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Review Article

Genotoxicity Hazards of Industrial Effluent Pollution in Ethiopia from a Public Health Perspective: A Review

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ABSTRACT

With the increase in the Economic performance of Ethiopia and associated structural economic transformation towards agricultural led industrialization it is a common sense industries are expanding in number and type. These, Industries release a substantial amount of waste. These wastewaters are a heterogeneous mixture including toxic chemicals which may have an impact on human health. Chemical residues present in industrial effluents discharged into the sewage system have adverse effects on the operation of biological sewage treatment plants or toxic effects on the natural ecosystems of receiving waters. Indeed, some of the substances found in industrial effluents are genotoxic and are suspected to be a possible cause of Cancers. Therefore special care in handling genotoxic waste is absolutely essential; any discharge of such wastewater into the environment for long time could have disastrous public health consequences. This article summarizes the genotoxicological scenario of industrial effluents and provides suggestions that wastewater should be handled and treated according to its type. Besides, there should be a regular monitoring tool for assessing the wastewater discharged from health care units to ascertain that industrial effluents discharged are safe. Eventually, this review suggests appropriate health policy framework with its technicality to prevent and medicate genotoxicity associated health risks in Ethiopia.

Introduction

Genetic toxicology is a multidisciplinary field of research involved in detecting compounds capable of causing DNA damage and/or protecting DNA, with the aim of understanding potential biological consequences and molecular mechanisms of genetic material (Uhl et al., 2003).

Industrial effluents are liquid wastes which are produced in the course of industrial activities. Over the years, the improper disposal of industrial effluents has been a major problem and a source of concern to both governments and industrialist. In most cases the disposal or discharges of effluents, even when these are technologically and economically achievable for particular standards, do not always comply with pretreatment requirement and with applicable tonic pollutant effluent limitations or prohibitions. The consequence of these anomalies is a high degree of environmental pollution leading to serious health hazards.

The effluents generated from domestic and industrial activities constitute the major sources of the natural water pollution load. This is a great burden in terms of wastewater management and can consequently lead to a point-source pollution problem, which not only increases treatment cost considerably, but also introduces a wide range of chemical pollutants and microbial contaminants to water sources (EPA, 1993, 1996; Eikelboom and Draaijer, 1999; Amir et al., 2004).

Therefore this paper tries to review the available information on genotoxicological impacts of industrial effluents on public health and provide a way forward solution specifically for public health stakeholders in the Ethiopian discourse.This paper is expected to fill the information gap ultimately serving as an input for awareness creation activities, occupational health packages and genotoxicity substances precaution.

Literature review

Overview of Ethiopia's industrial performance

The Ethiopian economy is among the fastest growing on the continent. The economy has recorded a double digit real GDP growth rate since 2004 (with the exception of 2009 as a result of the global financial crisis). Real GDP per capita growth has steadily increased from 3.3% in 2000 to 10.8% in 2004, reflecting improvements in purchasing power and standards of living, before slightly declining to 6.5% at the end of the period.

Sectoral composition of aggregate output in the country shows that the industrial sector has gained momentum in recent years.

An analysis of the industrial sector shows that value addition has consistently remained above 1 0% of GD P during the period from 2000 to 2009, as a result of increased industrial activity and coherent industrial policies. Based on the country's projections, the industrial sector is envisioned to be a major source of economic growth and job creation in the long run (MoFED, 20 10). However, the manufacturing sector has oscillated around 5 % o f GDP, as performance in the cut flower, textiles and apparel, as well as wood , paper and pulp sectors has slowed down(AA CCSA , 2015). Nevertheless, as discussed below, the leather and leather product sector continues to play a significant role in the country's manufacturing sector. In fact, according to Oqubay (2015: 5), industrial policy in the leather sector h as mirrored the experience of the East Asian countries, although it has been tailored to take into account industry specific factors such as its low industrial base, path dependency in the form of low value trap, and integration in to global value chains. In addition, 'the depth of political commitment to consistency of policy instruments and compatibility of policy institutions has been significant to the success of the leather sector'.

