer or monitor switch which account for 90% mercury (Akinseye, 2013).

Mercury's known genotoxicity is due to its ability to react with sulfhydryle group (SH) of tubulin and inhibit spindle function leading to chromosome aberrations such as polyploidy and aneuploidy (Silva-Pereira et al., 2005).The other mechanism of mercury genotoxicity is its ability to produce free radicals that can cause DNA damage(Schurz et al., 2000).Methyl mercury was reported to induce structural chromosome aberrations and increased sister chromatid exchange in Chinese hamster ovary (CHO) cells (Ehrenstein et al., 2002).

Nickel: Nickel is a compound found in environment in very low levels. It is classified as a human carcinogen by the International Agency for Research on Cancer and US Department of Health and Human Resources. There are five toxic forms for nickel: nickel powder, nickel sulfate, nickel chloride, nickel carbonate and nickel nitrate. The effect of nickel depends on the way of exposure by inhalation, oral or dermal and also by period of exposure: acute ((around 1 day)), subchronic (10-100 days) and chronic (>100 days) (Das et al., 2008). Nickel is found in batteries of many electronic equipment and CRTs of computer monitors (Akinseye, 2013) .The genotoxic effect of nickel include increased DNA strand breaks, sister chromatid exchange, DNA protein cross link, nucleotide excision, single gene mutation, micronuclei, nuclic acid concentration and cell transformation (Das et al., 2008).

Nickel induced oxidative stress and genotoxicity in normal rat kidney (Chen et al., 2010).

Nickel induced oxidative stress in culture, human lymphocytes but seemed less active in inducing reactive oxygen species ROS than such metals as Cr and Cd, this mean nickel less dangerous than chromium and cadmium (Schmid et al., 2007) .

Nickel can cause chromosome damage in vivo and in vitro. Also can cause DNA damage included: DNA strand break and cross link, impair of DNA replicate ,

inhibition of DNA repair, these happened by binding nickel ion to DNA and nuclear protein, also nickel induced DNA damaged by impaired DNA repair mechanism the interpretation of structural change in DNA or direct interaction with repair enzyme (Hartwig et al., 1994)

Arsenic: Arsenic is naturally present in ground water and present in small amount in electronic tools in a form of Gallium Arsenides (GaAs), where it is used because it have semiconductor properties(Akinseye, 2013). Arsenic is highly toxic in its inorganic form. It reaches human bodies by drinking contaminated water and food, smoking tobacco, and industrial processes, but the major mean of exposure is direct contact with dust containing this compound especially the worker in semiconductor manfacture (Akinseye, 2013).

Arsenic is classified as human carcinogenic class I agent by the International Agency for Research on Cancer. Inorganic arsenic increases the frequency of micronuclei, chromosome aberrations and sister chromatid exchanges both in humans and animals, but it does not seem to produce point mutations (Gradecka et al., 2001) .Higher dose of arsenic trioxide show significant inhibition of cell proliferation and significant increase in DNA damage measured by comet assay compared with non-exposed cells (Stevens et al., 2010)

Cadmium: Cadmium is normally present in the environment at low level human activity increased these level((WHO), 2010). It is found in many electronic equipment as switches, contact plate and used to prevent decay (Akinseye, 2013). Cadmium is used to produce nickel-cadmium batteries, paint pigments, in making polyvinyl chloride plastic, and in electroplating (WHO, 2010).The major exposure to cadmium occurs through inhalation, consumption of contaminated water and food (Akinseye, 2013). Cadmium is classified as human carcinogenic and has other toxic effect on

respiratory system, skeletal system and kidney which is a critical target organ. The mode of action of cadmium is induction of oxidative DNA damage and interference with DNA repair processes (Hartwig, 1994; Schwerdtle et al., 2010 ). Both CdO and CdCl increased the level of oxidative DNA damage and nucleotide excision repair (NER) of BPDE-induced bulky DNA adducts and UVC-induced photo lesions (Schwerdtle et al., 2010). One study done on the brain, kidney and liver cells of rats exposed to cadmium showed DNA damage. Of the three organs examined, kidney cells shown the most damage followed by liver cells (Fasanya-Odewumi et al., 1998).

Chromium: Chromium mainly exists in the environment in two forms, Chromium III and chromium VI, from natural or industrial source. Chromium III is less toxic than chromium VI (EPA, 2000). In other studies article chromium is only toxic form. But other studies show opposite results in vitro Cr (III) caused greater DNA migration than Cr (VI) in comet assay test (Blasiak and Kowalik, 2000). Chromosomal type breaks were almost two fold higher in exposed than in control individuals (Halasova et al., 2008). Chromium III is essential for human health, for that there many mechanisms in body to detoxify some amount of chromium VI to chromium III(EPA, 2000).

Chromium exists in small electronic equipment as plastic hardener and protection layers for some metal compounds. When electronic compounds are burned 99% of chromium VI may arrive in the air and soil and this contaminate may reach water supplies (Akinseye, 2013).

Poly cyclic aromatic hydrocarbon (PAH): PAHs are a group of chemicals that are formed during the incomplete burning of gas, oil, coal ,wood, garbage or other organic substances such as tobacco. PAHs exist as complete mixture as soot not as a single compound. PAH can enter the body through breathing contaminated air, cigarette smoking, wood smoke, coal smoke, and smoke for many industrial sites that contain

PAH. PAH produce many health problems depending on length of exposure, dose and if other chemical present (Akinseye, 2013). Some compounds of PAHs have been classified according to International Agency for Research on Cancer IRAQ to : carcinogenic group A or likely carcinogenic group 2A ((IARC), 2000).

The most common mechanism of carcinogenicity induced by PAH is DNA damage by forming of adducts, and the presence of free radical oxygen species induces DNA damage. Further, exposure to PAH changes expression of genes involved in apoptosis, cell cycle control and DNA repair (Munos and Albores, 2011). PAH maternal exposure increases chromosomal aberration in fetal cord blood) (Bocskay et al., 2005)

Poly chlorinated biphenyle (PCBs) compounds: PCBs are a group of 219 different organochlorine compounds (Akinseye, 2013) PCBs have been used in painting ink ,transformer oil, hydraulic fluid (Akinseye, 2013). Also, they are used to make coolant and lubricant for different type of electrical equipment (Akinseye 2013). PCBs are released to the environment by dumping old electrical equipment and e-waste and via recycling of old equipment (Akinseye, 2013). PCBs enter the air, soil, water from manufacture, accidental shed and carless recycling. They don't break down easily and can stay on environment for a very long time, transported as particles in water and air. The effect of PCBs on humans is dependent on duration, dose and type of exposure. They enter the bodies by inhalation through lung, ingestion via mouth or from direct skin contact. The mode of action of PCBs to induce DNA damaged, PCB can be metabolized to dihydroxyle compound and then oxidized to reactive metabolites which form DNA adducts and inducing free radical mediated oxidative DNA damaged (Oakley et al., 1996)

Poly brominated diphenyl ether (PBDE s): PBDEs is one of several classes of brominated compound that are used as flame retardant additive in the plastic cover of electronic equipment (Akinseye, 2013).They are chemically similar to PCBs. There are 209 possible types which dependent on brome positions (Costa et al., 2008).Commonly used derivatives are penta, octa, deca BDEs (Akinseye, 2013).The main source of PBDE is the diet and inhalation for example when people are near the workshop of recycling e-waste (Costa et al., 2008).

The major problem when PBDEs are burned, they produce brominated dioxin/furan or mixed bromochloro dioxin/furan which have great toxicity than chlorinated dioxin(Akinseye, 2013).

### 2.1.2 Ways of humans are exposed to e-waste

Sources of exposure to e-waste can be classified into three categories informal recycling, formal recycling and exposure to hazardous substances of e-waste(Grant et al., 2013).Informal recycling by is taking valuable material by using primitive technique without or with very little technology to decrease the effect of hazardous substances. Formal recycling uses specifically designed equipment to separate valuable material from other e-waste and protect worker from a negative effect on health, but these techniques are very expensive in this technique still exposure to low dose . Exposure to hazardous substances from environment can happen through inhalation, ingestion or dermal contact(Grant et al., 2013).

## 2.2 E-waste in Palestine

To our knowledge, this is the first work done in Palestine to show the effect of e-waste on the environment and human health. A report by ARIJ (2012) alerted us to E-waste on Idhna. It stated that Idhna receives 200-500 ton of e-waste every day. In order to

process these waste the people in Idhna established 55 main workshops in addition to many small workshops that run by women inside homes. Workshops are distributed next to residential areas and even schools. Many workshop operators also burn the material for recycling metals inside plastics and this is done next to residential areas and near water wells and thus contaminating the underground water. While there are other locations for e-waste, Idhna became a prime location and consumes much of the Israeli occupation e-waste because of its location near Tarqumia checkpoint and near the settlements Adora and Telem.

**2.3 Genotoxicity**

Genotoxicity is defined as the effect of the chemical on genetic material (chromosome and DNA). Chemicals that are released to the environment followed by occupational and industrial exposure can produce reactive oxygen species(ROS) and oxidative stress leading to genotoxicity (Erkekoglu and Kocer-Gumusel, 2014)

Genotoxicity is also considered a primary step in initiating cancer (Kang et al., 2013). Random mutations become significant in cancer development when they result in inactivation of tumor suppressor gene or activation of an oncogene. The Cytogenetics, micronucleus, and comet assays are useful methods for detection genetic damage (Kang et al., 2013).

DNA damage by chemical or other genotoxic agents includes single or double-strand breaks and possible rearrangements. If damage is not repaired, cell division may be arrested by apoptosis (checkpoint arrest) or could lead to mutations leading to cancer or other genetic abnormalities (Fox et al., 2012) .Thus, the negative health effect caused by genotoxins in vivo may not appear instantly(Clare, 2012).

After the discovery that ionizing radiation and other insults can induce mutation in *Drosophila* in 1940, the field of mutagenesis underwent a revolution (Natarajan, 2005). Most chemical mutagens induce chromatid type aberrations but most of these cells do not survive cell division or are arrested until repair is accomplished, but rare mutant cells may gain a selective advantage developing tumorogenesis (Ishidate Jr.M 1998). Cancer usually develops via a multi step process involving accumulations of DNA break or mutation (Major, 2000). Environmental insults increase frequency of DNA damage. For example smokers have a higher SCE frequency than non smokers (Ghada Ben Salah and Neila Belghith-Mahfoudh, 2011).

Hammad and Qumsiyeh (2013), found that the individual in Burqeen resident exposed to waste from industrial settlement had a higher chromosome break and DNA damage than control group. Borqeen village that subject to industrial waste product coming from Israeli settlement, these industrial by product are genotoxic agent that induce DNA damaged or chromosome break. I highlight in this studies because this studies done on Palestine authority in the north site which also effected by direct exposed to Israel pollution because it contain 73 factories the same as these studies that done on Idhna in the south part that effect by large amount of genotoxic agent which majority export from Israeli geoverment.

## 2.4 Review of Methods of Testing

There are many methods to test genotoxocity including comet assay, chromosomal aberration test , micronucleus (MN) and sister chromatid exchange (Kang et al., 2013). A wide range of methods are used nowadays for the detection of early biological effect of DNA damaging by environmental or occupational effects. These include well established methods for chromosomal damaged detected by chromosome aberration and SCE, but these methods are laborious and time consumes. The second method

micronucleus assay these methods has an easier technical procedure than CA and SCE assay. The third methods that detect specific adduct as P32 postlabling but these methods less frequently than cytogentic methods. For that increase the necessary for rapid and reliable test that detect DNA damage. The comet assay offers all these. It is fast , cheap, and require little biological material (one drop enough) . All these reasons above made the comet assay preferable during the last 10 years (Moller et al., 2000).

In this research, both alkaline comet assay and conventional cytogentic methods (chromosome aberration assays) were used. The comet assay is simple and sensitive assay for low level of DNA damageand can apply to different cell types such as lymphocytes, spermatocytes, epithelial cells, and fibroblast cells (Tice et al., 2000; Wong. et al, 2005).The chromosomal aberration assay is a useful tool to study for study the effect of chemical agent and can be used as a validation test for comet (Silva.et al, 2000).

