Toxicity and environmental risk assessment of Cosmetic Dye

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Toxicological study of hair dye
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Abstract
Hair dyes are immensely popular in modern society. However, impact of hair dye and its residual discharged to the environment in relation to human health and ecological imbalance have not been widely studied. The current study demonstrated the cytotoxicity and genotoxcity effect on aquatic microbes and *Allium cepa*. Cytotoxicity of dye was significantly noted on aquatic microbes. The subdued cell division, chromosome abnormality and lower mitotic index in *A. cepa* reflect the presence of cytotoxic and genotoxic compounds in dye. Spectroscopy studied exhibited positive DNA interaction with dye. Aquatic system can accumulate pollutants directly or indirectly from water contaminated with cosmetic effluents from domestic and hair spa.
Keywords: Hair dyes, Totoxicity, Chromosomal aberrations, Dye-DNA interaction.

Introduction

Since ancient periods, chemicals and plant pigments are being used as coloring agents in the textile, cosmetics, pharmaceutical, plastics, food, cosmetics, plastics, photographic and paper industries (Carneiro et al. 2007; Zollinger 1987). Dyes are classified according to their chemical structure and uses. Industrially, 10,000 different dyes and pigments are used with over 7 x 10^5 tons of synthetic dyes being produced annually all over the world (Ogugbue and Sawidis 2011; Robinson et al. 2001; Zollinger 1987). Dyes that are used in cosmetics (e.g. hair dyes, face cream, lipstick) should use FD&C (Colors that are certified and allowed for use in cosmetics, food and drugs by the US Food and Drug Administration) or From the very beginning of civilization, hairs have been playing an important role in attractiveness, youthfulness and beauty. In 3400 B.C. ancient Egyptians began using henna to color their gray hairs. A Roman doctor in 100 A. D**.** described a method for production of black dye. Scientists tested his formula in 2006 and it showed up that the formula actually worked (Nohynek et al. 2004). In this modern era not only the aged people cover their gray hairs with color, it has become a fashion, for both girls and boys to color their hairs. Today, hair dyes are used by millions of consumers and these products will always remain very important, the fact that human nature will always desire to improve his appearance (Corbett 1999). The chemical substances roughly are divided in direct colourants and oxidative hair dyes, the latter being formed during the hair dyeing process from a precursor and a coupler. In hair dyes, couplers and primary intermediates are mixed before use at an approximate ratio of 1:1(Brown 1997) and generally kept for 30 min to generate the dye on/in the hair by chemical reactions and after 30 min, hair is washed

with water to remove excess dye which is not entered into the hair shaft. So if only hair dye is considered among all hair care product, the effluent will be having the derivatives of phenylenediamines (PD), aminophenols, resorcinol, hydrogen peroxide and alkaline agents, the hair dye ingredients(Monnais 1995). In 1975, the results of Ames test on oxidative hair dye ingredients suggested that nearly 90% of permanent hair dye ingredients were mutagenic and might therefore pose a carcinogenic risk to consumers(Ames et al. 1975). After this observation, chemicals present in permanent hair dyes, particularly aromatic amines are studied vividly. Results have shown that they are mutagenic *in vitro*, (2007; Garrigue et al. 2006; Murata et al. 2006; Yoshida et al. 1998) carcinogenic to animals (Burnett and Corbett 1987; Haws et al. 1994) and able to penetrate human skin (Genina et al. 2002; Lademann et al. 2008). Several epidemiological studies have revealed the risk of bladder and lung cancer in hairdressers, barbers and beauticians who are occupationally exposed to hair dyes (La Vecchia and Tavani 1995), ovarian cancer is associated with the woman who dye their hair (Tzonou et al. 1993) and prolonged use of hair dye has increased risk of non-Hodgkin's lymphoma, multiple myeloma (Correa et al. 2000; Zhang et al. 2004). The case control studies to check the parental exposure and risk of malignancy in the unborn children have suggested avoiding all hair coloring products during pregnancy (Chen et al. 2006; Efird et al. 2005; McCall et al. 2005). Cytoxic and genotoxic effect of three commonly used hair dye in its useable form was reported (Maiti et al. 2017). So, taking into account the toxicological effect of hair dye, risk assessment must be focused on the colorants and its pollutants to determine the ecological effect and the role of it on human health. In this report cytotoxicity and genotoxcity of dye on aquatic microbes and *Allium cepa* was studied.

Materials and methods

Materials

All the chemicals used in the experiment are of analytical quality purchased from reputed brands and used without any further purification. Based on our previous report three oxidative hair dyes with dark shade are purchased from local market. Usable form of dye was prepared as per instruction for dye preparation. Then 100 mg/ml stock was prepared by adding phosphate buffer saline (PBS) as described in our previous report (Maiti et al. 2017).

Effect of dye on microbes

To check the effect of dye on aquatic microbes, pond water collected in front of the institute and cultured for 24 h. at 30 ^{o}C. Agar diffusional assay was used to check the toxicity of dye in aquatic microbes (Maiti et al. 2017). Freshly cultured microbes were spread on LB agar medium and then wells were made. 100 µl of different concentration of dye mixture (0-0.1 mg/ml) was added in each well and plate was incubated for 24 h at 30 ^{o}c. Zone of inhibition around each well were measured.

Effect of dye on plant

Genotoxic effect of environmental contaminants have been screened with different plant systems (Dong and Zhang 2010; Ozkara et al. 2011). To check the genotoxicity of dye *Allium cepa* was used as model system and experiment was done according to our previous report (Singh et al. 2013). *A. cepa* seeds are germinated in glass tubes containing autoclaved double distilled water and when the radicals reached 2 cm in length those were transferred to a glass tube containing dye of different concentration (0-1 mg/ml) in total volume of 50 ml and negative control (distilled water). After two days some of the rooted seeds were collected at random and prepared those for examining chromosomal aberration as our previous report (Singh et al. 2013) and then observed using compound light microscope (Olympus) using the 40 x objective.

Effect of dye on DNA

To check the effect of dye on DNA integrity gel electrophoresis was done with Salmon Sperm (SS) DNA purchased from Gene Link by standard procedure. DNA and dye (1: 0, 1: 5 and 1: 10) in PBS (pH 7.4) was incubated for 1 hour at 37 ^{o}C. After completion of incubation period the sample was run on 1 % agarose gel. Gel picture is collected from gel documentation system of Vilber Lourmat model no. ECX 20.M.

Results

Toxicity of hair dye was studied on microbial and plant system. The mechanism of interaction was studied using gel electrophoresis and spectroscopic analysis. From Figure 1 it is observed that with increasing concentration of dye zone of growth inhibition was increasing which indicates the toxicity of dye. Figure 2 shows various chromosomal aberration induced by hair dye. It is observed that most of the cells in interphase stage. Lower Mitotic Index (MI) indicates the genotoxic nature of dye. Figure 3 is the result of gel electrophoresis study based on the experiment of effect of hair dye on SS DNA. Figure 3a is the visual picture and Fig. 3b is the gel image collected from gel documentation system. From Fig. 3a it is seen that dye is moving towards the negative field, it implies that dye is positively charged. From Fig. b we have observed that mobility of DNA is retired with increasing concentration of dye. DNA in lane 1 without dye has moved towards the positive field. Where as in lane 2 some amount of DNA is trapped in well and an extra band is observed above the bulky DNA band. In lane 3 amount of DNA in well is increased with less movement of that new band. The dye bound DNA can't move as unbound due to charge neutralization. Similar experiment with calf thymus DNA exhibited interaction with DNA (Maiti et al. 2015) . From Fig. 3b it is seen that dye is moving towards the negative field, confirm that dye is positively charged.

Discussions

In the present study we explored the effect of hair dye on bacteria and plant system. Growth inhibition of microbes, subdued cell division and lower Mitotic Index reflect the presence of cytotoxic and genotoxic compounds in dye. Gel electrophoresis study and spectroscopic analysis implies the interaction of Dye with DNA. So the discharge of hair spa can pose a serious threat to the aquatic system. In summary, the whole aquatic ecosystem will be contaminated by the cytotoxic, clastogenic and genotoxic pollutants. So, taking into account the toxicological effect of hair dye, risk assessment must be focused on the colorants and its pollutants to determine the ecological effect and the role of it on human health.

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Figure Legends

Figure1. Different conc. of dye (0, 0.02, 0.04, 0.06 , 0.08, 0.1 mg/ml) on well (i-vi) on agar plate containing aquatic microbial consortium.

Figure 2. Cytological plate exhibiting various chromosomal aberrations in *A. cepa* root tips cell after hair dye treatment. (a) anaphase with fragmented chromosome. (b) Disturb metaphase (c) and (d) sticky metaphase (e) distorted aberration (f) nuclear bud.

Figure 3. Agarose gel profile of salmon sperm DNA treated with hair dye for 1 hour. (a) visual observation of gel, (b) gel image captured by gel documentation system.

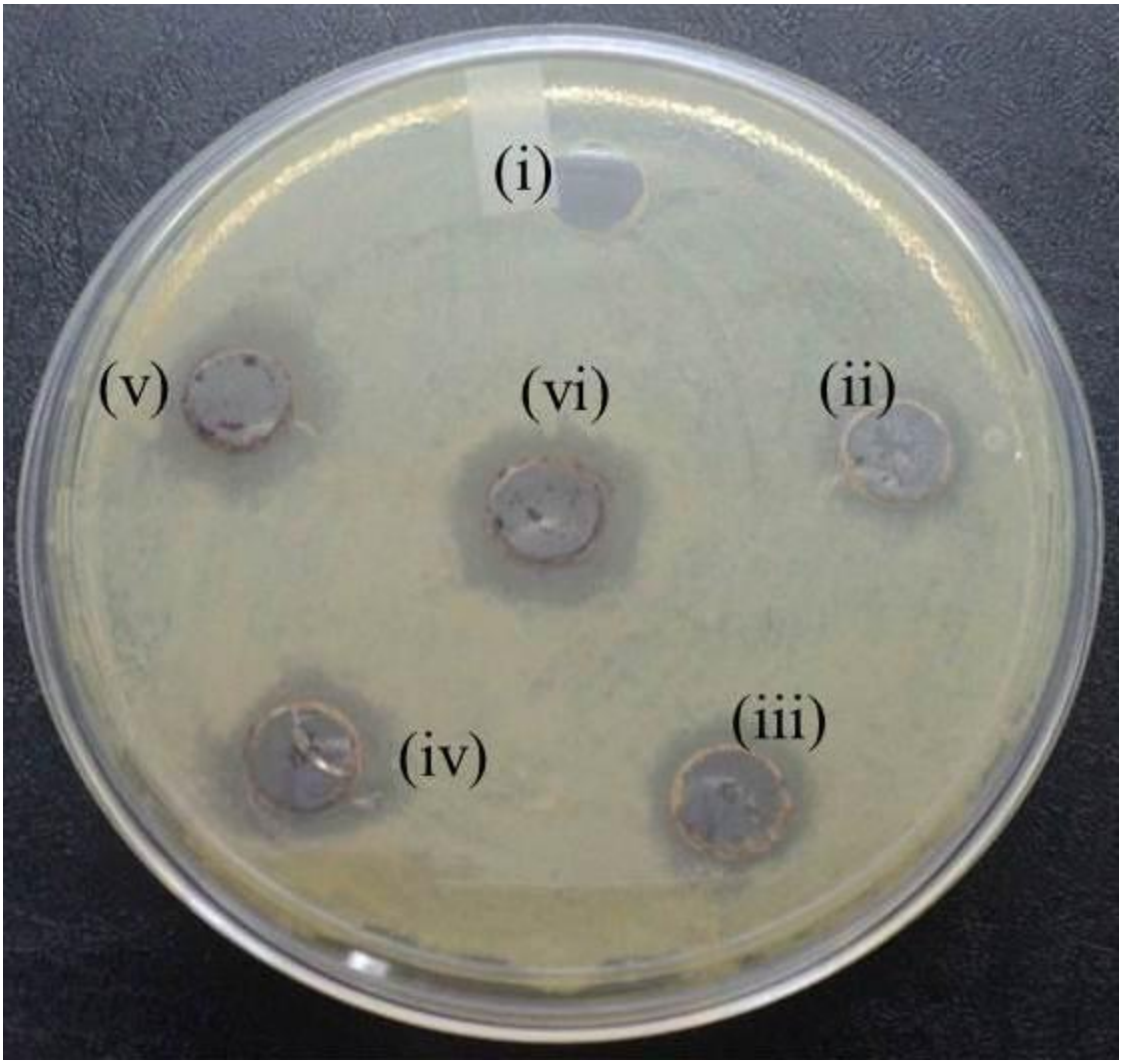

Figure 1.

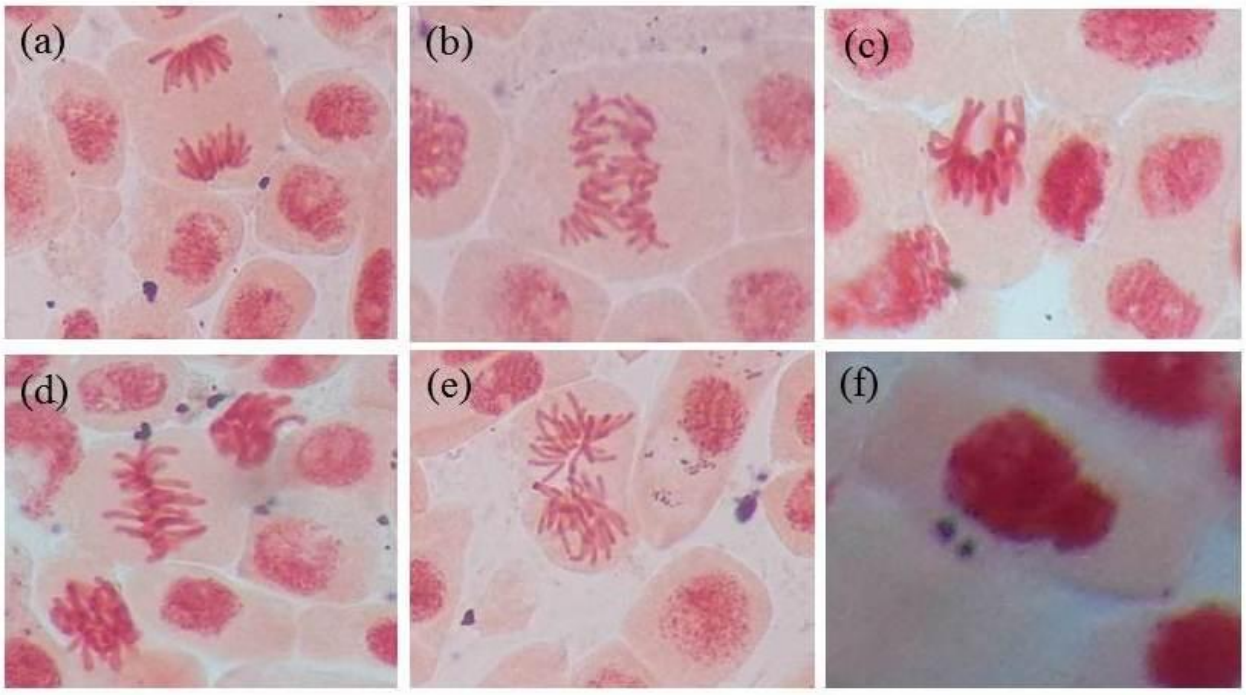

Figure 2.

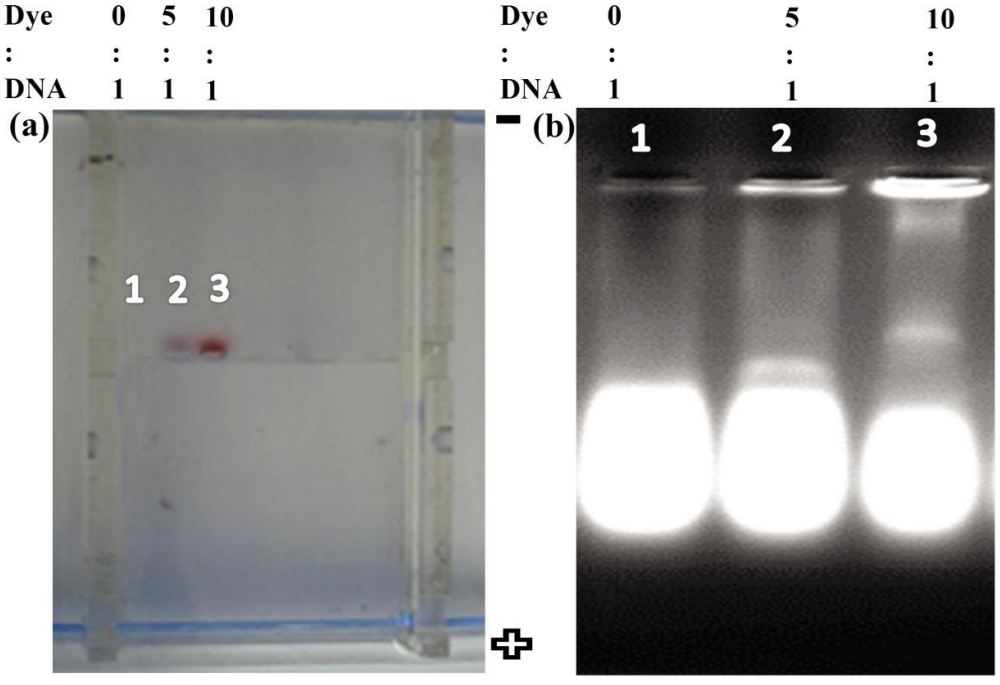

Figure 3.