Legislation for the Control of Discharge of Industrial Effluents in Ethiopia

According to the Ministry of Trade and Industry, there are over three thousand industrial establishments in the country. They vary considerably in terms of process, technology, size, and nature of products, characteristics of the wastes discharged and the receiving environment. Equally, among the industries, brewery industry is the largest manufacturing industry in Ethiopia. There are more than five major brewery factories. Environmental pollution derived from domestic and industrial activities is the main threat to the surface and groundwater qualities in Ethiopia (EEPA, 2003). It is reported that the majority of industries in the country discharge their wastewaters into nearby water bodies and open land without any form of treatment (EEPA, 2003). Likewise, in Ethiopia most of the brewery factories have no effluent treatment plants.

However, the survival of the ecosystem depends on the ability to manage wastes in an environmentally sound manner. This can only be achieved through establishment and enforcement of appropriate standards and guidelines set to ensure that one does not destroy the environment (EEPA, 2003). This necessitates the formulation of regulations and standards for discharge limits of the effluents before they are released into the environment (GOE, 2002).

Baseline information on the characteristics of the wastewater and the receiving environment is therefore the means and the primary point for discharge standards. However, lack of decisive technical information for various pollutants including priority pollutants renders compliance and enforcement difficult at all levels. Moreover, the fate and the impact of these pollutants in the receiving environment need to be determined for the definition of reliable numerical criteria for safe limits. Ideally, standards are set based on country specific baseline data and information, which are scanty in the present circumstances in Ethiopia (EEPA, 2003).Therefore, environmental quality standards are set with a goal of safeguarding public health and protecting the environment by indicating pollution limits.

Pollution Loads of Industrial Effluents in Ethiopia

Most of the high water consuming industries in the Awash basin in particular in the city of Ethiopia and in the Akaki area draw water for production purposes from water supply sources and discharge their by-product wastes in to streams and rivers without any kind of treatment. Besides this, there is no restriction on industrial plants discharging their wastewater into the rivers and watercourses. EPA and Ethiopia cleaner production centre (ECPC) realized that the tanneries built along the Awash river basin especially on the Akaki and little Akaki rivers carrying all the devastating pollutant wastes on their way to the neighboring peripheral Oromiya region needs due attention (ECPC, 2004). Investigation made on the presence and concentration of heavy metals including toxic hexavalent Chromium from tanneries, in vegetable leafs irrigated by the Akaki River was found to be more than the maximum limit that may induce gastrointestinal ulceration and cancer (Prabu et.al,2009). Findings from the Sheba tannery in Ethiopia also shows that the levels of hexavalent chromium in the downstream river and spring water samples exceed the World Health Organization (WHO) permissible limit of total chromium in drinking waters (0.05 mg/L) as opposed to the levels in the upstream waters. The increased concentrations of Cr (VI) in the water samples indicate the possible environmental pollution of the downstream water bodies by the tannery effluents, Sheba Tannery an instance in this case.

In view of the toxicity and related environmental hazards, the levels of hexavalent chromium from the tannery effluents must be reduced to a permissible limit before discharging into the downstream waters being used for domestic purposes by the nearby communities. (Abraha G, et.al.,2009) However, even tanneries in the city of Ethiopia and elsewhere in Ethiopia, which have treatment facilities, divert their raw wastewaters into the storm water drainage system or directly to the watercourses. The reason could be either for technical reasons related to the wastewater treatment plant operation or for practical reasons since there are no regulations and effective control regarding industrial effluent discharges by concerned parties. Some studies indicate that the industries equipped with some form of effluent treatment facilities have installations undersized and frequently inoperable. It seems that the main function of these facilities appeared to have been obtaining the permit required to build the factories. There are very few industries in the city of Ethiopia that use septic tanks for the disposal of industrial waste effluent. (UNESCO, 2004).

Eco-genotoxicity of Industrial effluents

Several reviews demonstrate the presence and potency of genotoxins from a broad range of industrial and municipal effluents (De Raat et al. 1990, Stahl 1991, Helma et al. 1994, White et al. 1996a, Helma and Knasmüller 1997, Claxton et al. 1998). In eco-genotoxicity, possible effects of mutagenic/genotoxic substances, present in the environment, are investigated on the population and ecosystem level (De Raat et al. 1990, Würgler and Kramers 1992). Contrary to human toxicology studies which focus on the fate of the individual, eco-genotoxicity tests evaluate the consequences for population size and structure. Investigations showing high prevalence of hepatic tumours in different fish species from contaminated areas initiated studies in the aquatic environment (Murchelano and Wolke 1991, McMahon 1994, Moore and Myers 1994). Several examples of neoplasms in fish due to waste water effluents have been described (Metcalfe and Sonstegard 1985, Metcalfe et al. 1985, Kimura et al. 1989).