### 2.4.1Chromosome aberration assay

The first drawing of human chromosome was published in 1879 by Toutain Arnold followed by many attempts to correctly count the number of chromosomes by Hanseman in 1891, De Winiwater in 1912, Painter in 1920s and Koller in 1937. With the development of techniques like using hypotonic solution by TC Hsu in 1952,Tijo and Levan were finally able to count correctly 46 chromosomes in 1956. Thus, trisomy 21was identified as the cause of Down syndrome by Lejeune in 1959 and the Philadelphia chromosome associated with CML in 1960. Early we can distinguish between each chromosome depending on size and centromer position only. But there are two factors that facilitate distinguishing between these chromosome: first, the banding of chromosome by using dye was described in late 1960 that depending on tyrosine. Second, staining technique, the most common staining technique is Giemsa

stain. We can also use a solid stain because there are many numeric and morphological abnormalities can observe clear by these stain as break and gap, multiple satellite, bisatelited marker and fragile sites.

Chromosomes are classified depending on size and centromere position into seven group: A,B,C,D,E,F,G.

Group A(1-3): Large metacentric chromosomes.

Group B(4-5): large submetacentric chromosomes.

Group C( 6-12+X): medium sized metacentric or submetacentric chromosomes.

The X chromosome look like the longest chromosome in this group.

Group D( 13-15): Medium sized acrocentric chromosome with satellites.

Group E(16-18): medium metacentric or submetacentric chromosome.

Group F(19-20): short metacentric.

Group G(21-22+Y) : short acrocentric chromosomes with satellites on 21 and 22 (not Y).

Karyotyping is the process of arranging chromosomes photographed or imaged during metaphase or late prophase stages to detect structural and numerical abnormalities. For routine studies, peripheral blood culture medium that includes phytohemagglutin in (PHA) stimulates white blood cell division. After three day culture, colcemid is added to arrest mitosis at the metaphase stage. We then use a hypotonic solution and then fixation with methanol and acetic acid to help spread and visualize chromosomes. (Qumsiyeh et al 2001; Clare 2012)

Ionizing radiation and other genotoxic agents can increase chromosome aberration including producing dicentric chromosomes, inversions, and ring chromosomes. These aberrations can happen with G0 or G1 prior to replication. Break and gaps induced by chemical mutagens happen mostly during S or G2 phase. .

A Chromosome aberrations are classified into two classes: structural aberrations such as gap, break, dicentric and ring and numerical aberrations such as hypoploidy, hyperploidy and polyploidy.

According to Major (2000) the most frequent aberrations seen in genotoxicity monitoring studies are divided into two parts: a) chromatid type aberrations such as chromatic lesions (gaps), terminal deletion (break) and exchange (quadriradial) and b) chromosome aberrations such as terminal deletion (acentric fragment), dicentric chromosome and ring chromosome.

There are many biomarkers that can be used to assess the effect of genotoxin agents such as a chromosome aberration assay, micronuclei assay, measurements of Sister chromatid exchange, DNA adduct assay, and hemoglobin adduct (Natarajan, 2005). SCEs for example show have been used in genoxicity as a test of increased liability of the genome (Dulout. F, 1992; Testa et al., 2002).

The chromosome aberration test is a sensitive methods to study genotoxicity because can detect different type of aberration chromosome break, chromatide break, ring, dicentric and PCS (Ishidate et al., 1998; Clare, 2012; Hartmann et al., 2003b; Dertinger et al., 2011)

The lymphocyte test until now is the most validated test available for monitoring population exposed to genotoxicity.

### 2.4.2 The Comet Assay

The Comet assay is technically a simple and fast method for detecting genotoxicity in mammalian cells without the need for cell culture or other elaborate techniques (Moller, 2006). The assay was used extensively in biomonitering studies for pollution studies including sewage and waste material, wood dust, ionizing radiation Nutraceuticals , organic solvents (Moller, 2006; Moller et al., 2000; Silva.et al, 2000;

Wong. et al, 2005). The comet assay is used for detecting DNA breaks/damage by measuring DNA migration in single cells by agarose gel electrophoresis . Longer strands of DNA (including intact chromsoomes) migrate little while damaged DNA moves faster through the agarose pores under electrical current. It has even been used in plants (Maluszynska and Juchimiuk, 2005).In addition, there are challenges for applying the comet assay in apoptosis is associated with increased level of DNA strand break. These cell seen under microscope normal or non founded head with long tail. These cell are called hedgehog, ghost cell with cloudy or non-detectable cell nucleus (Hartmann et al., 2003). Another challanges is to try to apply the comet assay on mitochondrial DNA which is small (16569 bp or 11*10/6 da) but some attempts used flourcent in situe hybridization with padlock probes (Collins et al., 2008).

The comet assay has many advantages: a) rapid simple and mainly cheap method, b) allows for collection of data at individual cell level, c) requires small number of cell <10,000 in each sample, d) offers high sensitivity for detection DNA damage, and e) can be applied on many cell types (Al-Salmani et al., 2011), (Heuser et al., 2002)(Tice et al., 2000).

Two basically different protocols were developed for the Comet assay: The neutral comet assay and the alkaline comet assay(Trevigen, 2010).

Singh et al (1988) developed to measure low level of strand break with high sensitivity. And Olive 1989 which suitable to detect sensitivity of cell to drug or radiation. But the version of Singh et al protocol of choice for biomontring studies (Moller et al., 2000).

The principle of the assay is the ability of denatured DNA fragments to migrate out under the influence of electrical current after lysing and treatment with alkaline medium (Moller et al., 2000)(Figure 2). The single cell gel electrophoresis (SGE) assay was developed by Cook et al in 1976 (as cited in (Piperakis, 2009). The assays included

cell lysis by nonionic detergent and high molarity sodium chloride to that histone become soluble and then the loose DNA can be subjected to electrophoresis in semi-intact cellular structures. Ethidium bromide is added to stain the DNA which appear as a comet with a varying length of "tail" depending on DNA size which is thus a measure of DNA damage (Tice et al., 2000).

Rydberg and Johnston in 1978 (cited in Piperakis2009) slightly modified the technique by adding lysis under mild alkaline condition. Technical developments in the 1980s (by Johnson and Singh et al.) and by Olive et al. in 1990 allowed the optimized procedure to be used and expanded in the two decades that followed(Tice et al., 2000), (Wonget al,2005),(Al-Salmani et al., 2011).

Examples of its use include

1. Measure DNA damage caused by oxidative stress in vivo (Al-Salmani et al., 2011)
2. To detect human mutagen and carcinogens (Tice et al., 2000). Comet assay is widely used nowadays to detect genotoxicity and can be useful in assessment after sufficient validation (Moller, 2006).
3. Comet assay , procedure for evaluating DNA lesion, single strand break and alkali-labil site (Heuser et al., 2002; Wong. et al, 2005)
4. In early 1990, comet assay was suggested to be a suitabl esay for monitoring occupational exposure (Moller et al., 2000).
5. Comet assay is a sensitive and multilateral methods for measuring single and double strand break. The sensitivity of the assay is high because hundred and thousand break per cell can be determined (Collins et al., 2008).
6. Comet assay can be used to detect the genotoxicity of industrial chemical, biocides, agrochemical and pharmaceutical (Hartmann et al., 2003).

Comet assay is a provide simple and effective methods for evaluating DNA damage (Trevigen, 2010). The advantages of comet assay sensitive , easy to use, inexpensive, short time wanted to take out result and its applicability to any eukaryotic organism and cell type (Heuser et al., 2002). Comet assay can detect different form of DNA damaged especially reparable damaged for that is useful for detect short term genotoxic damaged (Wong et al,2005). And the variation in damaged refere to repair mechanism.

DNA repair mechanism can be divided into five categories: base excision repair (BER), nucleotide excision repair (NER), mismatch repair (MMR) and double-strand break repair, which includes both homologous recombination (HR) and non-homologous end joining ( Dexheimer,2013; see Figure 3).

Excision repair (BER): repairs damage to a single nitrogenous base by action of enzyme glycosylases enzymes remove a single nitrogenous base to create an apurinic or apyrimidnic site (AP site). Then, AP endonucleases split damaged DNA backbone at the AP site. After that, DNA polymerase removes the damaged region using its 5' to 3' exonuclease activity to synthesizes the new strand using the complementary strand as a template (Dexheimer,2013; Sancar et al,2004).

Nucleotide excision repair (NER):this mechanism remove a wide variety of bulky, helix-distorting lesions from DNA. NER pathway is more complex, requiring some thirty different proteins to carry out a multi-step 'cut-and-patch'-like mechanism. Damaged regions are removed in 12-24 nucleotide-long strands in a three-step process :recognition of damage, excision of damaged DNA both upstream and downstream of damage by endonucleases, and resysnthesis of removed DNA region (Dexheimer,2013; Sancar et al,2004).

Mismatch repair (MMR): system plays an essential role in post-replication repair of bases that have escaped the proofreading activity of replication polymerases.

Inaddition to mismatched bases, MMR proteins also correct insertion and deletion loops. MMR pathway can be divided into three major steps: a recognition step where mispaired bases are recognized, an excision step where the error-containing strand is degraded resulting in a gap, and a repair synthesis step, where the gap is filled by the DNA resynthesis (Dexheimer,2013; Sancar et al,2004).

Double-strand break repair: Double-strand breaks (DSBs) are the most biologically dangerous types of DNA damage. which contain two main mechanisms: homologous recombination (HR) and non-homologousend-joining (NHEJ). HR-directed repair is largely an error-free mechanism as it utilizes the genetic information contained in the undamaged sister chromatid as a template. HR is limited to the late-S and G2 phases. NHEJ is normally error-prone and involves elimination of DSBs by direct ligation of the broken ends for that it consider the prevalent pathway in mammalian cells in all phases of the cell cycle (Dexheimer,2013; Sancar et al,2004).

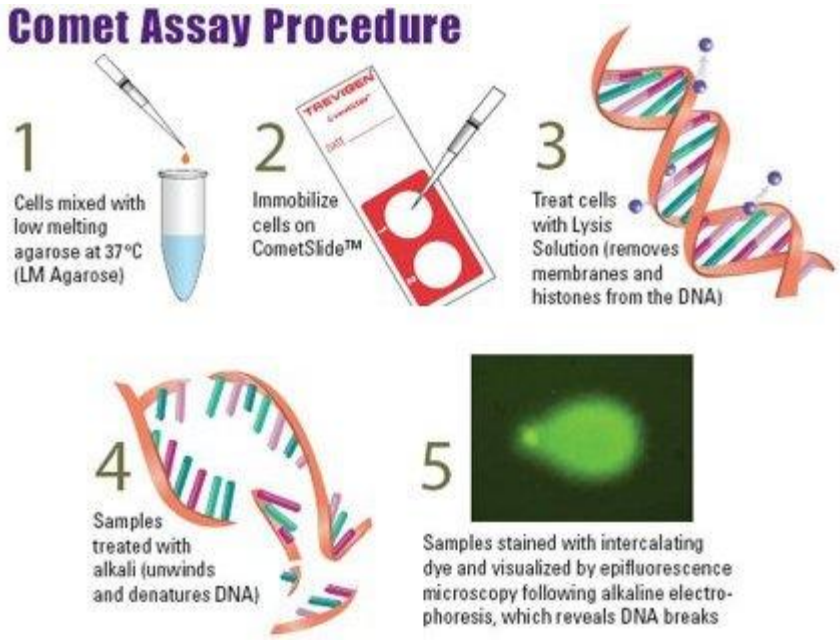


Figure 2: Comet principle

(fromAmsbio, http://www.amsbio.com/Comet-Assays.aspx )

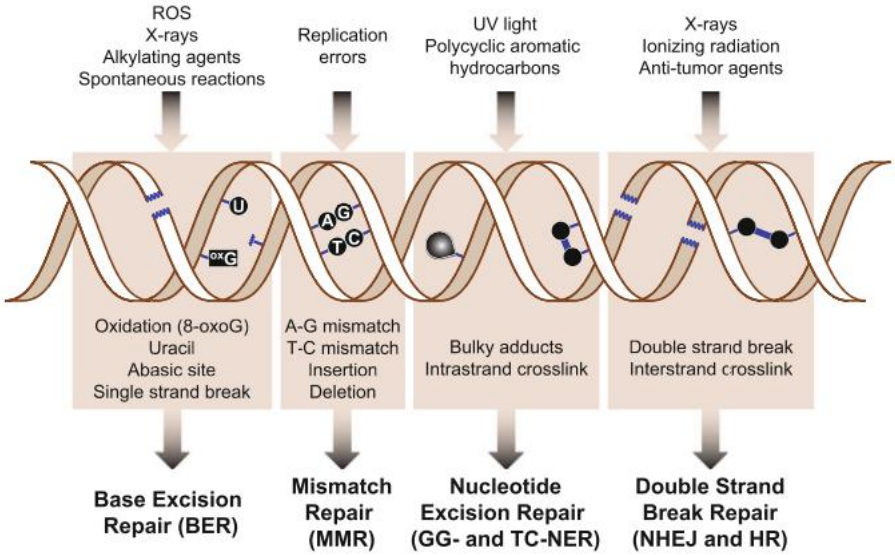


Figure 3: DNA repaire mechanisms(From Dexheimer,2013).

