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Original Research Article

Galaxolide® pollution of *Gracilaria bursa-pastoris* collected in Dardanelles: An ecotoxicological marker of Danube River water route to Aegean Sea

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In this work, Galaxolide® pollution is reported in the alga *Gracilaria bursa-pastoris* (S. G. Gmelin) P. C. Silva collected from Sogandere, Dardanelles. The identification was made by GC/MS analysis. Galaxolide®, widely used in perfumery was detected in water, river, marine organisms and algae. This pollutant was earlier detected in Danube River water, in the Bulgarian coast and in red alga *Laurencia pyramidalis* collected from the İğneada, western Black Sea coast of Turkey. These findings can generate a proof of Danube River water circulation to the Aegean Sea and the potential use of Galaxolide® as a chemical tracer for the assessment of hydrological pollution sources in the region.

Key words: Galaxolide®, pollution, seaweed, *Gracilaria bursa-pastoris*.

INTRODUCTION

The pollutant, Galaxolide® (HHCB) 1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta (alpha)-2-benzopyran (Figure 1), is a synthetic musk with widespread use in perfumery. It has been reported as an emerging contaminant in waters and may produce harmful effects on aquatic ecosystems (Parolini et al., 2015) and human health (Kumar and Xagoraraki, 2010). It was identified in ground water (Stuart et al., 2012), drinking water (Kumar and Xagoraraki, 2010), wastewater (Gomez et al., 2011; Antoniou et al., 2009; Machado et al. 2011; Yu et al., 2012), sediment (Tian et al., 2011; Spozhnikova et al., 2010; Che et al. 2011), rivers (Sengi et al., 2008; Schwarzbauer and Ricking 2010; Sang et al., 2012), marine organisms (Bulk and Ford, 1999; Franke et al., 1999; Subedi et al., 2011; Ramirez et al., 2009) and in air (Ramirez et al., 2010; Kubwabo et al., 2012).

Earlier ecotoxicological and pharmaceutical studies carried out on *Gracilaria* sp. include endogenic fatty acids and exogenic petroleum products, butylated hydroxytoluene, hexachloroethane from *G. bursa-pastoris* (Güven et al., 2014), lipase released by substances obtained from *G. verrucosa* (Aktin and Güven, 1969), β-phenylethylamine (Percot et al., 2009), sterol glycoside (Aydogmus et al., 2009), antiprotozoal and antimycobacterial (Selcuk et al., 2011).

The Black Sea' water circulation has two separate cycles; west and east. The west part of the circulation starts from the Ukrainian coasts down to Romania, Bulgaria and runs through the western part of Turkey's coasts including the opening of the Bosphorus (Ovchinnikov, 1991). There are two flows in Bosphorus; the upper flow (Black Sea water) and under flow (Mediterranean Sea water) (Marsilii, 1681). The amounts of these waters were calculated by Merz (1918). Black Sea water circulation was determined in the Bosphorus, the Sea of Marmara and the Dardanelles (Vyazilov and Michailov, 1999). Danube River waters flows into the Black Sea containing many pollutants from 17 countries. In addition, an accident occurred in Feb 2002 at the gold mines of Baia, Romania, where approximately 83 million liters of cyanide ran off into the Tissa River

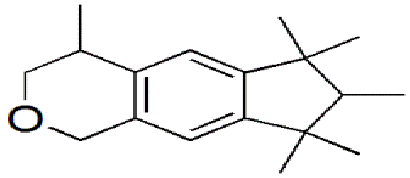

Figure 1: Chemical structure of Galaxolide. It has a molecular formula of $C_{18}H_{26}O$ and a molecular weight of 258.4 g. At room temperature it occurs as a highly viscous liquid. Its water solubility is very low (1.7 mg/L) but is metabolized rapidly into its lactone derivative and then hydroxyl acid derivative, hereby; it becomes 10 times more soluble in water and available to the marine organisms.