Furthermore, exposure to DNA damaging agents may result in the formation of carcinogen-DNA adducts. These adducts as possible indicators for carcinogens have been detected in mussels (Harvey et al. 1997) and fish from contaminated sites (Dunn 1991, Weisburger and Williams 1991, Pfohl-Leszkowicz et al. 1993, El Adlouni et al. 1995, Ericson and Larsson 2000).

Increased mutation rates, e.g. due to environmental pollution, might negatively affect populations. This is still controversially debated in the scientific community (Anderson and Wild 1995, Würgler and Kramer 1992) but evidence is growing that environmental mutagens can reduce the reproductive success of populations. Lynch et al. (1995) developed a mathematical theoretical approach for evaluating the risks of small populations to extinction via the accumulation of mutations. Even though an increasing number of studies involving eco-genotoxicity are available (Hose and Brown 1998, Hutchinson et al. 1998, Odorakis et al. 1998, Rodgers and Baker 2000), the identification of clear cause-effect relations is increasingly complicated, the higher the level of biological organisation.

For example Hose et al. (1998) performed a large-scale genotoxicity assessment in a coastal marine environment following the Exxon Valdez oil spill in 1989 using the anaphase aberration test with newly hatched herring fish eggs. Aberration rates were significantly elevated in the fish larvae from heavily contaminated sites and correlated with PAH concentration. In the following years the population of herring was reduced to one-third of the expected value. Nevertheless it could not be conclusively demonstrated that the spilled oil caused significant long-term damage to the herring population. While Anderson and Wild (1998) stated that genotoxicity could be correlated with reproductive effects using a polychaete worm, Hutchinson et al. (1998) found, that municipal sewage effluent disinfected with sodium hypochlorite, although causing strong developmental effects, did not increase chromosome aberration in larval stages of a marine polychaete worm.

According to Anderson and Wild (1994) endpoints in eco-genotoxicity studies include frequencies of gamete loss due to cell death, embryo mortality caused by lethal mutations, abnormal development, cancer, and mutation frequencies affecting the gene pool of exposed populations. Up to now only endpoints like gamete loss or teratogenic effects as well as cancer incidences can be measured. Effects for exposed populations might be estimated, in cases where these populations are ecologically characterized. But knowledge about consequences of genotoxic exposure on the gene pool of exposed species is still scarce. As mentioned earlier Kurelec and coworkers described a genotoxic disease syndrome that, in combination with loss in reproduction, can pose a threat to population survival. For populations with a large reproductive surplus loss of individuals due to mutational changes might not be critical (Würgler and Kramers 1992). Newer approaches to describe genetic effects of contaminants on the population level focus on the genetic diversity, examining the current status and the history of populations by molecular genetic techniques. But these effects were not necessarily caused by mutagenicity. They depend also on chronic effects and population size (Bickham et al. 2000, Belfiore et al. 2001).

Genotoxicity Evaluation of Industrial effluents

Live organisms are frequently exposed to environmental agents that induce mutations. Therefore detection of these products are important (Costa and Menk 2000; Silva et al., 2003; Paz et al., 2006). Specifically genotoxins in water are of interest because epidemiologic investigations have shown a link between genotoxic drinking water intake and a rise in cancers (Koivusalo *et al.*, 1994, 1995, 1997). The impacts caused by toxic agents on the genetic material, often are not capable of being observed and measured directly.

Information obtained through biomonitors permits estimating and comparing these impacts (Bagatini *et al.*, 2009). Therefore, genotoxic chemical emissions from anthropogenic activities into environmental compartments require genotoxicity assays for the assessment of the potential impact of these sources on the ecosystems and on human health (Rank 2003).

Review of some of the Bio Assays for Genotoxicity test of an industrial effluent

Although more than a hundred "test systems" for investigating genotoxicity have been described in the literature or used in practice, ranging through the biological phyla from bacteriophage to mammals, less than 20 are in regular use and some of these are only available in specialized laboratories. The most widely-accepted systems are summarized below.