# CHAPTER THREE

## Study area

Idhna is a town in the Hebron Governorate located 15 km southwest of Hebron City in the southern part of the West Bank (figure 4). Idhna town was traditionally an area of high agricultural productivity and many local Palestinians maintained their livelihood from the land.

Israeli Expension and Segregation Wall was built on the fertile land and resulted in the destruction of 2,500 trees (ARIJ, 2012). Now, many town people make a living from extracting and selling material from e-waste which flows into Idhna on trucks loaded with old appliances mostly originating from Israel including refrigerators, computer monitors, and cell phones. Idhna receive roughly 200-500 tons of e-waste everyday according to Ta'awan Centre for Conflict Resolution ((TCCR), 2012).In order to process the large quantities of e-waste, Palestinians have established 55 workshops that are located within the town of Idhna. Most of these workshops are located in the populated areas and even near schools (see Figure 5b). Workshops are usually small employing as few as 2 people and as many as 30 with an estimated total workers in this area over 1000 many of which are under the age of 16 (TCCR, 2012).Workers process the e-waste to extract useful or worthy materials such as copper, nickel and lead which is then sold and re-used. This process involves dismantling and burning components of electronic material in order to separate the useful materials. Burning is common methods for process e-waste 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 or in the occupied territory in general. Being close to the green line and to the illegal Israeli settlements made Idhna prime location for such industry. The Tarqumiya checkpoint next to Idhna was built in 2006 for the purpose of monitoring

and facilitating the passage of commercial goods and increasingly plays a significant role in the dumping of Israeli waste into areas like Idhna and beyond (ARIJ, 2008).

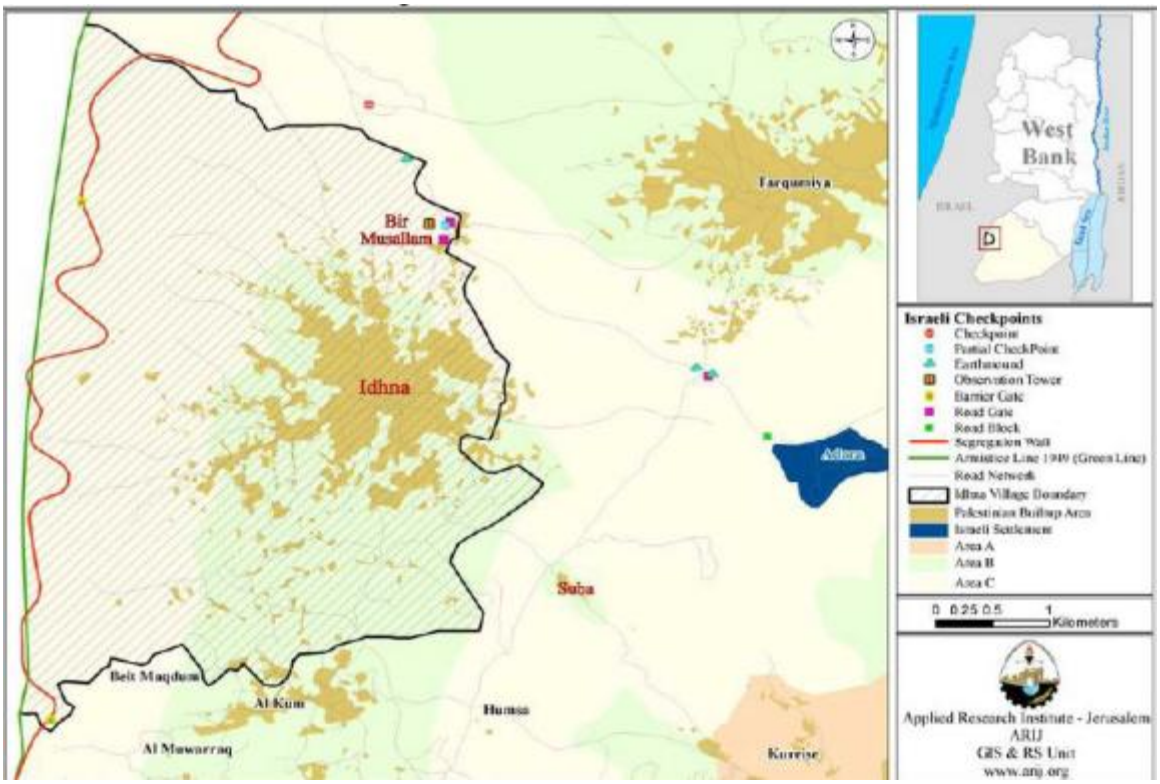


Figure 4: Map the location of Idhna in Hebron government byARIJ,2009

A previous study from Bethlehem University on genotoxicity used the Barkan industrial complex served as a model to show the impact of industrial discharge on human health in Palestine (Hammad and Qumsiyeh, 2013). Similarly, we thought of picking an area that addresses a particular, but different, problem (in this case 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 is nearby and no local other industries).

A

B

C

Figure 5: E.Waste in Idhna is located near busy streets (A), near schools (B), and near residential buildings (C).

# CHAPTER FOUR

# MATERIALS AND METHODS

Blood sample were collected in 9 ml sodium heparin tubes (Greiner bioone, Germany,95057-415) from 45 subjects from Idna and 16 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 control area 17-65 (average 24). People from Idhna were chosen to meet the following criteria: non-smokers, residing in Idhna, working in or living next to workshops processing e-waste. They involved individuals with different occupations (housewives, teachers, municipal employees and worker in e-waste workshop) see figure 6. A consent form and questionaire were obtained from all participating prior to blood donation (see Appendix). The consent forms included some information about the aim of the study, while the questions were intended to get some basic background on health status, family history and lifestyle.

The first sampling trip from Idna village was 28 of September 2013 (23 samples), while the second trip was in 11 of January 2014 (23samples).The blood sample was returned to the laboratory at Bethlehem University where we split it to do both tests (Comet and Cytogenetics). The chromosome aberration test was possible for all samples while one sample from the first trip produced no comet data because of a technical error.

Peripheral blood samples (volume3-5 ml ) were collected by venipuncture into sodium heparinised tube (9ml Sodium Heparin, Greiner Bio-one Corp.,Germany,95057-415) . After collection of blood sample were coded, transported in dark cool container ( room temperature to cytogenetic lab in Bethlehem University and processed as soon as possible to prevent additional damage for DNA.

**4.1 Cytogenetic analysis (chromosomal aberration test):**

0.5 ml whole blood was mixed with 5ml blood culture medium containing PHA (Biological Industries, Cat # 01-201-1A) and incubated at$37^{o}$C for three day.50ul Colcmid solution (Biological Industries, 10ug/ml in DPBS, cat.#12-004-1D) was added 45 min prior to harvest to arrest dividing lymphocyte in metaphase. The tubes were centrifuged at 1000 RPM for10 min. The supernatant was removed with a pipette and 10 ml hypotonic solution was added (0.75M KCl), the cells suspended, and then incubated at $37^{0}$C for 18 min.

About 2 ml fresh Carnoys 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 finger tip or gently with pipette). 10 ml of fix was added to the tube (slowly at first) since this is critical step. 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 two times (Total 3 time) until the pellet was white. The last step included suspending pellet in small volume of fixative (few drop) and dropping onto clean wet microscopic slide (frosted ends) held at $45^{0}$ angle. The slides were put on a warm and humid environment (40 C, 80% humidity) for spreading for about 30 seconds , then kept on warm plate(about 45C) until completely dry. Each slide was kept on hot plate at 90C for one hour, or at room temperature over night for chromosome hardening . Slides were stained using 3% stock Giemsa solution in pH 6.8 Gurr's Bufferfor 3 min then washed with water. Each slide was coded and scored . 100-200 metaphases scored per subject for chromosomal break using a light microscope (Leica ATC2000) with 100x magnification (immersion oil).Total number and types of aberration for each sample were evaluated.

**4.2 Comet assay(SCGE)**

The comet assay was done according to Tice & Vasquez (1999) with modifications depending on trial and error in cytogenetics lab at Bethlehem University lab as in Hammad and Qumsiyeh (2013).

1-Preparation of base slide:

Clean dry microscopic slides (Approx.76*26mm./3*1 inch, SB ground edge, forested ends, Electron microscopic science, USA, 72250-03 were dipped and then immediately removed in nearly hot 1.0% normal melting point agarose (NMPA). 500mg NMPA (Gibco, Cat.#15510-019) per 50ml in double distilled water . The underside of each slide was cleaned with dry tissue to remove agarose then the slide were put on flat surface and left to dry at room temperature. It preferred that is done at least one day before conducting the assay. Slides can be stored on slide box at room temperature and far from high humidity condition. Lysing solution, stock alkaline buffer and 10x TBE buffer were prepared a day before conducting the assay.

2- Preparing of lysing solution :

- 7.305 g NaCl (2.5M)(Bio lab, Cat #:19030291),1.86g EDTA (100m M)( Ethylene diamine tetra acetic acid, Sigma ,Cat.# ED), 0.06 g Trizma base (10m M)(Sigma ,Cat .# T4661) were added to 35 ml dH2O, then stringing the mixture
- About 0.4 gNaOH (Bio lab,Cat.#19080391) was added until EDTA dissolve,.
- The mixture was left to dissolve about (30min).
- The mixture was adjusted to 50ml by adding d H2O After that , the pH also was adjusted at 10pH by using NaOH (Bio lab,Cat.#19080391) or conc .HCL (Sigma, Cat.#2584). It was prepared a day before conducting the assay.
- On the same day of doing the experiment, we adding fresh 0.5 ml 10% Triton X-100 (Triton X-100, Sigma.Cat.#T8532) and 5 ml 10% DMSO (Dimethyl

sulfoxide, Sigma,Cat.#D84118) to the lysing solution then refrigerated or placed on ice at least 30 min prior to use.

3-Preparing the alkaline buffer:

The Stock solution of alkaline buffer could be prepared every two weeks. The stock solution is made from 10N NaOH solution (10g/25ml d H2O), and 200m MEDTA solution (0.7445g/10ml d H2OpH 10) prepared and stored at room temperature until usage.

At day of usage 1x buffer was made fresh as follows per 250 ml: mix 7.5 ml of the NaOH stock and 1.25 ml of the EDTA stock then adjust the pH to 13.

4- Preparing the electrophoresis solution:

Stock 10X TBE buffer was prepared as follows: 5.4g Trisma base (Sigma ,Cat .# T4661), 2.75g Boric acid (Bio Chemika,Cat#15665) and 0.465g EDTA (Ethylene diamine tetra acetic acid, Sigma ,Cat.# ED) were dissolved in 45ml d H2O then the volume adjusted to 50ml and stored at room temperature. The working solution was prepared by diluting 10X to 1X with dH2O.

5- Cell isolation and treatment:

All the steps here are done away from direct light to avoid additional DNA damage, In eppendorf tubes add

1) 100ul of 0.8% low melting point agarose (LMPA) (prepared by dissolving 0.2g LMPA (Sigma, Cat #A9414-5G) in 25 ml 1x PBS (Dulbecco's Phosphate Buffered Saline,10X, Sigma, Cat. #D1408).
2) 15 ul of whole blood.