Figure 2: Location of alga sample (Sogandere, Dardanelles, 40° 06' 05" N / 26° 18' 53" E) and arrows indicate the circulation of Danube River water.

to Danube, found in the Bulgarian coasts (Laszlo, 2006) and fish collected in İğneada and Bosphorus by using spectrophotometric method (Guven et al., 2001). Galaxolide® was found in Danube River (Sengi, 2008) and in *Laurencia pyramidalis* collected from İğneada, Turkish Black Sea coasts in 2007 (Guven et al., 2013). A small proportion of sediment influx and heavily polluted river waters, most importantly from Danube river waters which are relatively enriched in trace metals (Zeri et al., 2000; Zeri and Vautsinou, 2003) and organic pollutants (Sempere et al., 2002) successively empties into the Aegean Sea (Yuce, 1995; Ehrmann et al., 2007) by means of the strong current in the Black Sea through the Romanian coasts, Bulgaria and western part of Turkey, the Straits and the Sea of Marmara.

A pollutant accumulated in marine algae can be used as a chemical tracer to survey water circulation using the Danube River water as an example. This is the first proposition in this area after cyanide accident in Romania (Laszlo, 2006; Guven et al., 2001) and Gallaxolide detected in Igneada, Turkish Coast of the Black Sea. In this paper, the same pollutant, Gallaxolide®, was detected in marine alga *Gracilaria bursa-pastoris* in the same year in the Dardanelles thereby revealing the Danube River water route in sea water.

MATERIALS AND METHODS

The *Gracilaria bursa-pastoris* (S. G. Gmelin) P. C. Silva sample was collected in September 2007 in Sogandere, the Dardanelles (Figure 2).

Extraction

60 g of powdered alga samples were mixed with 20 g anhydrous sodium sulfate and extracted with dichloromethane in Soxhlet apparatus for 8 h. The extract was distilled at 36°C. The residue was mixed with 1 ml hexane and applied to GC/MS.

All solvents and chemicals were of analytical grade (Merck, Darmstadt, Germany) while sodium sulfate was supplied by BASF (Baden, Germany).

GC/MS analyses

The gas chromatography mass spectrometer {HP 6890 Series GC System; Hewlett Packard, Willmington, DE, (USA)} was fitted with an electronic pressure control and a mass selective detector {(HP 5972 A; ionization energy: 70 eV; HP-PONA capillary column (50 m × 0.25 mm film thickness)}. The chromatographic conditions were: sample size 2 microliters, injection port temperature 280°C, configured for split injection; initial oven temperature 40°C rising to 280°C at 8°C/min, final hold for 20 min. Helium was used as carrier gas (1 ml/min).

RESULTS

Identification of Galaxolide® was based on the comparison of its mass spectra in literature (Serrano et al., 2011; Mercier et al., 2012; Kubwabo et al., 2012; Berdié et al., 2012; Guven et al., 2013). The GC/MS spectrum of