Plant assay: Because of the tremendous advances made in the use of microbial and mammalian cell procedures in genetic toxicology, plant material is less often used for studying mutagenic chemicals than previously. However, the use of plants such as the bean (*Vicia faba*), onion (*Allium cepa*), spiderwort (*Tradescantia paludosa*), Maize (*Zea mays*), Barley (*Hordeum vulgare*), and Soyabean (*Glycine max*) may have significant advantages over other systems and their value in screening chemicals for mutagenic activity has still to be fully explored. Investigation of genetic changes at both the gene and chromosomal level can be conducted in plants without the complicated laboratory facilities required for other types of assay and this may be a great advantage under certain circumstances.

(a) Allium cepa Test:

Allium cepa test is one of the most used plant species in toxicity and genotoxicity tests, particularly when monitoring effluents. Although a number of species of *Allium* have been used for genetic studies, the common onion, *Allium cepa*, has proved to be the species of choice for root-tip chromosome studies (Grant, 1982).

(b)**Tradescantia paludosa** Compared to Allium, only a few chemicals have been tested for mitotic chromosomal aberrations in Tradescantia, but it has the advantage that both meiotic and mitotic chromosomal damage and gene mutations can be tested in the same species. Dividing cells in the root tip of Tradescantia contain 12 large metacentric chromosomes. A large number of roots can be obtained from cuttings from mature plants in about a week. These rooted cuttings can then be used for chromosome studies in much the same way as those of Allium (Ahmed & Grant, 1972).Besides a number of animals and fishes have also been used for genotoxicity evaluation of a number of environmental samples like surface water (Glos et al. 2000: Reifferscheid, G. et al., 2000: Schnurstein et al. 2001: Scalon, MCS. et al., 2010) industrial effluents (Sumathi et al., 2001: Gauthier, 2003).

Microbial Assay:

One of the cornerstones of modern toxicological investigations is the concept of the three R's reduce, refine, and replace, put forward by Russell (2005). The intention is to reduce the numbers of animals used in toxicological experiment, to refine the methods by which they are used, and to replace the use of animals as appropriate alternative methods become available. Therefore microbial assays are promising system designed to evaluate the genotoxicity of environmental substances.

Assays that involve the use of bacteria for detecting mutagenic chemicals are the most extensively used and, in general, the most thoroughly validated. Unlike higher organisms, in which the DNA is organized into complex chromosomal structures, bacteria contain a single circular molecule of DNA that is readily accessible to chemicals that can penetrate the cell wall. Bacterial tests also have the advantage that a population of many millions of cells with a relatively short generation time can be tested in a single assay (WHO-IPCS, 1985). These bioassays are generally performed using cultured organisms & measure the toxic responses after exposure under controlled conditions in the laboratory.

Results from these genetic bioassays are relevant to human health because the toxicological target is DNA, which exists in all cellular life forms. Thus, it can be extrapolated that compounds shown to be reactive with DNA in one species have the potential to produce similar effects in other species (Mathur *et al.*, 2007). Some of the most commonly used short term microbial bioassays are:

AMES test:

This test is based on a strain of *Salmonella typhimurium* that cannot synthesize histidine. It lacks the enzymes to repair DNA so that mutations show up readily, and has leaky cell walls that permit the ready entrance of chemicals. Many potential carcinogens are mutagenic agents only after being acted on by mammalian liver enzymes, so an extract of these enzymes is added to the test medium. In the control setup, bacteria are plated on a histidine-free medium containing liver enzymes but lacking the test agent. The experimental plate is prepared the same way except that it contains the test agent. After incubation, plates are observed for colonies. Any colonies developing on the plates are due to a back mutation in a cell, which has reverted it to his (+) strain. The degree of mutagenicity of the chemical agent would be calculated by comparing the number of colonies growing on the control plate with the number on the test plate (Ames *et al.*, 1975).