The mixture was placed on the coated slides (see above) and covered with a coverslip on ice for ~5-10 min. The coverslip was gently removed, then an 50-75 ul of LMPA

was added, cover slipped again for 5-10 min on ice until agarose layer hardened. The coverslips were then removed and the slide was gently placed into cold, freshly made lysing solution as above and left in refrigerator (~4C) away of light overnight.

The slides were gently removed from the lysing, then placed in horizontal container filled with freshly made pH 13 alkaline buffer for 20min at room temperature the slide must completely covered with alkaline buffer (to enable DNA unwinding and expression of alkaline –labile damage). The slides were removed gently from alkaline buffer and washed with 1XTBE buffer before being put in gel box.

6- Electrophoresis of microgel slides :

A horizontal gel box was filled with 1XTBE buffer, then the slides were placed side by side as close together as possible in an electrophoresis box. The power supply was turned on 75 volts and 300mA for 30 min. The power supply was turned off and the slides were gently removed from the buffer.

If the slide were to be scored directly, the slides were stained with 50ul 1X Ethidium Bromide (Sigma, Cat.#E7637) (10X: 100 mg in 50 ml d H2O, 1X 1ml mixed with 9 ml dH2O), left 5min and then dipped in chilled distilled water to remove excess stain, then the coverslip was placed for each slide and scored .

Time did not enable us to examine all slides instantaneously, so a number of slides were dried by going through 100% cold methanol (Bio lab, Cat.# 13680521) about 20 min for dehydration. Then the slides were left to dry , then stored in dry dark area (slide box). When we wanted to examine them, they were rehydrated with chilled distilled water for 30 min and stained with ethidium Bromide as before. Then we add anti fade (50ml PBS buffer +20mg phenldiamine) that suppress photo-bleaching.

8-Evaluation of DNA Damage:

Observation and scoring were made for stained DNA using 10X objective on fluorescent microscope (Olympus BX 41). Infinity analyzer camera (Lunenera Corporation ,Ottaw, Canada) was used for taking picture for cell sample, then (infinity software, Canada)was used to measure tail and head length (Figure 7).50-100 randomly selected cells were analyzed per sample.

**2.3 Satistical analysis was done using SPSS 15**

First descriptive statistic as (mean, standard deviation and frequency ) was done to summarize the data . Then the null hypothesis was put (H0:There no effect for e-waste in human chromosome and DNA).To test these hypothesis the normality test was done to show which test are used to chick hypothesis by depending on the ( Shapiro-Wilk test) because the sample size < 50. The normality test was rejected for that we used 2-independent sample test Mann Whitney test to check if theses difference between control and test site are significant (Marusteri and Bacarea,2010).

Figure 6: Blood collection

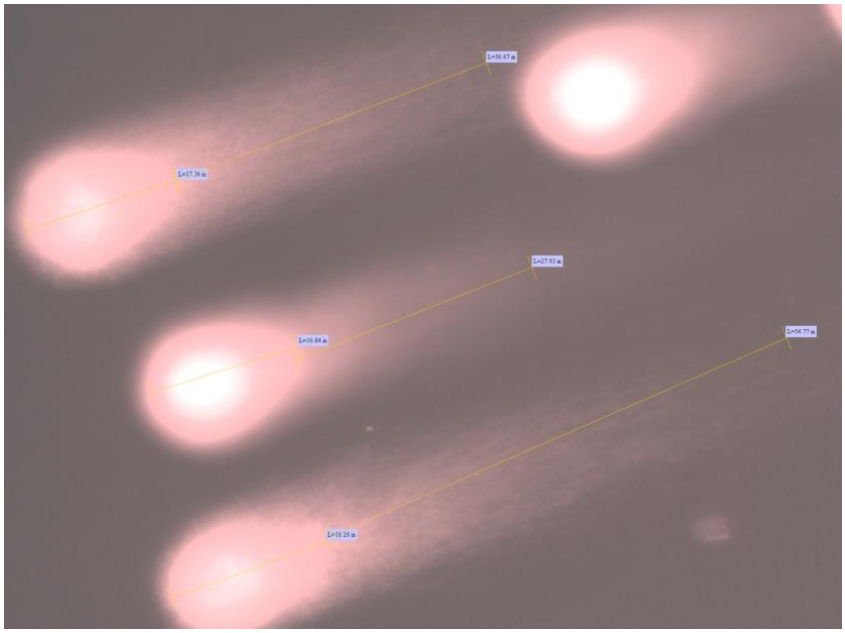

Figure 7: Comet head and tail

# CHAPTER FIVE

# RESULTS

### 5.1. Chromosome analysis

The data is divided into two groups: the exposed group (people in Idhna) (n=46) and control(n=16). For each subject the following variables were examined: chromosome breaks (chrB), chromatide breaks (CtB), dicentrics, rings, tetraploidy, and PCS.

The total chromosome abnormalities is measured the sum of (chrb+ ctb+ dicentric + ring + tetraploidy + PCS) for the 100 metaphases analyzed for each subject.

A B

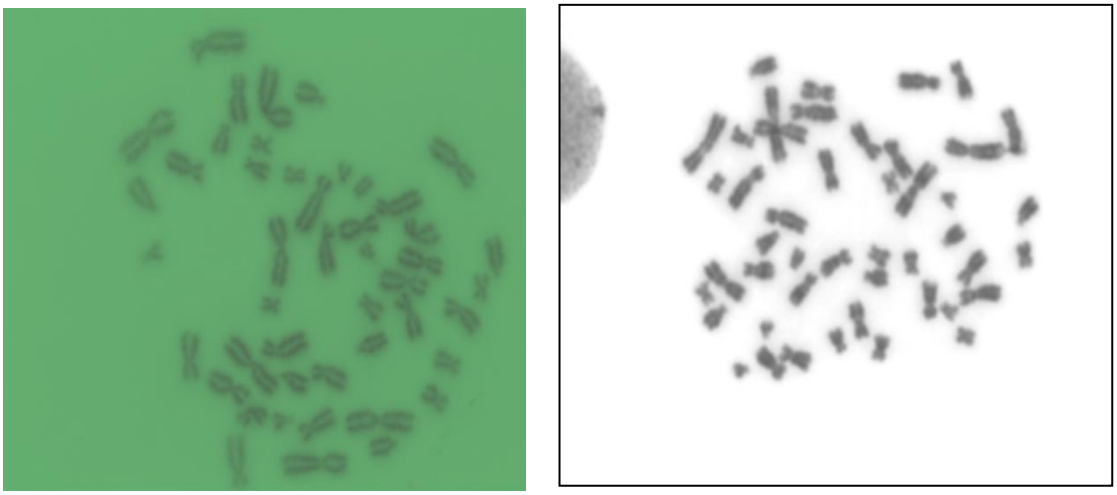

Figure 8: Normal metaphases showing no chromosome damage

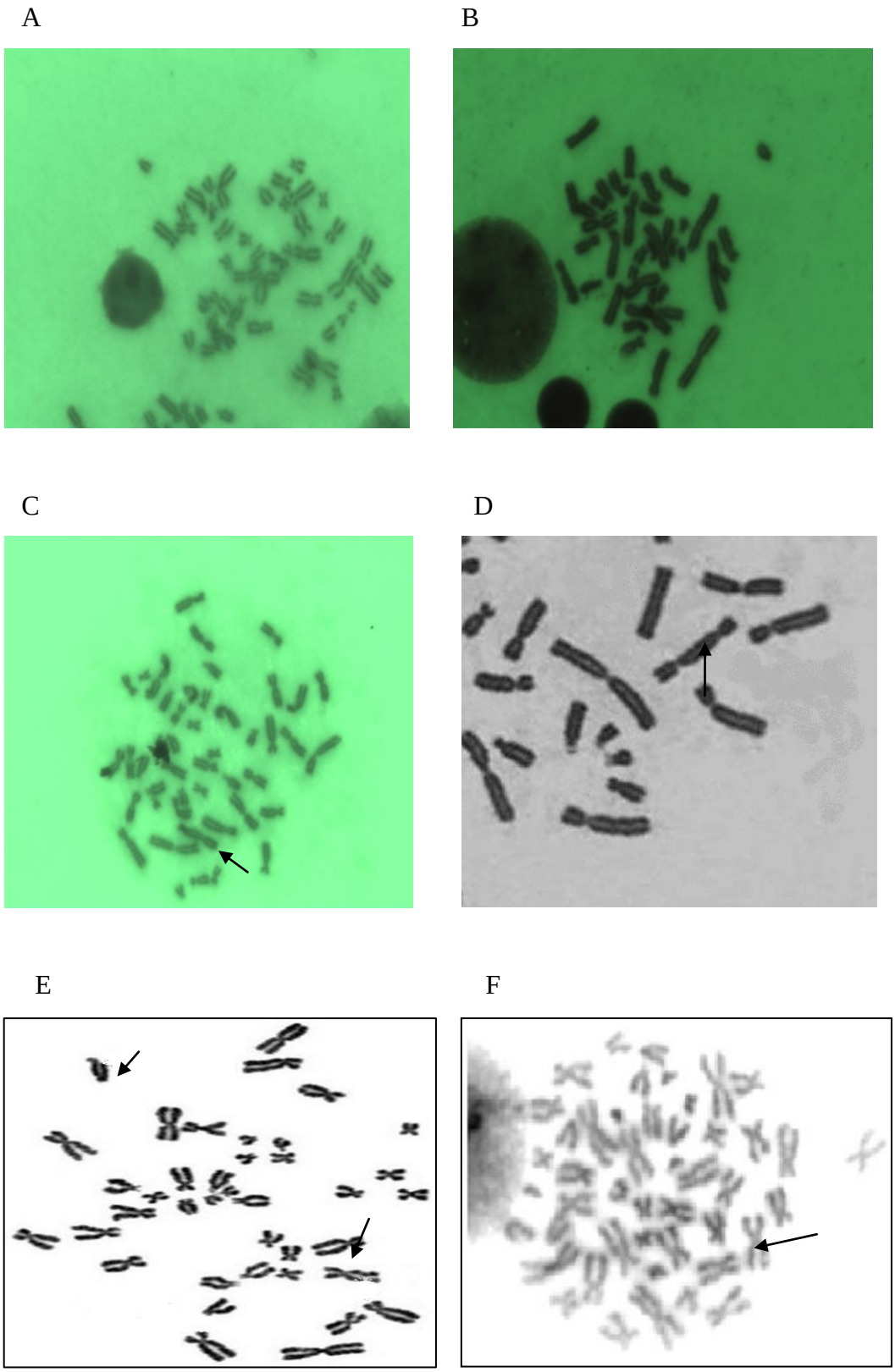


Figure9: Picture for metaphases with some aberrations: A+B PCS, C+D dicentric, E chromosome and chromatid breaks, F chromatid break.

