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Assessing the concentration of phthalate esters (PAEs) and bisphenol A (BPA) and the genotoxic potential of treated wastewater (final effluent) in Saudi Arabia

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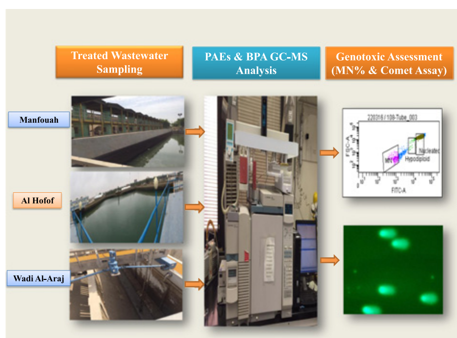
HIGHLIGHTS

- This study detected phthalate esters (PAEs) and bisphenol A (BPA) in Saudi treated wastewater.
- Both PAEs and BPA were found in secondary and tertiary treated wastewater.
- Most treated wastewater samples exhibited genotoxicity using comet assay and micronucleus test.
- In spite of the low levels of BPA and some PAEs in treated wastewater, they induced genetic damage.

GRAPHICAL ABSTRACT



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ABSTRACT

Plasticizers such as phthalate esters (PAEs) and bisphenol A (BPA) are highly persistent organic pollutants that tend to bio-accumulate in humans through the soil-plant-animal food chain. Some studies have reported the potential carcinogenic and teratogenic effects in addition to their estrogenic activities. Water resources are scarce in Saudi Arabia, and several wastewater treatment plants (WTPs) have been constructed for agricultural and industrial use. This study was designed to: (1) measure the concentrations of BPA and six PAEs, dimethyl phthalate (DMP), diethyl phthalate (DEP), dibutyl phthalate (DBP), butyl benzyl phthalate (BBP), bis (2-ethylhexyl) phthalate (DEHP) and dioctyl phthalate (DOP), in secondary- and tertiary-treated wastewater collected from five WTPs in three Saudi cities for four to five weeks and (2) test their potential genotoxicity. Three genotoxicological parameters were used: % tail DNA (%T), tail moment (TM) and percentage micronuclei (%MN). Both DBP and DEHP were detected in all treated wastewater samples. DMP, DEP, BBP, DOP, and BPA were found in 83.3, 84.2, 79, 73.7 and 97.4% of the samples, respectively. The levels of DMP ($p < 0.001$), DOP ($p < 0.001$) and BPA ($p = 0.001$) were higher in tertiary- treated wastewater than secondary-treated wastewater, perhaps due to the influence of the molecular weight and polarity of the chemicals. Both weekly sampling frequency and WTP locations significantly affected the variability in our data. Treated wastewater from Wadi Al-Araj was able to induce DNA damage (%T and TM) in human lymphoblastoid TK6 cells that was statistically higher than wastewater from all other WTPs and in untreated TK6 cells (negative control). %MN in samples from both Wadi Al-Araj and Manfouah did not differ statistically but was significantly higher than in the untreated TK6 cells. This study also showed that the samples of tertiary-treated wastewater had a higher genotoxicological potential to induce DNA damage than the samples of secondary-treated wastewater. BPA

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and some PAEs in the treated wastewater might have the potential to induce genetic damage, despite their low levels. Genotoxicity, however, may also have been due to the presence of other contaminants. Our preliminary findings should be of concern to Saudi agriculture because long-term irrigation with treated wastewater could lead to the accumulation of PAEs and BPA in the soil and ultimately reach the human and animal food chain. WTPs need to remove pollutants more efficiently. Until then, a cautious use of treated wastewater for irrigation is recommended to avoid serious health impacts on local populations.

1. Introduction

The US EPA (The United States Environmental Protection Agency) reported an emerging concern of low levels of contaminants, such as endocrine disruptor chemicals (EDCs), pharmaceuticals and personal-care products, in surface water that may have an impact on aquatic life (US EPA, 2008). The US EPA defined an environmental EDC as "an exogenous agent that interferes with the production, release, transport, metabolism, binding, action or elimination of natural hormones in the body responsible for the maintenance of homeostasis and the regulation of developmental processes". (Kavlock et al., 1996).

Large amounts of water are used daily in agriculture and industry and by the general population that release many compounds into wastewater and aquatic ecosystems (Deblonde et al., 2011; Bondarczuk et al., 2016). These compounds are still detected and can be released into the environment regardless of the method of wastewater treatment (Wu et al., 2015; Semblante et al., 2015). The introduction of contaminants to crops *via* plant uptake leading to food contamination and potential health risks to humans is a major public concern for agricultural applications of treated wastewater (Fatta-Kassinos et al., 2011; Dodgen et al., 2013). Typical and routine wastewater treatment does not include suspended particulate matter due to further preparation requirements and analysis, which results in the under-reporting of several contaminants entering wastewater-treatment facilities and the aquatic environment (Petrie et al., 2015). Becerra-Castro et al. (2015) also reported that the use of treated wastewater in irrigation might alter the physicochemical and microbiological properties of the soil and/or introduce or contribute to the accumulation of chemical and biological contaminants in soil. The uptake of EDCs by plants has received an increasing amount of attention in the last decade, due to their potential to accumulate and to their long-term effects (Weber et al., 2006; Diamanti-Kandarakis et al., 2009; Shenker et al., 2011; Grassi et al., 2013; Sun et al., 2013). Phthalate esters (PAEs) and bisphenol A (BPA) are industrial chemicals classified as EDCs (Heudorf et al., 2007; Oehlmann et al., 2008; Vandenberg et al., 2009).