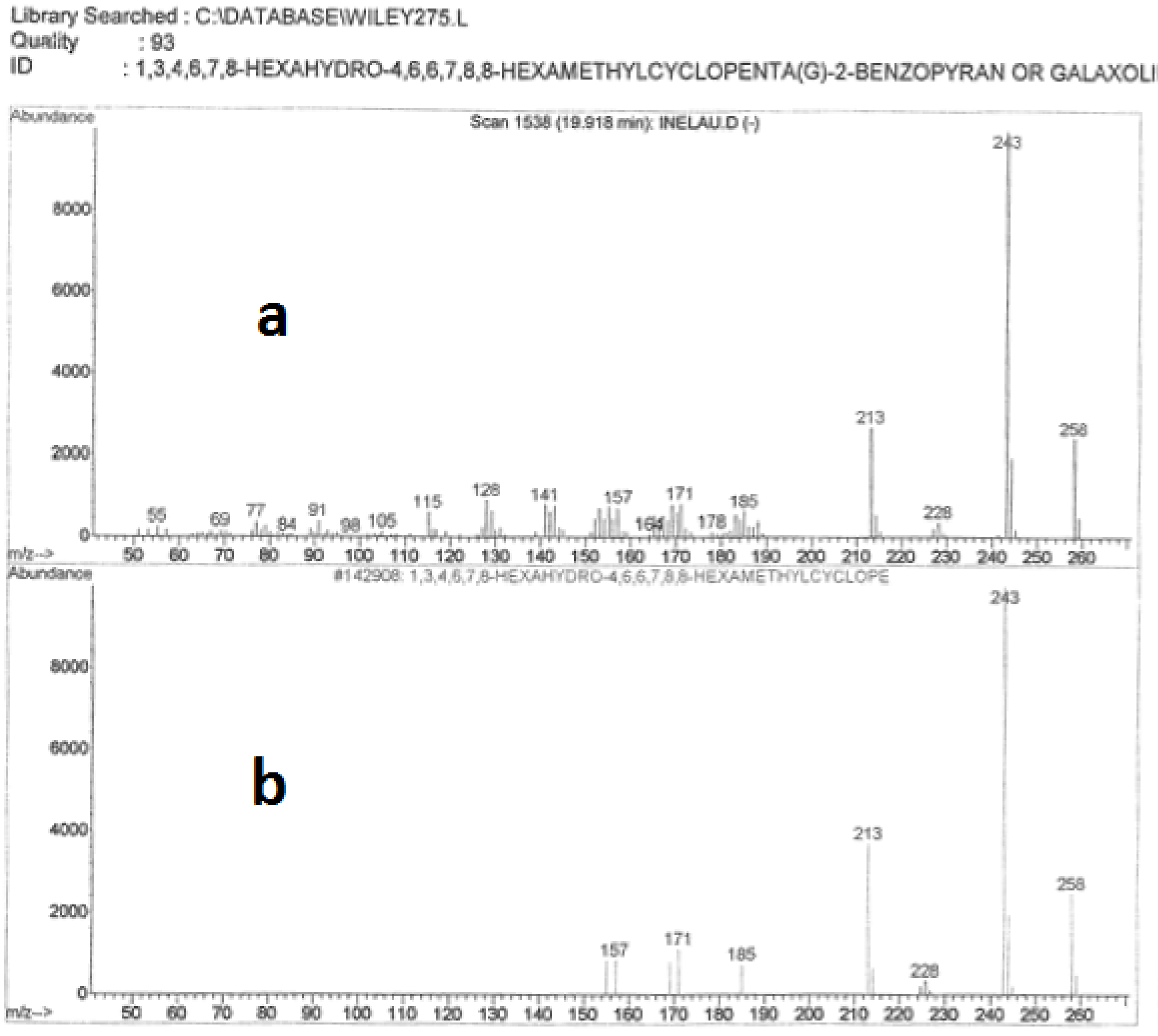

Figure 3: GC/MS spectrum of Galaxolide® from alga sample (a) and the spectrum taken from the memory of the GC-MS apparatus (b).

Galaxolide® from Sogandere, Dardanelles alga sample (a) and the spectrum taken from the memory of the GCC-MS apparatus (b) are shown in Figure 3. The relative ion abundance closely matached and was measured as mass 258 (M^+, relative intensity: 23), mass 243 ($[M-15]^+$, rel. int.: 100) and mass 213 ($[M-45]^+$ rel. int.: 39). The fragmentaion pattern of the sample has a 96% similarity with the corresponding library search result.

GC/MS Alga sample: 258 (M^+), 244, 243, 228, 213, 185, 171, 157.

GC/MS Memory: 258 (M^+), 244, 243, 228, 213, 185, 171, 157.

GC/MS from literature: 258 (M^+), 243, 213 (Serrano et al. 2011; Kubwabo et al., 2012).

Quality-retention time: 96%-19.9 min

Galaxolide® was found first time in alga *Laurencia pyramidalis* taken from Igneada in 2007 (Western Black Sea coast). This research is the second study of Galaxolide® pollution in red alga *Gracilaria bursa-pastoris* collected from Sogandere, Dardanelles in 2007.

DISCUSSION

Heavily polluted Danube River and the other rivers flow into and contaminate the Black Sea with many pollutants. The exchange of the Black Sea water with the Aegean and Mediterranean Seas occurs via the upper and under currents of the Bosphorus and the Dardanelles. In an earlier study, the cyanide contaminant was found in fish collected from İğneada and Bosphorus. Another contaminant Galaxolide, after its determination in the Danube River, was found in algae collected from İğneada and in the Dardanelles in this study. Zeri and co-workers found similar results on dissolved trace elements in the Black and Aegean seas (Zeri et al., 2000; Zeri and Vautsinou, 2003). Similarly in this work, we demonstrated that the pollutant Galaxolide was found in algae samples from Iğneada (Guven et al., 2013) and the Dardanelles. Thus, this paper showed that an ecotoxicological marker can be used for water circulation in seawaters by means of a strong current in the Black Sea through the coasts of Romania, Bulgaria and western part of Turkey, the Straits and the Sea of Marmara.

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