Table 1: Descriptive data of Idhna sample ( exposed sample) and control data (people in Bethlehem and Al-'Aizarya) including the data detected about chrb, ctb, dicentric, ring, tetraploidy, PCS, and total CA. The data is scored per100 cell per subject.

| Number of sample | Gender | Age | Chr B | Cr B | Dicentric | Ring | Tetraploidy | PCS | CA |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Male | 33 | 3 | 4 | 2 | 2 | 0 | 0 | 11 |
| 2 | Male | 18 | 2 | 1 | 0 | 0 | 0 | 0 | 3 |
| 3 | Male | 46 | 1 | 3 | 1 | 1 | 0 | 0 | 6 |
| 4 | Female | 33 | 1 | 1 | 0 | 0 | 0 | 0 | 2 |
| 5 | Female | 37 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| 6 | Female | 25 | 0 | 1 | 0 | 1 | 0 | 0 | 2 |
| 7 | Male | 18 | 2 | 2 | 1 | 0 | 0 | 0 | 5 |
| 8 | Male | 45 | 0 | 1 | 0 | 1 | 0 | 0 | 2 |
| 9 | Male | 51 | 5 | 2 | 0 | 0 | 0 | 0 | 7 |
| 10 | Female | 24 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| 11 | Male | 18 | 2 | 0 | 0 | 2 | 0 | 0 | 4 |
| 12 | Male | 48 | 2 | 1 | 0 | 1 | 0 | 0 | 4 |
| 13 | Male | 18 | 2 | 0 | 0 | 1 | 0 | 0 | 3 |
| 14 | Male | 51 | 5 | 1 | 2 | 2 | 0 | 0 | 10 |
| 15 | Male | 32 | 5 | 0 | 1 | 0 | 0 | 0 | 6 |
| 16 | Male | 24 | 4 | 1 | 0 | 0 | 0 | 0 | 5 |
| 17 | Male | 19 | 2 | 0 | 3 | 0 | 0 | 0 | 5 |
| 18 | Male | 19 | 5 | 0 | 1 | 0 | 3 | 0 | 9 |
| 19 | Male | 27 | 2 | 0 | 1 | 2 | 0 | 1 | 6 |
| 20 | Male | 58 | 2 | 0 | 1 | 1 | 2 | 2 | 8 |
| 21 | Male | 29 | 4 | 1 | 0 | 0 | 0 | 3 | 8 |
| 22 | Male | 27 | 4 | 0 | 2 | 1 | 0 | 0 | 7 |
| 23 | Male | 19 | 3 | 0 | 1 | 2 | 0 | 0 | 6 |
| 24 | Male | 35 | 1 | 0 | 1 | 1 | 2 | 4 | 9 |
| 25 | Male | 19 | 1 | 2 | 1 | 0 | 4 | 3 | 11 |
| 26 | Male | 27.8 | 5 | 2 | 0 | 2 | 1 | 0 | 10 |
| 27 | Male | 27.6 | 3 | 0 | 0 | 0 | 0 | 0 | 3 |
| 28 | Male | 27.3 | 3 | 1 | 0 | 1 | 1 | 0 | 6 |
| 29 | Male | 27.1 | 0 | 1 | 1 | 1 | 0 | 0 | 3 |
| 30 | Female | 26.9 | 1 | 1 | 1 | 1 | 0 | 2 | 6 |
| 31 | Female | 26.6 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| 32 | Male | 26.4 | 4 | 0 | 0 | 0 | 1 | 2 | 7 |
| 33 | Male | 26.1 | 2 | 0 | 0 | 0 | 1 | 2 | 5 |
| 34 | Male | 25.9 | 3 | 0 | 0 | 0 | 0 | 0 | 3 |
| 35 | Male | 25.7 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| 36 | Male | 25.4 | 2 | 0 | 0 | 1 | 0 | 0 | 3 |
| 37 | Male | 25.2 | 0 | 1 | 0 | 0 | 1 | 0 | 2 |
| 38 | Male | 24.9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 39 | Male | 24.7 | 3 | 0 | 1 | 1 | 0 | 0 | 5 |
| 40 | Male | 24.5 | 1 | 1 | 0 | 0 | 0 | 1 | 3 |
| 41 | Male | 24.2 | 1 | 0 | 1 | 0 | 0 | 2 | 4 |
| 42 | Male | 24 | 1 | 0 | 0 | 0 | 0 | 1 | 2 |
| 43 | Male | 23.7 | 1 | 3 | 0 | 0 | 0 | 1 | 5 |
| 44 | Male | 23.5 | 5 | 1 | 0 | 0 | 0 | 1 | 7 |
| 45 | Male | 23.3 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| 46 | Male | 23 | 2 | 0 | 0 | 0 | 0 | 2 | 4 |
| | | 28.4 | 2.15 | 0.695652174 | 0.47826087 | 0.54347826 | 0.347826087 | 0.6086955 | 3.774194 |
| control 1 | Male | 21 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| control 2 | Female | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| control 3 | Female | 28 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| control 4 | Female | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| control 5 | Female | 21 | 3 | 0 | 0 | 0 | 0 | 0 | 3 |
| control 6 | Female | 20 | 0 | 0 | 1 | 1 | 0 | 0 | 2 |
| control 7 | Female | 28 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| control 8 | Female | 29 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| control 9 | Female | 20 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| control 10 | Male | 33 | 2 | 0 | 0 | 0 | 0 | 0 | 2 |
| control 11 | Male | 28 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| control 12 | Male | 22 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| control 13 | Male | 15 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| control 14 | Male | 45 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| control 15 | Male | 15 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| control 16 | Female | 19 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| | | 24 | 0.44 | 0 | 0.125 | 0.0625 | 0.125 | 0 | 0.733333 |

Table 2: A) Total aberrations in the 46 test subjects varied from zero aberrations (1 case 2.2%) to having 11 aberrations. B) Aberrations in control subjects.

| Aberrations | | Case | Percent | Valid Percent | Cumulative Percent |
|---|---|---|---|---|---|
| Valid | 0 | 1 | 2.2 | 2.2 | 2.2 |
| | 1 | 5 | 10.9 | 10.9 | 13.0 |
| | 2 | 5 | 10.9 | 10.9 | 23.9 |
| | 3 | 7 | 15.2 | 15.2 | 39.1 |
| | 4 | 4 | 8.7 | 8.7 | 47.8 |
| | 5 | 6 | 13.0 | 13.0 | 60.9 |
| | 6 | 6 | 13.0 | 13.0 | 73.9 |
| | 7 | 4 | 8.7 | 8.7 | 82.6 |
| | 8 | 2 | 4.3 | 4.3 | 87.0 |
| | 9 | 2 | 4.3 | 4.3 | 91.3 |
| | 10 | 2 | 4.3 | 4.3 | 95.7 |
| | 11 | 2 | 4.3 | 4.3 | 100.0 |
| | Total | 46 | 100.0 | 100.0 | |

**B)**

| Aberration | | Cases | Percent | Valid Percent | Cumulative Percent |
|---|---|---|---|---|---|
| Valid | 0 | 8 | 50.0 | 50.0 | 50.0 |
| | 1 | 5 | 31.3 | 31.3 | 81.3 |
| | 2 | 2 | 12.5 | 12.5 | 93.8 |
| | 3 | 1 | 6.3 | 6.3 | 100.0 |
| | Total | 16 | 100.0 | 100.0 | |

Table 3: Descriptive information (Mean, Standard deviation) of exposed and control data for chrb, ctb, dicentric, ring tetrapliody, PCS and total CA (number of any aberration or sum of aberration /100 metaphases /person).

| Parameter | GROUP | Mean | Standard Diviation |
|---|---|---|---|
| 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 |
| CA | Exposed | 4.83 | 2.9 |
| | Control | 0.75 | 0.931 |

According to mean and standard deviation exposed group are higher than of control group for all parameter tested (Table 3). 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 normal distribution.

Table 4: Result of normality tests of exposed and control of CA data (chrb, Ctb, dicentric, ring, tetraploidy, PCS, and total CA).

| | Group | Kolmogorov-Smirnov(a) | | | Shapiro-Wilk | | |
|---|---|---|---|---|---|---|---|
| | | Statistic | Df | Sig. | Statistic | df | Sig. |
| Chr B | Exposed | .191 | 46 | .000 | .898 | 46 | .001 |
| | Control | .438 | 16 | .000 | .575 | 16 | .000 |
| Ct B | Exposed | .308 | 46 | .000 | .729 | 46 | .000 |
| Dicentric | Exposed | .376 | 46 | .000 | .681 | 46 | .000 |
| | Control | .518 | 16 | .000 | .398 | 16 | .000 |
| Ring | Exposed | .361 | 46 | .000 | .712 | 46 | .000 |
| | Control | .536 | 16 | .000 | .273 | 16 | .000 |
| Tetraploid | Exposed | .463 | 46 | .000 | .480 | 46 | .000 |
| | Control | .518 | 16 | .000 | .398 | 16 | .000 |
| PCS | Exposed | .398 | 46 | .000 | .656 | 46 | .000 |
| CA | Exposed | .128 | 46 | .057 | .955 | 46 | .076 |
| | Control | .290 | 16 | .001 | .786 | 16 | .002 |

From Table 4 the p-value for ChrB, CtB, dicentric, ring, tetraploidy and PCS is < 0.05, so normality is rejected. So we used Mann whitney U test that used to compare differences between two independent groups.

Table 5: Descriptive information (Mean rank) of exposed and control data: (ChrB, CtB, dicentric, ring, tetraploidy, segregation, and total CA).

| | Group | N | Mean Rank | Sum of Ranks |
|---|---|---|---|---|
| Chr B | Exposed | 46 | 36.92 | 1698.50 |
| | Control | 16 | 15.91 | 254.50 |
| | Total | 62 | | |
| Ct B | Exposed | 46 | 35.15 | 1617.00 |
| | Control | 16 | 21.00 | 336.00 |
| | Total | 62 | | |
| Dicentric | Exposed | 46 | 33.54 | 1543.00 |
| | Control | 16 | 25.63 | 410.00 |
| | Total | 62 | | |
| Ring | Exposed | 46 | 34.37 | 1581.00 |
| | Control | 16 | 23.25 | 372.00 |
| | Total | 62 | | |
| Tetraploid | Exposed | 46 | 32.15 | 1479.00 |
| | Control | 16 | 29.63 | 474.00 |
| | Total | 62 | | |
| PCS | Exposed | 46 | 34.11 | 1569.00 |
| | Control | 16 | 24.00 | 384.00 |
| | Total | 62 | | |
| CA | Exposed | 46 | 38.35 | 1764.00 |
| | Control | 16 | 11.81 | 189.00 |
| | Total | 62 | | |

Table 5 shows that mean rank for all parameter of exposed sample are higher than control.

To show if the value of mean rank are significant we used 2-independent sample test (Mann- Whitney Test) for comparison between exposed for all variable, the Null hypothesis.

Because normality was rejected we chose to use Mann-Whitney test for statistical differences between test and control subjects.

Table 6: The result of Mann-Whitney Test for exposed and control group CA data: (chrb, Ctb, dicentric, ring, tetraploidy, PCS, and total CA).

| | Chr B | Ct B | Dicentric | Ring | 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) | .000 | .001 | .061 | .010 | .468 | .010 | .000 |

From the table 6 above the P-value for Chr B, CtB, ring , PCS and total CA < 0.05 so there are significant differences between exposed and control. While the p-value in dicentric and tetraploidy was >0.05 so there are no significant differences between exposed and control for these variables.

To show if there any effect of age in chromosome aberration we do mean rank test.

Table 7: Descriptive information (Mean rank) show the effect of age group on chromosome break by divided the population into two group: first group < 25 (n=25) and second >26(n=21)

| | age1 | N | Mean Rank | Sum of Ranks |
|---|---|---|---|---|
| Chr B | <25 | 25 | 24.54 | 613.50 |
| | >26 | 21 | 22.26 | 467.50 |
| | Total | 46 | | |
| Cr B | <25 | 25 | 22.66 | 566.50 |
| | >26 | 21 | 24.50 | 514.50 |
| | Total | 46 | | |
| Dicentric | <25 | 25 | 21.28 | 532.00 |
| | >26 | 21 | 26.14 | 549.00 |
| | Total | 46 | | |
| Ring | <25 | 25 | 22.34 | 558.50 |
| | >26 | 21 | 24.88 | 522.50 |
| | Total | 46 | | |
| Tetraploid | <25 | 25 | 24.48 | 612.00 |
| | >26 | 21 | 22.33 | 469.00 |
| | Total | 46 | | |
| PCS | <25 | 25 | 21.32 | 533.00 |
| | >26 | 21 | 26.10 | 548.00 |
| | Total | 46 | | |
| CA | <25 | 25 | 21.86 | 546.50 |
| | >26 | 21 | 25.45 | 534.50 |
| | Total | 46 | | |

From the table 7 above the result no great difference between two aged group according to result of mean rank .To test whether these difference are significant, the following Null hypothesis was put under examination: Mean rank for all aged group are equal.

Table 8: Result of 2-independent sample test (Mann-Whitney Test) on two aged groups first group <25 (n=25)and second>26(n=21).

| | Chr B | Ct B | Dicentric | Ring | 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) | .558 | .607 | .151 | .467 | .435 | .148 | .363 |

Table 8 above shows that p-values for all parameter (Chr b, Ctb, dicentric, ring, tetraploidy, PCS and total CA) were > 0.05, so there are no significant difference between by age.

To show if there any excessive effect for the people who work in e-waste workshop we divided the population in Idhna into work in work shop (n=33)or only living nearby (n=13).