SOS Chromotest:

The SOS Chromotest is a simple bacterial colorimetric assay for genotoxicity. It is based on the measure of the induction of sfiA, a gene controlled by the general repressor of the SOS system in E. coli. Expression of sfiA is monitored by means of a gene fusion with lacZ, the structural gene for beta-galactosidase. The SOS chromotest is one of the most rapid and simple short-term test for genotoxins and is easily adaptable to various conditions, so that it could be used as an early-- perhaps the earliest--test in a battery (Quillardet, Hofnung, 2003). This test is used to evaluate the genotoxicity of many environmental substances such as industrial effluent (Legault et al., 1998), domestic effluent and healthcare waste effluent (Jolibois et al., 2003: 2005). Waste water studies using the SOS chromotest were performed in Canada (Legault et al., 1996:

White et al., 1996a: White et al., 1996b, White and Rasmussen 1998, White et al., 1998b: White et al., 1998a), Austria (Helma et al., 1996), Finland (Suominen et al., 1998), and Germany (Janz et al., 1990). Sorption of genotoxins to effluent suspended particulate or detection of genotoxic substances in bivalve molluscs has also been studied (White *et al*., 1996b: White *et al*., 1997).

E. coli WP2:

The Escherichia coli WP2 test has been adopted in one EC test guideline (67/548/EEC, B.13/14). The principle of the test is that an E. coli strain deficient to synthesise tryptophane reverts to its "wild" type and recovers its ability to grow on tryptophane free agar plates under the influence of mutagens. Compared with others this test has not achieved any considerable importance. Therefore only few data with waste water are documented (Fracasso et al., 1992: Codina et al., 1994)

Yeast:

The budding and fission yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, respectively, are among the most extensively studied of the eukaryotes and provide convenient tools for use in genetic toxicology studies of environmental chemicals. The internal structure of the yeast cells shows strong similarities to that of the cells of higher organisms, in that they possess a differentiated nucleus containing a nucleolus. The accurate functioning of cell division depends on the synthesis of a spindle apparatus; however, unlike mammalian cells, yeasts and other fungi maintain their nuclear membrane during cell division. Yeast and fungi occupy a position between bacteria and animal cells in terms of genetic complexity. The structure of fungal DNA and its organization into chromosomes is similar in many ways to that of mammals. Both haploid and diploid forms can be used in genetic assays. Tests using yeasts, such as *Saccharomyces cerevisiae*, are available for detecting both forward and reverse mutations and a variety of other genetic changes. Certain strains of yeast can be used to detect chemicals that induce aneuploidy (i.e., unequal distribution of chromosomes during cell division) and there is some evidence that non-genotoxic carcinogens can be identified using these strains.

The primary advantages of yeasts in genotoxicity studies are eukaryotic chromosome organization; variety of genetic end-points can be assayed, cost-effective assays requiring limited technical and laboratory facilities using a "robust" organism (WHO-IPCS, 1985).

Recent developments in Genotoxicity assay

In the field of genotoxicity evaluation of environmental samples similar developments as in classical toxicology have been undertaken. Amanuma established a transgenic zebrafish for the detection of mutagens; it carries plasmids that contain the rpsL gene of *Escherichia coli* as a mutational target gene (Amanuma et al. 2000). Winn et al. (2000) prepared a transgenic fish that carries multiple copies of a bacteriophage lambda vector that harbours the cII gene as a mutational target, a technique originally developed for lambda transgenic rodents. The p53 tumour supressor gene, which is known to be implicated in cancer development, has been investigated as a possible biomarker for genotoxins in fish cells (McMahon 1994, Bhaskaran et al. 1999, 2000). The amplification of DNA by the Polymerase Chain Reaction technique enabled the detection of mutations at specific sites and the development of electrochemical DNA-based biosensors (Kennerley and Parry 1994, Parsons and Heflich 1998, Mascini et al. 2001). Polyak et al. (2000) developed a whole-cell biosensor with genetically engineered bacteria. The reaction to target toxicants is detected by the induction of a selected promoter and subsequent bioluminescent light through a recombinant lux reporter. A genotoxicity and cytotoxicity test kit based on genetically modified yeast (*Saccharomyces cerevisiae*) which uses the green fluorescent protein as reporter system has been developed for drug screening (Anonymous 2001).

Conclusion

As a conclusion, we need a well framed policy packages that addresses the adoption and refining of appropriate bioassay techniques, integration of complementary stakeholders, awareness creation and basic occupational health education, the appropriate treatment of industrial effluents before they are treated and Medical treatments for genotoxicity hazard assessment risks. Eventually, it needs further focused research and innovation and connecting the present scientific information with that of the genomic tools.

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