PAEs are fat-soluble synthetic chemicals used as additives in many consumer products containing polyvinyl chloride (PVC) to soften vinyl plastic, so they are also called 'plasticizers'. Since PAEs are not chemically bound to the PVC polymer or other matrices, ~~so~~ they may leach from products during use or after disposal. They can also reach sewage through urban runoff and drainage and domestic and industrial discharges of wastewater (Barnabé et al., 2008; Dargnat et al., 2009; Clara et al., 2010; Çifci et al., 2013), which can be serious threats to the environment, biota and human health (Huang et al., 2013; Net et al., 2015). The US EPA categorized the following six phthalate compounds as priority environmental pollutants under the Clean Water Act to regulate: dimethyl phthalate (DMP), diethyl phthalate (DEP), di-*n*-butyl phthalate (DBP), butyl benzyl phthalate (BBP), bis (2-ethylhexyl) phthalate (DEHP) and di-*n*-octyl phthalate (DOP) (US EPA, 1982).

BPA is a component of polycarbonate plastics and epoxy resins, which are extensively used in the manufacture of many consumer goods (Vanderberg et al., 2009). It has a short half-life, but it is a ubiquitous pollutant because of its continual release (Staples et al., 1998), which can occur during chemical manufacture, transport, and processing. BPA discharge in urban wastewater from industrial and domestic sources is therefore expected, and many studies have reported its presence in raw water, sewage sludge and wastewater (Sidhu et al., 2005; Ying et al., 2009; Flint et al., 2012; Chen et al., 2013; Ding et al., 2014; Lee et al., 2015; Pookpoosa et al., 2015). Fent et al. (2003) showed that BPA rapidly dissipated in soil with a half-life of less than three days. Canada was the first country to declare BPA as a 'dangerous substance', and add it to the country's toxic substances list, as a precautionary approach to restrict its use and in preparation for its ban (Buka et al., 2009; Vandenberg, 2011). The US EPA began an initiative in 2010 to include BPA on its Concern List as a substance that may present an unreasonable risk of injury to the environment on the basis of its potential for long-term adverse effects on growth, reproduction and development in aquatic species at concentrations similar to those found in the environment (US EPA, 2010). Recently, some European Union (EU) Member States have introduced national bans on the use of BPA in both plastic food contact materials under these safeguard measures, as well as for other materials such as coatings (EFSA, 2015).

PAEs and BPA (and other pollutants) are still detected in effluent (Deblonde et al., 2011; Corrales et al., 2015), despite the efforts to introduce different strategies of wastewater treatment. Several *in vivo* and *in vitro* studies have examined the carcinogenic and mutagenic effects of PAEs and BPA (Kozumbo et al., 1982; Yoshikawa et al., 1983; Schrader et al., 2002; Zeiger et al., 1985; Fic et al., 2013; Ahbab et al., 2014; Erkekoglu and Kocer-Gumusel, 2014). Many studies have shown that exposure to PAEs and BPA can induce oxidative stress, leading to genotoxicity that can be the underlying factor of several health conditions such as early puberty, inflammatory and reproductive diseases and cancer (Rusyn et al., 2006; Muncke, 2011; North et al., 2014; Mathieu-Denoncourt et al., 2015; Poursafa et al., 2015). The cytotoxicity and genotoxicity of untreated and treated wastewater were assessed for monitoring water quality using plant, aquatic and mammalian-cell-line bioassays (Prasse et al., 2015; Iqbal, 2016). Ohe et al. (2004) suggested that single-cell gel electrophoresis (comet) and micronucleus (MN) induction should be used to monitor environmental pollution to efficiently assess the presence of mutagens in the water, in addition to more sensitive chemical analyses such as ^{32}P–postlabeling. Many studies have used these methods for evaluating the genotoxicity of effluents but without identifying and quantifying the chemical components causing the DNA damage (Rodrigues et al., 2010; Richard et al., 2014; Gupta et al., 2015; Manzano et al., 2015; Hemachandra and Pathiratne, 2016). Most of our knowledge of the environmental fate of PAEs and BPA in effluents is based on chemical analyses and not biological effects. A combination of both will allow the proper assessment of risk.

Water is a scarce resource in Saudi Arabia, so several wastewater treatment plants (WTPs) were installed to recycle water for irrigation and industrial applications. Approximately 170,000–200,000 m^3/d of the treated wastewater are used in Riyadh for landscaping and agricultural irrigation, 15,000–20,000 m^3/d are used by industries and the remainder is discharged into Wadi Al-Batha, which contributes to groundwater recharge (Al-Jasser, 2011). A recent study by Balkhair and Ashraf (2016) reported elevated levels of heavy metals in the edible portions of crops cultivated in Saudi Arabia irrigated with treated wastewater. The authors also observed that irrigation substantially affected the chemical properties of the soil, especially in the 0–30 cm layer, and the plant nutrients. Al-Jassim et al. (2015) detected antibiotic-resistant bacteria and genes in treated wastewater collected