Table 9: Descriptive information (Mean rank) for two group: first group the people in work shop (n=33), second group only living (n=13).

| | Work | N | Mean Rank | Sum of Ranks |
|---|---|---|---|---|
| Chr B | Work | 33 | 25.56 | 843.50 |
| | not work | 13 | 18.27 | 237.50 |
| | Total | 46 | | |
| Ct B | Work | 33 | 22.27 | 735.00 |
| | not work | 13 | 26.62 | 346.00 |
| | Total | 46 | | |
| Dicentric | Work | 33 | 24.06 | 794.00 |
| | not work | 13 | 22.08 | 287.00 |
| | Total | 46 | | |
| Ring | Work | 33 | 22.42 | 740.00 |
| | not work | 13 | 26.23 | 341.00 |
| | Total | 46 | | |
| Tetraploidy | Work | 33 | 24.64 | 813.00 |
| | not work | 13 | 20.62 | 268.00 |
| | Total | 46 | | |
| PCS | Work | 33 | 23.85 | 787.00 |
| | not work | 13 | 22.62 | 294.00 |
| | Total | 46 | | |
| CA | Work | 33 | 24.89 | 821.50 |
| | not work | 13 | 19.96 | 259.50 |
| | Total | 46 | | |

No great difference between two work group according to result of mean rank. To test whether these difference are significant, the following Null hypothesis was put under examination: Mean rank for two group are equal.

Table 10: Result of 2-independent sample test (Mann-Whitney Test) on two group (first group work on e-waste workshop second living nearby).

| | Chr B | Ct B | Dicentric | Ring | Tetraploid | PCS | CA |
|---|---|---|---|---|---|---|---|
| Mann-Whitney U | 146.500 | 174.000 | 196.000 | 179.000 | 177.000 | 203.000 | 168.500 |
| Wilcoxon W | 237.500 | 735.000 | 287.000 | 740.000 | 268.000 | 294.000 | 259.500 |
| Z | -1.696 | -1.097 | -.529 | -.985 | -1.323 | -.338 | -1.129 |
| Asymp. Sig. (2-tailed) | .090 | .273 | .597 | .325 | .186 | .736 | .259 |

Table 10 above shows that P-value for all parameter (Chrb, Ctb, dicentric, ring, tetraploidy, segregation and total CA) >0.05, so there is no significant difference between the two groups and pollution effects both workers and residents nearby.

### 5.2 The comet assay

The data is divided into two group: exposed group (people in Idhna) (n=45), and control group (n=16). For each group we measure the nucleus and tail length and do the ratio of (tail length/ total length) (TL/(TL+NL).

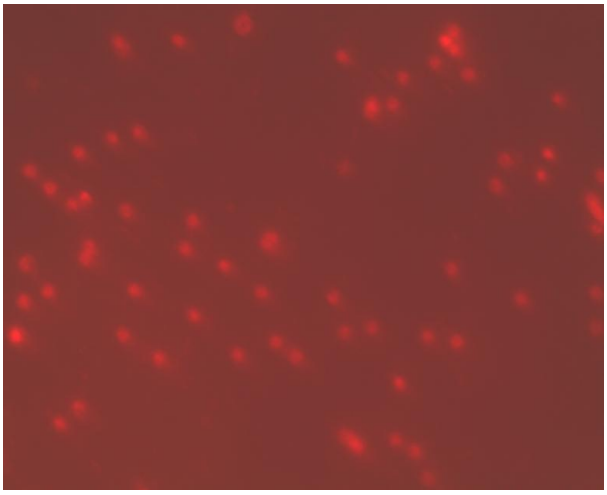

Figure (10): Representive picture for comet sample with damaged and non damaged DNA with 10X magnification

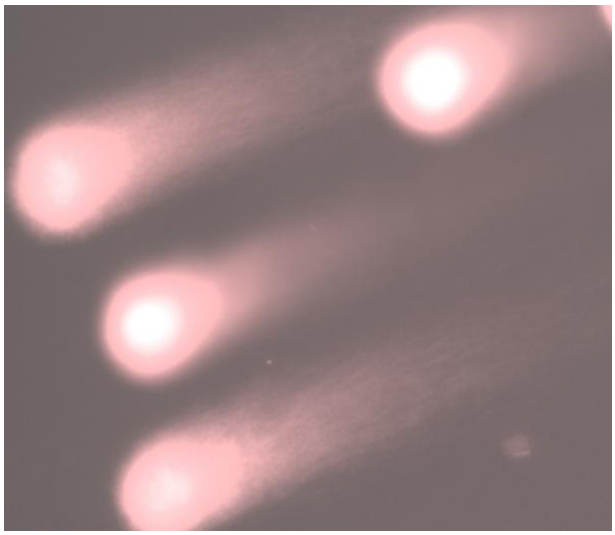

Figure (11): Representative picture for sample comet with different degree of damaged with: 50X magnification

Table 11: descriptive data about ratio (TL/TL+NL) for exposed and control and result of independent sample test.

| Group | N | Mean | Standard diviation |
|---|---|---|---|
| Exposed | 45 | 0.7088 | 0.5595 |
| Control | 16 | 0.52 | 0.0498 |

From the table above both mean and standard diviation show increased in DNA damaged in the test compared with control .To evaluate if these different in mean in two groups are significant or not, the data was first tested for normality (the null hypothesis: the data is normal)

Table 12: Normality test on exposed and control data for the ratio of (TL/TL+NL) in the comet assay.

| | Group | Kolmogorov-Smirnov(a) | | | Shapiro-Wilk | | |
|---|---|---|---|---|---|---|---|
| | | Statistic | Df | Sig. | Statistic | Df | Sig. |
| DNA | Exposed | .159 | 45 | .006 | .955 | 45 | .016 |
| | Control | .312 | 16 | .000 | .837 | 16 | .009 |

From table above the P-value <0.05 so the normality is rejected.

Table 13: Descriptive information (Mean rank) about the ratio of (TL/TL+NL).

| | Group | N | Mean Rank | Sum of Ranks |
|---|---|---|---|---|
| DNA | Exposed | 45 | 38.78 | 1745.00 |
| | Control | 16 | 9.13 | 146.00 |
| | Total | 61 | | |

The descriptive Table 13 above shows the mean rank for ratio of( TL/TL+NL) of exposed higher than of control. To show if these difference are significant w 2-independent sample test (Mean-whitney Test) was used for comparison between exposed and control.

Table 14: Result of 2-independent sample test ( Mann-whitney Test) of on exposed and control data for the ratio of (TL/TL+NL).

| | DNA |
|---|---|
| Mann-Whitney U | 10.000 |
| Wilcoxon W | 146.000 |
| Z | -5.748 |
| Asymp. Sig. (2-tailed) | .000 |

Table 14 shows that P-value <0.05, so there significant difference between exposed and control.

Table 15:Descriptive information (Mean rank) show the effect of age group on DNA damaged by divided the population into two group :first group <25 (n=25)and second >26(n=21)

| | age1 | N | Mean Rank | Sum of Ranks |
|---|---|---|---|---|
| group | <25 | 25 | 23.00 | 575.00 |
| | >26 | 20 | 23.00 | 460.00 |
| | Total | 45 | | |

From the table 15 above the result no great difference between two aged group according to result of mean rank .To test whether these difference are significant, the following Null hypothesis was put under examination: Mean rank for all aged group are equal.

Table 16: Result of 2-independent sample test (Mann-Whitney Test) on two aged group first group <25 (n=25) and second >26 (n=21).

| | Group |
|---|---|
| Mann-Whitney U | 250.000 |
| Wilcoxon W | 460.000 |
| Z | .000 |
| Asymp. Sig. (2-tailed) | 1.000 |

These table above show there no effect of age in increase the DNA damaged because P-value >0.05.

To show if there any excessive effect for the people who work in e-waste workshop we divided the population in Idhna into work in work shop (n=33)or only living (n=13).

Table 17: Descriptive information (Mean rank) for two group: first group the people in work shop (n=33), second group only living (n=13).

| | VAR00003 | N | Mean Rank | Sum of Ranks |
|---|---|---|---|---|
| group | Work | 32 | 23.00 | 736.00 |
| | not work | 13 | 23.00 | 299.00 |
| | Total | 45 | | |

From table above No great difference between two work group according to result of mean rank. To test whether these difference are significant, the following Null hypothesis was put under examination: Mean rank for two group are equal.

Table 18: : Result of 2-independent sample test (Mann-Whitney Test) on two group (first group work on e-waste workshop second living nearby).

| | Group |
|---|---|
| Mann-Whitney U | 208.000 |
| Wilcoxon W | 299.000 |
| Z | .000 |
| Asymp. Sig. (2-tailed) | 1.000 |

The table shows that the P-value >0.05 so there no significant different between working in e-waste workshop and not work " people who live in Idhna" this mean all the population in Idhna at risk.

# CHAPTER SIX

# DISCUSSION

In this research the total number of chromosome aberration was ranging from 0-11 (average 3.78%) for exposed and 0-3% for control sample (average 0.75). There was no effect for age or for being a worker in e-waste recycling or simply living near the e-waste recycling plants Some authors did find some effect for age (e.g. Mustahapa et al. 2004) while others did not (Anwar,1994; Moller et al 2000; Zeljzic and Garaaj-vrhovac 2001). The conflicting data maybe explained by types and duration of exposure or technical issues (sample size, methpodology) as well as sample size. We chose to divide the data into those two age groups: less or equal 25 years and more 26 years to roughly equal sample. However if we had enough sample size it would have been good to see the difference between those at a much older cut off value (like 45 or 50 years old).

For the worker versus nearby residents, from our visit, we noted the strong odor of the air that is inhaled by both workers and residents. It is likely that this is the primary source of genotoxic effect for the people of Idhna

The results of chromosomal aberration test data showed thata chromosome aberration increase in test subjects compared with control subjects. In the case of Chr B, CtB, ring , PCS and total CAthis mean was statistically significant (P-value <0.05 ), It was not significant between exposed and control for incidents of dicentric and tetraploidy (P-value >0.05; Table 6).

Studies by Hammed and Qumsiyeh (2013) similarly showed that Burqeen villagers in Palestine subjected to industrial waste had total aberration was ranging from 1-12% (average 3.86) for control and 0.5-4% (evarage 1.91) for control subjects.

Kopjaret al. (2006) conducted study about chromosome aberration and comet assay in healthy person which as a reference or control value for monitoring studies. This study showed that total percentage of aberrant cells was in the range of 0 –2.5% with an average of 0.48%. Gundy and Varga (1983) concluded that the chromosomal aberration frequency was between 1-6%with an average 0.88% in healthy subjects similar to our studies.

Liu et al. (2009), showed the effect of e-waste on chromosome in Jinghai County of Tianjin in China with total chromosome aberrations averaging 5.5% which exceed our result (3.78%). The difference may related to sample size (their studies included 171 test subjects also contain smoking individuals). Testa et al. (2002) showed that medical technologists (lab workers) have total CA aberration averaging 2.68 a bit less than our studies (3.78%)

Zeljzic and Garaaj-vrhovac (2001) showed the effect of pesticides exposure on chromosome and DNA showed that exposed group have significantly increase the chromosomal aberrations and DNA damage compared with the control group. The mean value of total number of aberration found in exposed group was 6.7 +/-1.75 while in control was 1.02+/-0.77. Both control and exposed group have more aberration than our results.

Anwar (1994) studied traffic policemen of Cairo in Egypt showing there was significant difference between exposed and control groups in chromosome aberrations with percent 7.7+/-3.1 and control 2.8+/-2.1 these result in combined individual(smoker and non smoker) but in non smoker individual the percent of chromosome aberration was 4.5+/11.4. These results also slightly exceed our result.

In our data by comparing the different types of chromosome aberrations between exposed samples we found that the frequency of chromosome break was 87% (which

seen in 40 person) and the frequency of chromatide break was 55.6% (which seen in 21 person). The percentage of dicentric chromosome was 37% (which seen in 17 person). In addition ,41% had the ring form (which seen 19 person) and 19.6%, tetraploidy (which seen on 9 person) and 33% segregation (which seen in 15person). This mean that the most frequent type of aberration was chromosome breaks. Similarly Au et al .(1994) studies on residenst near the uranium mining showed significant increases in chromosome aberation.

Type and frequent of chromosome aberration dependent on time of genotoxin exposure and time of exposure in cell cycle (Natarajan, 1993).

Kopjar et al 2006 studies showed that the most frequent type of aberration in normal control subjects were chromatid breaks. These aberration type is mostly induced by chemicals during S phase or post replicative stages (Kopjar et al., 2006; Major, 2000). In both our control and test data, the frequency of chromatid breaks was lower than chromosome break. Chromosome type aberration caused by double strand breaks can be induced in G1 phase and these aberration replicated during S phase so both sister chromatide carry aberrations in the same position and produced chromosome breaks. On the other hand entrance of double strand break during G2 phase effect one sister chromatid and produced chromatid breaks (Pfeiffer et al., 2000).

Alkylating agents usually produce DNA adducts or crosslinks and cannot directly break a the sugar-phosphate DNA backbone but can induce DNA repair and that mis-repaired strand breaks can lead to the chromatid type aberrations (see review by Major 2000). It is possible that the unusual high number of chromosome breaks compared with chromatid breaks in both test and control data in our case reflects susceptibility to other agents that cause chromosome but not chromatid breaks (i.e. other than e-waste).

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 group with P-value <0.05 (Table 14). These results are in line with the chromosome aberration results. Similar finding was found in Hammad and Qumsiyeh (2013) for genotoxic effects of Israeli industrial settlements.

The chromosomal aberration test did not show significant difference for all parameters. The comet assay detects all DNA lesions, that damage DNA while chromosome aberration tests detect changes that pass through to metaphase stage. The comet assay reflexs short term genetic damage that happened over few weeks while the chromosome aberration test gives information about gentic damage that happened over longer period (Kopjar et al., 2006). Further, the comet assay includes all nucleated peripheral blood cells while the data in chromosomal aberration test (conventional cytogentic) is focuses mostly on T-lymphocytes (Kopjar et al., 2006). In un stimulated peripheral blood cells almost all nucleated cells are in G0 phase. The frequency of chromosome aberration may only lead to mutagenic effect many years after exposure to chemical mutagen and cells have to successfully pass through the cell cycle to replicate a genomic change (Zeljezic and Garaj-Vrhovac, 2001). Carrano and Natarjian (1988 cited in Zeljzic and Garaaj-vrhovac 2001) 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 G0 phase of cell cycle and if miss-repaired happened theses DNA lesion converted to chromosome break (Zeljezic and Garaj-Vrhovac, 2001).

Crebelli and Caiola (2009) mentioned in their report that the chronic exposure to air pollution can be discovered by peripheral blood lymphocyte which same extent to show

DNA damage. This is because the DNA repaired ability is reduced because of deficient intracellular deoxyribonucleoside pool and lower efficiency in nucleotide excision repair.

Some of the genetic damage may be reversible after time away from further chemical mutagen exposure. Studies of subjects away from pesticide exposure for 8month showed that CA formation decreased significantly (Zeljezic andGaraj- Vrhovac, 200). These result may give hope to people to reverse some of genetic damaged if the environment is improved.

# Conclusion and Recommendations

People in Idhna have higher chromosome break and DNA damaged than the control group this mean there significant effect for e-ewaste on genetics material. This is the second study of genotoxicity in the Palestinian population after Hammad and Qumsiyeh (2013). Both showed health effects (one for Israeli industrial pollutants and one for e-waste). We recommend doing other studies for other potential pollutants. As for these pollutants, we recommend governmental (including municipal actions) and non-governmental civil society action to create alternative healthy circumstances. While it may be economically and politically unfeasible to remove 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 alteranative system of separating beneficial substances. Another possibility is to move e-waste recycling to an area away from the populated areas and sins it that workers wear appropriate protective gear especially masks. Education and working with schools in the area (young children can help in behavioral 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. It is recommended to perform some studies on the health history and diseases status of the targeted people linking each case to the finding of this research concerning the DNA damage and chromosome aberration.

# Chapter Seven

## References


Akinseye, V.O. 2013. Electronic Waste Components in DevelopingCountries:

Allsopp.M,D. Santillo and P.Johnston. 2006. Enviromental and human health concerns in the processing of electrical and electronic wast.*GreenpeaceResearch Laboratory, Technical Note*.25 pp. Available at

Al-Salmani, K., H.H. Abbas, S. Schulpen, M. Karbaschi, I. Abdalla, K.J. Bowman, K.K. So, M.D. Evans, G.D. Jones, R.W. Godschalk, and M.S. Cooke. 2011. Simplified method for the collection, storage, and comet assay analysis of DNA damage in whole blood. *Free Radic Biol Med*. 51(3):719-725.

Anwar, W.A. 1994. Monitoring of Human Populations at Risk by Different Cytogenetic End Points. *Environmental Health Perspectives*. Volume 102(4):131-134.

Applied Research Institute–Jerusalem (ARIJ). 2012. The impact of electronic waste disposable into inviroment and puplic health in the occupied palestinian territory: case study from Idhna, Hebron Goverment.v

ARIJ. 2008. Repercussion of the Israeli segregation wall start to materialize: An Israeli decision to rearrange fule delivary procedure to Bethlehem governorate from Tarqumiya comercial terminal in Hebron Governorate.Report by the Applied Research Institute-Jerusalem http://www.poica.org.

Bakare , A.A., O.A. Alabi, A. M. Gbadebo , O. I. Ogunsuyi, and C.G. Alimba. 2013. In Vivo Cytogenotoxicity and Oxidative Stress Induced by Electronic Waste Leachate and Contaminated Well Water. *Challenges*. 4(2):169-187.

Ben Salah, G., H. Kamoun, A. Rebai, A. Ben Youssef, H. Ayadi, N. Belghith-Mahfoudh, A. Fourati, and F. Fakhfakh. 2011. Sister chromatid exchange (SCE)

and high-frequency cells (HFC) in peripheral blood lymphocytes of healthy Tunisian smokers. *Mutat Res*. 719(1-2):1-6.

Blasiak, J., and J. Kowalik. 2000. A comparison of the in vitro genotoxicity of tri- and hexavalent chromium. *Mutat Res*. 469(1):135-145.

Bocskay, K.A., D. Tang, M.A. Orjuela, X. Liu, D.P. Warburton, and F.P. Perera. 2005. Chromosomal aberrations in cord blood are associated with prenatal exposure to carcinogenic polycyclic aromatic hydrocarbons. *Cancer Epidemiol Biomarkers Prev*. 14(2):506-511.

Center for Disease Control (CDC).**A**gency for Toxic Substances and Disease Registry (ATSDR). 1995. Puplic Health statement: Polycyclic Aromatic hydrocarbon (PAHs). http://www.atsdr.cdc.gov/phs/phs.asp?id=120&tid=25.

Chen, C.Y., T.K. Lin, Y.C. Chang, Y.F. Wang, H.W. Shyu, K.H. Lin, and M.C. Chou. 2010. Nickel(II)-induced oxidative stress, apoptosis, G2/M arrest, and genotoxicity in normal rat kidney cells. *J Toxicol Environ Health A*. 73(8):529-539.

Clare, G. 2012. The in vitro mammalian chromosome aberration test.Chapter 5 pp. 69-91, in *Methods Mol Biol*. (edited by J M Perry and E M. Perry), Springer, New York.

Collins, A.R., A.A. Oscoz, G. Brunborg, I. Gaivao, L. Giovannelli, M. Kruszewski, C.C. Smith, and R. Stetina. 2008. The comet assay: topical issues. *Mutagenesis*. 23(3):143-151.

Costa, L.G., G. Giordano, S. Tagliaferri, A. Caglieri, and A. Mutti. 2008. Polybrominated diphenyl ether (PBDE) flame retardants: environmental contamination, human body burden and potential adverse health effects. *ACTA BIOMED*. 79:172-183.

Crebelli, R.,S.Caiola. 2009. Genetic effects of air pollutants: insights from human biomonitoring studies. *Indian J Med Res*. 130:pp 501-503.

Das, K.K., S.N. Das, and S.A. Dhundasi. 2008. Nickel, its adverse health effects & oxidative stress. *Indian J Med Res*. 128(4):412-425.

Dertinger, S.D., D.K. Torous, M. Hayashi, and J.T. MacGregor. 2011. Flow cytometric scoring of micronucleated erythrocytes: an efficient platform for assessing in vivo cytogenetic damage. *Mutagenesis*. 26(1)139-145.

Dexcheimer,T.S,2013.DNA Repair Pathway and Mechanism,L.A Methwas,SM Cabarcas,E Hurt(eds). Text book of DNA repair of cancer stem cell,Ch.2,pp19-32.

Dulout,F.N., C.J.S.Lopez, H.N.V.Guradze. 1992. Analysis of sister chromatid exchanges (SCE) in human population studies. *Brazil .J. Gentics*. 15(1):169-182.

Ehrenstein, C., P. Shu, E.B. Wickenheiser, A.V. Hirner, M. Dolfen, H. Emons, and G. Obe. 2002. Methyl mercury uptake and associations with the induction of chromosomal aberrations in Chinese hamster ovary (CHO) cells. *Chem Biol Interact*. 141(3):259-274.

Enviromental Production A gency (EPA). 2000. Chromium Compounds. http://www.epa.gov/ttnatw01/hlthef/chromium.html

Erkekoglu, P., and B. Kocer-Gumusel. 2014. Genotoxicity of phthalates. *toxicol Mech Methods*. 24(9):616-626.

Fasanya-Odewumi, C., L.M. Latinwo, C.O. Ikediobi, L. Gilliard, G. Sponholtz, J. Nwoga, F. Stino, N. Hamilton, and G.W. Erdos. 1998. The genotoxicity and cytotoxicity of dermally-administered cadmium: effects of dermal cadmium administration. *Int J Mol Med*. 1(6):1001-1006.

Fox, J.T., S. Sakamuru, R. Huang, N. Teneva, S.O. Simmons, M. Xia, R.R. Tice, C.P. Austin, and K. Myung. 2012. High-throughput genotoxicity assay identifies antioxidants as inducers of DNA damage response and cell death. *Proc Natl Acad Sci U S A*. 109(14):5423-5428.

Garcia-Leston, J., J. Mendez, E. Pasaro, and B. Laffon. 2010. Genotoxic effects of lead: an updated review. *Environ Int*. 36(6):623-636.

Gradecka, D., J. Palus, and W. Wasowicz. 2001. Selected mechanisms of genotoxic effects of inorganic arsenic compounds. *Int J Occup Med Environ Health*. 14(4):317-328.

Grant, K., F.C. Goldizen, P.D. Sly, M.N. Brune, M. Neira, M. van den Berg, and R.E. Norman. 2013. Health consequences of exposure to e-waste: a systematic review. *Lancet Glob Health*. 1(6):e350-361.

Halasova, E., T. Matakova, L. Musak, V. Polakova, and P. Vodicka. 2008. Chromosomal damage and polymorphisms of DNA repair genes XRCC1 and XRCC3 in workers exposed to chromium. *Neuro Endocrinol Lett*. 29(5):658-662.

Hammad K., and M.B.Qumsiyeh. 2013. Genotoxic Effects of Israeli Industrial Settlement Pollutants on residents of Bruqeen Village (Salfit District, Palestine). *Intl. Journal of Environmental Studies*, 70(4):655-662.

Harmless Substances or PotentialCarcinogen. *Annual Review & Research in Biology*. 3(3):131-147.

Hartmann, A., E. Agurell, C. Beevers, S. Brendler-Schwaab, B. Burlinson, P. Clay, A. Collins, A. Smith, G. Speit, V. Thybaud, and R.R. Tice. 2003. Recommendations for conducting the in vivo alkaline Comet assay. 4th International Comet Assay Workshop. *Mutagenesis*. 18(1):45-51.

Hartwig, A., I. Kruger, and D. Beyersmann. 1994. Mechanisms in nickel genotoxicity: the significance of interactions with DNA repair. *Toxicol Lett*. 72(1-3):353-358.

Hartwig, A., Role of DNA repair inhibition in lead- and cadmium-induced genotoxicity: a review.1994. Environ Health Perspect.102( 3): 45–50.

Heuser, V.D., J. da Silva, H.J. Moriske, J.F. Dias, M.L. Yoneama, and T.R. de Freitas. 2002. Genotoxicity biomonitoring in regions exposed to vehicle emissions using the comet assay and the micronucleus test in native rodent Ctenomys minutus. *Environ Mol Mutagen*. 40(4):227-235.

http://greenpeace.to/publications/Ewastetreatment-May2006-FINAL.pdf

International Agency for Research on Cancer (IARC). 2000. Chemical carcinogen. http://monographs.iarc.fr/ENG/Classification.

Ishidate, M., Jr., K.F. Miura, and T. Sofuni. 1998. Chromosome aberration assays in genetic toxicology testing in vitro. *Mutat Res*. 404(1-2):167-172.

Kang, S.H., J.Y. Kwon, J.K. Lee, and Y.R. Seo. 2013. Recent advances in in vivo genotoxicity testing: prediction of carcinogenic potential using comet and micronucleus assay in animal models. *J Cancer Prev*. 18(4):277-288.

Kopjar, N., D. Zeljezic, and V. Garaj-Vrhovac. 2006. Evaluation of DNA damage in white blood cells of healthy human volunteers using the alkaline comet assay and the chromosome aberration test. *Acta Biochim Pol*. 53(2):321-336.

Liu, Q., J. Cao, K.Q. Li, X.H. Miao, G. Li, F.Y. Fan, and Y.C. Zhao. 2009. Chromosomal aberrations and DNA damage in human populations exposed to the processing of electronics waste. *Environ Sci Pollut Res Int*. 16(3):329-338.

Major, M. 2000. Cytogenetic Biomarkers Applied Foroccupational Cancer Risk Assessment. *Semmelweis University, Faculty Of Medicine, And The National Institute Of Chemical Safety, Budapest, Hungary.*

Maluszynska, J., and J. Juchimiuk. 2005. Plant genotoxicity: a molecular cytogenetic approach in plant bioassays. *Arh Hig Rada Toksikol*. 56(2):177-184.

Marusteri.M, V. Bacarea.2010 Comparing groups for statistical differences: how to choosthe right statistical test? Biochemia Medica . 20(1): 15-32. http://dx.doi.org/10.11613/BM.2010.004.

Massey, K. A.. and J. Eunson. 2013. Electronic Wastelands: Decomposing Computers and Communities Around the World. *UBC Medical Journal* 2(1): 30-32.

Moller, P. 2006. The alkaline comet assay: towards validation in biomonitoring of DNA damaging exposures. *Basic Clin Pharmacol Toxicol*. 98(4):336-345.

Moller, P., L.E. Knudsen, S. Loft, and H. Wallin. 2000. The comet assay as a rapid test in biomonitoring occupational exposure to DNA-damaging agents and effect of confounding factors. *Cancer Epidemiol Biomarkers Prev*. 9(10):1005-1015.

Munoz.B , A.Albores. 2011. DNA Damage Caused by Polycyclic Aromatic Hydrocarbons: Mechanisms and Markers. pp. 125-146 , in Selected Topics in DNA Repair(Edited by Prof. Clark Chen). Available from: http://www.intechopen.com/books/selected-topics-in-dna-repair/dna-damage-caused-by-polycyclic-aromatic-hydrocarbons-mechanisms-and-markers.

Musson.S, Y-C.Jang,TG. Townsend and ,II- H. chung. 2000. Characterization of Lead Leachability from Cathode Ray Tubes Using the Toxicity Characteristic Leaching Procedure. *Environ. Sci. Technol.* 34(20): 4376–4381.

Musthapa MS, M. Lohani, S Tiwari, N Mathur, R. Prasad, Q Rahman. 2004.Cytogenetic biomonitoring of Indian women cooking with biofuels: micronucleus and chromosomal aberration tests in peripheral blood lymphocytes. Environ Mol Mutagen.43(4):243-9.

Natarajan, A.T. 2005. Chemical mutagenesis: From plants to human. *Current Science*. 89(2):312-317.

Natarajan, A.T.1993. Technique for biomontring of human population for gentic effect.*Brazil .J. Gentics*. 16(3):841-847.

Oakley, G.G., U. Devanaboyina, L.W. Robertson, and R.C. Gupta. 1996. Oxidative DNA damage induced by activation of polychlorinated biphenyls (PCBs): implications for PCB-induced oxidative stress in breast cancer. *Chem Res Toxicol*. 9(8):1285-1292.

Pfeiffer, P., W. Goedecke, and G. Obe. 2000. Mechanisms of DNA double-strand break repair and their potential to induce chromosomal aberrations. *Mutagenesis*. 15(4):289-`302.

Pinto, V.N. 2008. E-waste hazard: The impending challenge. *Indian J Occup Environ Med*. 12(2):65-70.

Piperakis, S.M. 2009. Comet assay:A breif history. *Cell biology toxicol*. 25(1):1-3.

Qumsiyeh, M.B., S.G.Adhvaryu,M.N.Ahmed, and A.S. Teebi, 2001. Cytogenetics. In: A.Y. Elzouki, H.A. Harfa, H.M. Nazer, eds. 2001. Textbook of clinical pediatrics. Philadelphia: Lippincott, Williams& Wilkins, Inc., Ch.2, pp.20-32.

Robinson, B.H. 2009. E-waste: an assessment of global production and environmental impacts. *Sci Total Environ*. 408(2):183-191.

Sancar, A., L. A. Lindsey-Boltz, et al. (2004). "Molecular mechanisms of mammalian DNA repair and the DNA damage checkpoints." Annu Rev Biochem **73**: 39-85.

Schmid, M., S. Zimmermann, H.F. Krug, and B. Sures. 2007. Influence of platinum, palladium and rhodium as compared with cadmium, nickel and chromium on cell viability and oxidative stress in human bronchial epithelial cells. *Environ Int*. 33(3):385-390.

Schurz, F., M. Sabater-Vilar, and J. Fink-Gremmels. 2000. Mutagenicity of mercury chloride and mechanisms of cellular defence: the role of metal-binding proteins. *Mutagenesis*. 15(6):525-530.

Schwerdtle, T., F. Ebert, C. Thuy, C. Richter, L.H. Mullenders, and A. Hartwig. 2010. Genotoxicity of soluble and particulate cadmium compounds: impact on oxidative DNA damage and nucleotide excision repair. *Chem Res Toxicol*. 23(15):432-442.

Silva.J.D, T.Freitas,G. Marinno,G. Speit andB. Erdtmann. 2000. An alkaline single - cell gel electrophoresis (comet) assay for enviromental biomontring with native rodent. *Gentic and Molecular biology*. 23(1):241-245.

Silva-Pereira, L.C., P.C. Cardoso, D.S. Leite, M.O. Bahia, W.R. Bastos, M.A. Smith, and R.R. Burbano. 2005. Cytotoxicity and genotoxicity of low doses of mercury chloride and methylmercury chloride on human lymphocytes in vitro. *Braz J Med Biol Res*. 38(6):901-907.

Stevens, J.J., B. Graham, A.M. Walker, P.B. Tchounwou, and C. Rogers. 2010. The effects of arsenic trioxide on DNA synthesis and genotoxicity in human colon cancer cells. *Int J Environ Res Public Health*. 7(5):2018-2032.

Ta'wun Center for Conflic Resolutution (TCCR) 2012. Burning of copper and toxic waste in Idhna town'. Partners of Change Project in cooperation with the popular committee for combating the environmental pollution phenomenon in Idhna. Unpublished Report.

Testa, A., R. Ranaldi, L. Carpineto, F. Pacchierotti, D. Tirindelli, L. Fabiani, A.R. Giuliani, M. Urso, A. Rossini, F. Materazzo, M. Petyx, and V. Leoni. 2002. Cytogenetic biomonitoring of workers from laboratories of clinical analyses occupationally exposed to chemicals. *Mutat Res*. 520(1-2):73-82.

Tice, R.R., E. Agurell, D. Anderson, B. Burlinson, A. Hartmann, H. Kobayashi, Y. Miyamae, E. Rojas, J.C. Ryu, and Y.F. Sasaki. 2000. Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing. *Environ Mol Mutagen*. 35(3):206-221.

Trevigen. 2010. CometAssay Reagent Kit for Single Cell Gel Electrophoresis Assay.http://www.trevigen.com/cat/1/3/0/CometAssay.

Truta.E., C.M.Rosu and I. C. Bara. 2011. Lead induced genotoxicity in wheat. *Molecular biology*. 12:51-58.

United Nations Environmental Program (UNEP). 2007. Global Partnership on Waste Management. http://www.unep.org/gpwm/InformationPlatform/CountryNeedsAssessmentAnalysis/Nepal/tabid/106535/Default.aspx.

United Nations Environmental Program (UNEP). 2013. Policy Brief on E-Wate. http://www.unep.org/ietc/Portals/136/Other%20documents/PolicyBriefs/13052013_E-Waste%20Policy%20brief.pdf.

Widmer, R., H. Oswald-Krapf, D. Sinha-Khetriwalb, M. Schnellmannc, and H. Boni. 2005. Global perspectives on e-waste .Environmental Impact Assessment Review.25(2005): 436–458.

Wong. V.W.C., Y.T.Szeto.,A. R.Collins, and I.F.F.Benzi. 2005. The Comet Assay: a biomonitoring tool for nutraceutical research. *Current Topics in Nutraceutical Research*. 3(1):1-14.

World Health Organization (WHO) 2010. Exposure To Cadmium: A Major Public Health Concern.http://www.who.int/ipcs/features/cadmium.pdf.

World Health Organization (WHO) 2014. Dioxins and their effects on human health. http://www.who.int/mediacentre/factsheets/fs225/en/.

World Health Organization 2000. Chemical carcinogen. WHO International agency for research on cancer (IARC).

Zeljezic, D., and V. Garaj-Vrhovac. 2001. Chromosomal aberration and single cell gel electrophoresis (Comet) assay in the longitudinal risk assessment of occupational exposure to pesticides. *Mutagenesis*. 16(4):359-363.

# Appendix

برنامج الدراسات العليا المشترك بين جامعه بيت لحم وجامعه بولتكنيك فلسطين

برنامج الماجستير في التكنولوجيا الحيوية

نموذج موافقة

صمم هذا البحث العلمي بهدف دراسة وتقيم مدى التأثيرات السلبيه للفضلات الالكترونية (من حرق ودفن) على صحة المواطنين.

يتضرر الانسان بهذه المخلفات من خلال الهواء الملوث, أ, وجود هده الملوثات في مياه شرب المواطنين أو في المياه التي تمتصها النباتات المزروعة وبالتالي تصل لجسم الانسان سواء بتناول هده النباتات أو الحيوانات التي ترعى هده النباتات. التأثيرات الضارة لا تظهر فورا ولكنها تعمل على احداث تغيرات غير طبيعيه في الحمض النووي الوراثي والكرموسومات . وهده التغيرات تؤثر على احتماليه الاصابة بالسرطان .

كافه النتائج ستبقى سريه ويبقى لكم خير المشاركه في هذا البحث.

الاسم :

1-الجنس:1- ذكر 2-انثى

2-العمر:

3-مكان السكن:----------

4- الحاله الاجتماعية: 1- اعزب 2-متزوج 3-غير ذلك

5- مكان العمل : في اذنه 1-نعم 2-لا

6- اذا نعم مدى القرب من ورشات فصل النفايات الكترونيه ----------

7 -ساعات العمل----------

8-مصادر مياه الشرب----------

10-نسبه استهلاك منتوجات الحيوانات التي ترعى وتتربى في إذنه----------

11-مدخن 1-نعم 2-لا

12- هل تعاني من أمراض : نعم 2-لا

اذا نعم

ما نوع المرض----------

13- هل يوجد امراض وراثية في العائله 1-نعم 2-لا

اذا نعم

ما هو المرض----------

في حاله الموافقة ستؤخذ عينه دم ليتم فحصها في مختبر جامعه بيت لحم.

مع تقديرنا لتعاونكم ومشاركتكم لإنجاح البحث.

أنا الموقع/الموقعه أدناه اوافق على المشاركه

الاسم:

التوقيع:

التاريخ: