THE USE OF GAS CHROMATOGRAPHIC ANALYTICAL METHODS FOR THE DETERMINATION OF POLLUTANTS IN THE MARINE ENVIRONMENT.

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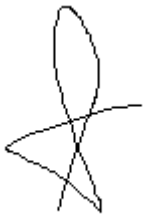

ABSTRACT

The current project decribes and analyzes the way which we can use to determine pollutants using gas chromatographic analytical methods in the marine enviroment. Chromatography is an analytical substance separation technique and is characterized by a great variety and number of analysis and separation techniques. All various chromatographic techniques are based on the same principle: the mixture to be separated is dissolved in a solvent called the mobile phase (eluent), and allowed to pass through or across an adsorbent material, called a stationary phase. They differ in the device, the eluent, the stationary phase or the type of field that is responsible for the separation of components of the sample used for analysis. The chromatographic separation and the analysis of the mixture's components is the result of repeating equilibria of the components between the two phases during movement in the stationary phase, and is attributed to the different values of the distribution constants of the components which results in their separation as they exit (elute) from the end of the chromatographic column. Gas chromatography has many applications and there are significant advantages and some disadvantages. Over 40 elements of the environment have been detected in the environment Category of heavy metals. Dangerous are the so-called heavy metals Such as beryllium, cadmium, lead, mercury, nickel, chromium, mercury Zinc, copper, etc., as they have an adverse effect on organisms And very small gatherings. Hydrocarbons are a large group of compounds that, as their name states, consist only of atoms of carbon and hydrogen. Metals shall not be assimilated, nor readily disposed of by the body and this results in selectively accumulating in certain tissues showing high concentrations. However, the pollution of soils with heavy metals is directly linked to Water pollution and therefore the reduction of aquatic organisms as Result of the accumulation of a large quantity of heavy metals in water Ecosystems.

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INTRODUCTION

POLLUTION

Although marine pollution has a long history, as the years go by and the population of the planet increases drastically, we notice the dramatic increase of the environmental pollution. General, pollution is the introduction of pathogenic microorganisms into the natural environment that can cause adverse changes which have harmful or poisonous effects in the environment. There are many types of pollution depending upon the environment, such as:

- air pollution is the pollution which occurs in atmosphere. The main source of air pollution results from burning fossil fuels mainly of industrial areas. This can cause many problems in human lives like respiratory problems such as asthma, lung cancer, chronic bronchitis.
- soil pollution is the pollution which occurs in land. The main source of soil pollution is the chemical such as pesticides or fertilizes which absorbs into soil surface after human agricultural activities. The most common problems are to injure or poison animals or plants through the absorption of chemicals.
- water pollution is the pollution which takes place in oceans, sea, rivers or lakes, estuaries, and streams. The main source of water pollution is runoff from agricultural fields, industrial sites, or urbans areas due to toxics which use people in the soil.
- light pollution refers to the large amount of light produced by most urban and other most populated areas. This type of pollution can occur problems largely in nocturnal animals or animals which hunt at night.
- noise pollution comes from humans who produce massive amounts of loud noise including industrial and vehicle sources. The certain pollution occurs impacts on the movement of sea mammals such as dolphins or whales, and birds which built nests.

Generally, there are two mainly sources of pollution, the point, and nonpoint sources. As far as point sources are considered all the sources of industrial and local wastewater emissions. On the other hand, nonpoint sources are considered agriculture, storm runoff and another runoff. (Jimenez B 2009 a)

POLLUTANTS

Pollutants are the elements, molecules and particles which are harmful on humans and plants when these exposed to these materials. Pollutants can be introduced into the environment in many ways, both naturally and by human activities and these can cause many health effects depending on pollutants' amounts. So, there are many notable pollutants such as: mercury (Hg), Ozone (O_3), Particular matter (PM), Nitrogen oxides (NOx), Sulfur oxides (SOx), Volatile organic compounds (VOCs), Polycyclic aromatic hydrocarbons (PAHs). However, the most usual pollutants which entered in sea water are:

a) Heavy metals (Hg, Pd, Cd)
b) Toxic substances and compounds (As, Se, CN^- etc.)
c) Inorganic compounds (NO_3^-, PO_4^{-3}, NO_2^-, etc.)
d) Organic compounds (phenol, chlorinated hydrocarbons, pesticides, paint colors, petroleum products)
e) Radioactive substances
f) Pathogenic microorganism (bacteria and virus)

To understand what type of pollutants we have, we could study its physicochemical properties such as: (Theodoros Georgiadis, Ioannis Zioias, Lydia Ignatiadou, Georgios Papatheodorou, and others, 2004)

•Solubility, the maximum amount of a substance that can be dissolved in a certain amount of solvent.

•Volatility, the ability of boundaries to escape from the solid or liquid phase to gases.

•Adsorption, the ability to be adsorbed by particles.

•Degree of decomposition, the time it takes for a substance to chemically change and give other compounds.

•Distribution factor describes how to distribute between two media: solid-liquid, liquid gas, atoms-liquid.

•Atomic pressure, the pressure exerted by the atoms of the fluid when it is in equilibrium with them.

•Bioconcentration indicators, the amount of substance that can accumulate in aquatic animal's organizations.

•Toxicity expresses its lowest concentration so as not to cause tissue damage and in the various bodies of the organizations.

CHROMATOGRAPHY

Chromatography use in term of technique, in decade of 1900 by the Russian biologist Mikhail S. (Tswett M., 1906). The breakthrough scientific studies of Russian biologist have like target to separate compounds (pigments of leaves) which abstracted from plants, using one solvent and one column plenty of particles.Tsvet was mainly concerned with the separation of dyes green leaves, such as chlorophylls, carotenes and xanthophylls, dissolving their extract in an organic solvent and allowing the solution to passes into a vertical pipe, a column, with filler material that is filled with powdered calcium carbonate. When passing the solvent through of the filler the various dyes were passed through the column with different speed, so separated appeared as different chromatic strips (green, orange, and yellow respectively) on the white material of the column. So, the technique got its name from describing the phenomenon. (R.S. Gohlke, 1959).

Tswett coined the name of chromatography by the Greek words' "color" and "graph" in order to describe his colorful experiment. Chromatography is the best tool of Analytical Chemistry. Chromatography includes the body of analytical techniques which separates mixture of compounds with same chemical qualifies and based on their separating due to different allocation between two no mixed phases: one mobile and one stationary phase. Mobile phase is the solvent of the mixture which causes the elution and therefore the movement of the mixture on the adsorbent material. The adsorbent material is stationary and is usually coated on some surface (e.g., paper, thin layer, inner capillary column) and for this it is also the stationary phase. They differ in the device, the eluent, the stationary phase, or the type of field that is responsible for the separation of components of the sample used for analysis. Components that are retained more strongly by the static phase move slowly during the mobile phase flow. On the contrary, the ingredients which they are retained weaker than the static phase, they move faster. As a result of these differences in agility, the components are separated.

The different color methods differ in their nature mobile phase (liquid or gaseous) or static (solid or liquid on a solid substrate), as to the mechanism to which the separation is due (adsorption, ion exchange, distribution) and in the medium in which it has place the static phase (column, thin layer on glass plate, paper). The state of the mobile phase determines whether it is a gas method chromatography or liquid chromatography. There are four main types of chromatography: high liquid chromatography (HPLC), thin-layer chromatography (TLC), paper chromatography and gas chromatography (GS). (Themelis and Zotou 2017).

CLASSIFICATION OF CHROMATOGRAPHY

The classification of chromatography is very difficult if we categorized it with only one criterion. These techniques differ in the nature of mobile phase, the nature and aspect of stationary phase, the mechanism which is accountable for the segregate, and the way of introduction to sample in the stationary phase. So, the classification is based on:

a) The nature of mobile and stationary phase.
   In this case classified to Liquid chromatography and Gas Chromatography depending on mobile phase if it is liquid or gas. If the nature of stationary phase is solid or liquid, the Liquid Chromatography and Gas Chromatography separated to Liquid- Solid Chromatography (LSC), Liquid- Liquid Chromatography (LLC) and Gas-Solid Chromatography (GSC), Gas-Liquid Chromatography (GLC).
b) The mechanism of separation, the mechanism with the components of mixture retained of stationary phase and occurred separation.
   In this case classified to adsorption chromatography, ion-exchange chromatography, partition chromatography, molecular chromatography, and affinity chromatography. Absorption Chromatography is the oldest chromatographic technique in which the components of the mixture interact on the surface or at certain positions of the solid, usually static phase surface. The equilibrium restored between the adsorbed particles in the mobile phase achieves separation. The mobile phase could be liquid or gas. In the ion-exchange chromatography use ion-exchanging resins or brawn in the solid stationary phase and one liquid like mobile phase. The ionic components of the mixture are held electrostatically to a different degree than the ionically charged ionic groups of the static phase. In the partition chromatography the components of the mixture are distributed between a thin layer of liquid static phase formed on the surface of a solid substrate, and a liquid mobile phase. If the liquid static phase is more polar than the mobile phase, it is a normal phase chromatography, while in the opposite case we have a reversed phase chromatography. In the molecular exclusion chromatography, we have not interaction between the components of the mixture and stationary phase, in normal conditions. The liquid or gas mobile phase passes through a porous gel, the pore size of which is too small to allow small molecules to enter the gel network, excluding large molecules. So, the large molecules pass quickly without entering the gel network, while the small molecules entering the network are slow to leave the column as they need a larger volume of mobile phase to entrain them. Thus, the molecules are separated based on their size with large molecules coming out first. This technique called gel filtration chromatography or gel permeation chromatography. Final, the affinity chromatography is based on the highly specialized interaction of a molecule of the mixture with a molecule, which is chemically bound to the solid static phase.
c) Physic form of stationary phase.

The stationary phase restrained in the column, and the mobile phase come inside to column with pressure or due to gravity. This chromatography called column chromatography and separated to packed columns chromatography and planar chromatography.

d) way of introduction and movement mixture.

In this case we have three chromatography, the frontal chromatography, the displacement, and elution chromatography. In the frontal chromatography, the dilution is introduced into the column continuously and its solvent also acts as a mobile phase. The components of the sample come out of the column in the form of fronts. The technique does not achieve complete separation and is used only for large cleaning volumes of liquid or gaseous samples. In the displacement chromatography we use the mobile phase which strongly combines with the static phase, thus displacing to a different degree the components of the sample through the column. This technique generally achieves imperfect separations but has the advantage that large sample volumes can be used, as required in preparative and industrial scale separations. In the elution chromatography, which is the most important, the sample components are transferred from the mobile phase at different speeds along the static phase, at which point they leave the column at different times, while the column is ready, usually for the next separation. (Chatsioannou Th. P, Kouppari, Athens 2015)

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Table 1: Classification of chromatographic technique. (Chatsioannou Th. P, Kouppari M.A., Athens 2015)

Mobile phase	Stationary phase	mechanism	Form of stationary phase	Technique chromatography
Liquid		absorption	Column	Absorption chromatography
			thin layer on a plate	Thin layer chromatography
			Map	Absorption map chromatography
		Ion- exchange	Column	Ion-exchange chromatography
			Map	Ion-exchange chromatography in map with ion-exchanges
		Molecular exclusion	column	Liquid-gas molecular exclusion chromatography
		Eclectic reaction	Column	Affinity chromatography
	liquid	allocation	Column	Partition chromatography in column
			Map	Partition chromatography in map
Gas	Solid	absorption	Column	Gas-solid chromatography
		Molecular exclusion	Column	Gas-luquid chromatography or gas chromatography
	liquid	allocation	Column (packed or open capillary)	Gas-liquid chromatography or gas chromatography

THEORETICAL ANALYSIS OF CHROMATOGRAPHY

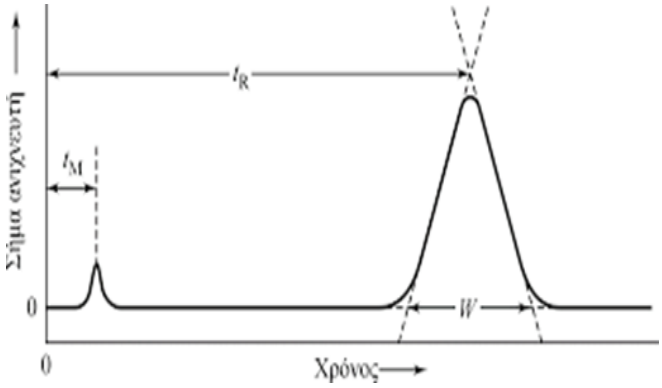
For each substance which is eluting in a particular column, the characteristic size is the retention time of the substance, t_R, or the retention volume, V_R. For a substance that is

not retained by the column, it takes as long to elute as the solvent-carrying molecules to move from one end of the column to the other. The time it takes for this movement is called dead time, t_M. (Ettre L.S. and J. V. Hinshaw J. V., 1996) Thus, we can define the mean linear displacement velocity of the substance, w, as:

$$W=\frac{l}{TR} \quad (1)$$ L is the length of column.

The speed of mobile phase, v, is:

$$\nu=\frac{L}{tM} \quad (2)$$



Picture 1: Typical chromatogram of a mixture of two substances. The small peak on the left is due to an unrestrained substance, which reaches the detector almost immediately after the onset of elution.

Gas or liquid or supercritical fluid can be used as the mobile phase. Liquids in relation to gases do not show compressibility, while supercritical fluids have intermediate properties. Due to the fact that many columns contain materials that achieve separation of the components of a mixture or have a long length, they present a pressure difference between the inlet gas carrier (pi) and the outlet (p). In these cases, a correction factor (j) is used to correct the calculation of the average linear velocity of the gas along the column. (Skoog D.A., Holler F. J., Nieman T.A., 2002). The type is:

$$J=\frac{3}{2}\frac{[\left(\frac{pi}{p}\right)^2-1]}{[\,(pi/p)^3-1]} \quad (3)$$

The choice and use of the corrective factor have, however, caused some controversy, because the type of mobile phase also plays an important role and the type of column.

The volumetric flow measured with a bubble flowmeter at the column outlet is given by the type: (Scott R.P.W., 2003)

$$F = Fm\frac{Tc\ (p-pH2O)}{T\ (p)} \quad (4)$$

Fm: the flow velocity measured by the flowmeter, Tc: the temperature of column, T: the environmental temperature, P: the pressure of gas out of column. This temperature is equal with air pressure and P_{H2O}: air vapor of water in counting temperature.

So, the type of retention volumes is:

$$V_R = j\ T_r\ F\ (5) \text{ and } \quad V_M = j\ T_m\ F\ (6)$$

The molecules of the analyte as they are transferred from the mobile phase interact with the static phase and separate. The random components of random and ataxic motion of their molecules are subjected to a kind of dilution as they move through the column. The main process for this dilution is diffusion. (Dallas E. 2002). Diffusion as defined by the first law of Fick is defined:

$$J = -\frac{RT}{f}\frac{dc}{dx} = -D\frac{dC}{dx} \quad (7)$$

J: the molecular flow with moles per surface area and time, R: the global gas constant the viscosity coefficient of the medium, T: temperature, D: the diffusion coefficient with surface units per year, C: the concentration of the substance at the point where we measured the flow and x: the distance variable.

Equation (7) holds for a linear change in concentration across a column. It therefore gives a constant flow value. The latter applies to variable concentration Fick's law, assuming that the diffusion coefficient is independent of concentration, and is defined by the formula:

$$\frac{dC}{dT} = D\ \frac{d^2C}{dx^2} \quad (8) \qquad \text{The time is variable.}$$

Equation (8) is a 2nd degree differential equation, the solution of which is one Gaussian function, assuming that the input is pulsed and instantaneous, so that in input to be described as an infinitely narrow band approaching a function of "d" Dirac. Subtracting from the system the movement of the belt, along the column, we come to a relation that describes the expansion of the zone, due to diffusion, with time. (Felinger A., 2008).

The formula is:

$$C = (4\pi Dt)^{1/2}\ e^{\left\{\frac{-(\psi)2}{4Dt}\right\}} \ (9)$$

$$\text{With } \psi = x\text{-}Wt. \ (10)$$

Each distribution function is described by certain statistical moments. The first torque describing the center of gravity of the belt is zero, since its center of gravity band on the ψ axis is zero and generally all unnecessary torques are zero. The torque that is the most useful and of the greatest importance is the second torque and is denoted by σ2, is called the scatter, while the square root of the scatter, s, is called the standard deviation and for the Gaussian curve is a measure of the distance from the center of the band to the turning point. It turns out that 68.3% of the area of a Gaussian curve contained between points –c and s, 95.6% of the area between points -2s and 2s and 99.7% between points -3s and 3s. The distance 4s between points -2s and 2s arbitrarily taken as the effective bandwidth. (Heftmann E., 2004).

The definition of the ninth statistical torque can be described by the formula:

$$<\psi^n> = \frac{\int_{-\infty}^{\infty}\psi^n c(\psi,t)d\psi}{\int_{-\infty}^{\infty}c(\psi,t)d\psi} \quad (11)$$

If we replace in the above relation n = 2, and after operations we have:

$$\sigma^2 = 2\,Dt \quad (12)$$

$$\text{Or } \sigma = (2Dt)^{1/2} \quad (13)$$

As it moves through the column, the molecules of the analyte exist interactions with either the walls or the column filler, affecting their smooth course. If in the previous random processes, we add the molecular diffusion we will have, at the exit of the column an expanded concentration distribution of the substance, which is in the form of a Gaussian distribution function. Since every factor, mentioned before, contributes either more or less to expansion of the distribution zone, it makes sense to assume that each factor is described alone from a dispersion. But according to statistics, we can add them dispersions of each factor, so we get a total dispersion σ_T^2, which according to ratio (12) corresponds to a total diffusion coefficient DT. So, it will be given by the relationship:

$$\Sigma_t^2 = 2\,D_T\,t \quad (14)$$

For time t the distance x that the substance will travel is Wt, where W is the constant velocity displacement of the substance. So, the relation (14) is transformed into:

$$\sigma_T^{\,2} = (2\,Dt\,/W)\,x \quad (15)$$

The term (2D / W) expresses its growth rate σ_T^2 (Moldoveanu S.C., 2013).

ELUTION OF THE SUBSTANCE FROM THE CHROMATOGRAPHIC COLUMN.

There are two theories that investigate its retention and dispersion substance upon its elution. These are the plate theory and the velocity theory, the which were proposed before the 1960s. In the years that followed these theories received some improvements aimed at expanding their applications to new chromatography techniques as well as their further confirmation. As a reference point is the recommended movement of a mixture along a column. During its movement mixture inside the column, its components interact with the stationary phase, which it can be some filler, coated on the walls or some exterior field, a possibility is created for the separation of the individual components of the mixture. For the theoretical analysis of this separation, we can first assume that individual components of the mixture move independently. Then through interaction with the separation domain, in a different way for each type of component, the individual separation takes place and then the separated components are subject to dilution process when displaced from the column. Thus, the optimal design of a chromatograph should consider the affinity of the solvent for its components of the mixture as well as the separation field in such a way that the substance is not retained much in the column, reducing the phenomenon of enlargement and at the same time giving the ability to separate the ingredients adequately.

The first theory proposed was the theory of plates by Martin and Synge 1941, who borrowed data from the slab model during its process distillation. According to this

model the movement of a substance in chromatography column can be thought of as a movement through successive, unreal, equilibrium zones, called theoretical plates. In each of these plates the researchers found that a kind of balance was restored between the carrier solvent and of the field responsible for the separation. It is understandable that the larger the coefficient of distribution of the substance with respect to the stationary phase, the longer the times elution of the substance will result, with all that entails. The theory accepted several improvements and became easier to use and at the same time more able to calculate the various retention factors and analyze the effects of elution.

The basic premise of the theory is the following relation in equilibrium C_S:

$$Cs = K\, C_M \quad (16) \text{ and } d\,Cs = K\, d\, C_M \quad (17)$$

where C_S is the concentration of the substance in the static phase, C_M is the concentration of the substance in mobile phase and K the distribution coefficient for the substance between the two phases.

If during the movement between two theoretical plates we have the change of volume of the mobile phase by dv, due to the retention of some of it by the static phase, then the relation that will connect the change in mass of the solvent dm with respect to the change of the concentration between two consecutive theoretical plates n - 1 and n, and the change of volume, will be:

$$Dm = (C_{M(n-1)} - C_{M(n)})\, dv \quad (18)$$

The mass balance in n theoretical plate, concerns the distribution of the solvent in static and in the mobile phase, so in equilibrium, the relationship will be valid:

$$Dm = v_s\, d\, C_s(n) + v_M\, d\, C_M(n) \quad (19)$$

But the relationship is valid $d\, C_ς = K\, d\, C_M$, so by substitution and by operations, we have:

$$Dm = (v_M + K\, v_s)\, Dc_{m\,(n)} \quad (20)$$

Recombination of Ex. (20) with Ex. (19), gives us:

$$\frac{d\, C_{M(n)}}{dv} = \frac{Cm(n-1) - C_{M(n)}}{Vm + K v_ς} \quad (21)$$

To make it easier to use, we define a new size:

$$dV = \frac{dV}{V_M + K_{Vs}} \quad (22)$$

which indicates the change in the volume of the solvent per volume of theoretical plate:

$$V_M + K\, v_s \quad (23)$$

$$\text{So: } \frac{d\, C_{M(n)}}{dV} = C_{\,M(n-1)} - C_{\,M(n)} \quad (24)$$

The above relation describes the change in the concentration of the mobile phase, as to the volumetric flow of the mobile phase. The above relationship is solved by completion, so for the ninth theoretical plate and for C0 initial concentration, we get:

$$C_{M(N)} = \frac{C_o e^{-V} V^N}{N\,!} \qquad (25)$$

The above relation is a Poison function and denotes a distribution, which for large N gives us a typical Gaussian error function. The chromatographs elution peaks are in the form of the Gaussian function, however a few factors that have to do with physical and chemical phenomena within the column, as well as construction imperfections disrupt the shape of the top. From the last relation it is possible to calculate the VR holding volume. This can be done by differentiating Ex. (25):

$$\frac{dC_{M(N)}}{dV} = C_o \frac{-e^{-V}V^N + e^{-V}NV^{(N-1)}}{N!} = C_o \frac{-e^{-V}V^{(N-1)}}{N!}\,(N-V) \qquad (26)$$

The maximum peak, which gives the solvent holding volume, is obtained when the derivative is equal to zero and this happens when N = V. This result shows that maximum is obtained when the mobile phase runs through a volume equal to N theoretical plates. After each theoretical plate has a volume VM + K vs, then for N we will have volume:

$$V_R = N\,(V_m + Kv_s) = N_v M + KN_{vs} \quad (27)$$

However, N_{vM} is the total volume of the mobile phase and KN_{vS} its total volume solvent held by the stationary phase, therefore:

$$V_R = V_M + KVs \quad (28)$$

In fact, the measured retention volume has both the contribution and other factors, such as the volume of the sample injected into the column, the volume of the cell of the detector, etc. If we denote this contribution of the volumes by V′, then the actual holding volume will be:

$$V'_R = V_M + KVs + V' \quad (29)$$

For a substance that is not retained by the column we will have the dead volume of the column:

$$V'_0 = V_M + V' \quad (30)$$

So, for a substance retained by the column, this retention will be due to:

$$V'_R - V'_0 = V_M + KVs + V' - V_M - V' = KVs \quad (31)$$

For two different substances A and B should apply:

$$K_A Vs\,(A) \neq K_B Vs\,(B) \quad (32)$$

That is, to separate and distinguish the elution peaks, the two must be substances A and B to be held differently in the stationary phase. This can be done with different coefficients of distribution in relation to the stationary phase and in addition to its amount which will affect the total volume of each substance you hold by the stationary phase [30].

The other important theory proposed for the study of the performance of a Chromatographic column is the theory of velocity. According to this theory, the shape of chromatographic peaks is directly affected by the elution rate. It depends on the different paths that the analyte takes as it travels between the particles of the static phase. The theoretical study began with the work of Lapidus and Amundson (1952) and expanded by Glueckauf and Tunitski (1954). Decisive The study of Van Deemter had a contribution to the theory, who also introduced the equation that bears his name (1956). This equation originally concerned paid gas columns chromatograph (GC) and then (’60) extended to paid liquid columns chromatograph (LC). Van Deemter's equation is the simplest and is used for design of a chromatographic column, in optimizing its efficiency and in study of the expansion of chromatographic zones [31].

Theoretical calculation of height H is difficult, as they will have to be obtained several factors into account. From time to time, various equations have been advanced, the most notable of which is the Van Deemter equation [16]. Its mathematical expression is given by relationship:

$$H = A + \frac{B}{u} + (Cs + Cm)u \quad (33)$$

where H is the height of the slab in cm, A term that is an indication of the number of pathways that the molecule of the substance can choose, moving through the static phase, B a term describing the longitudinal diffusion of a substance due to a difference in the concentration of the substance at the center of the moving mixture from the ends and the CS and CM coefficients mass transfer in the static and mobile phase, respectively, after achieving equilibrium between of the two phases are not instantaneous and in addition these coefficients affect together with the speed of the mobile phase, the distribution of substances between the two phases and the widening of the peaks. Van Deemter's theory was extended by Golay on capillary columns, who and he also introduced the homonymous equation (1958). This equation can be extended to describe the contribution of other parts of the chromatograph as expansion factors of the chromatographic peak, the sample entry, the connections, its cell detector, etc. For capillary columns, which do not contain material inside, A takes price zero and so the Ex. (33) takes the form:

$$H = \frac{B}{u} + (Cs+Cm)\,u \quad (34)$$

NUMBER OF THEORITICAL PLATES

Although in the process of moving the substance from one end of the column to the other, there are no distinct stages, however from Ex. (2.15) the σ_T^2 is proportional to x. So according to plate theory, it has been developed for processes which then contain distinct stages, we can consider the term ($2D_T$ / W) is the equivalent of a theoretical plate, i.e.: (Poe D.P., Martire D.E., 1990)

$$H = (2D_T / W) \quad (35)$$

$$\text{So } \sigma_T^2 = Hx \quad (36)$$

If we divide the length of column L by the theoretical height of a slab, we will have the number of theoretical plates:

$$N=\frac{L}{H} \quad (37)$$

If we take account the ex 15, so we have:

$$N=(\frac{L}{\sigma})2 \quad (38)$$

In non-uniform chromatographic columns, the local plate height is used: (Nagy B.,1960).

$$H =\frac{d\sigma^2}{dx} \quad (39)$$

Because the bands at the output of the column have a finite width, you will also need some time for the whole section to appear. If we denote by t the time that is required for a part of the zone equal to a constant deviation to appear, then the type: (Chen Y., 2007).

$$T= \frac{\sigma}{W} \quad (40)$$

If we combine the ex 34 and 38, for x=L we have:

$$H = \frac{\sigma^2}{L} = \frac{\tau^2 W^2}{L} \quad (41)$$

If we combine the ex 1 and 39, we have:

$$H = \frac{L\tau^2}{t_r^2} \quad (42)$$

Instead of size τ, which is difficult to determine in practice, size is used $\tau_{½}$, which expresses the width of the belt, if time is taken as dashed, at half the height (see Figure 2.4). Then the Ex. (40) reads:

$$H = L\frac{\tau_{1/2}^2}{2\sqrt{ln2t_r^2}} = \frac{0.72135\, L\, T_{1/2}^2}{T_r^2} \quad (43)$$

The number of theoretical plates, based on Ex. (2.35), for x = L, will be given by

relationship:

$$N = \frac{t_r^2}{T^2} = 1.38629\frac{t_r^2}{T_{1/2}^2} \quad (44)$$

Instead of times we can measure distances on the recorder paper, since the sizes are proportional.

Also, the number of theoretical plates is proportional to the ratio t_R/w_H, with Wh = 4σ. So, from the combination of Ex. (2.40) and (2.42) any factor that increases the ratio will reduces the H. Although the Wh, is affected in practice by factors outside the column, such as the range of the input distribution of the substance and the constant distribution, its value reflects the elongation of the substance band as it passes through the column. One of the reasons for elongation of the substance zone are the diffusion of molecules in the gases and in static phase. For the gas phase the diffusion coefficient takes values between 0.15 - 0.6 $cm^2\ s^{-1}$, while in the static phase it receives values of

the order 10^{-7} cm^2 s^{-1}, which is why many times can be ignored. The diffusion coefficient is affected by the density of the carrier gas and in fact varies inversely according to, as well as by its size molecule of the carrier gas, under the same conditions. Also plays a role column temperature, after elevated temperature, means that the molecules have more kinetic energy, and therefore move and propagate in space with higher speed. However, with increasing temperature we have an increase in its viscosity mobile phase something that acts competitively in the diffusion of molecules. Finally, from the above equation it is understood that each column behaves as if it were different number of plates for different substances in a mixture, analyzed by the chromatograph, because of the different t_r of each substance. (Robards K., 2004).

DIFFUSION OF GASES INTO LIGUIDS

For a gas component A in contact with a liquid phase, a flow of it will occur component in the liquid phase due to a difference in concentration. Component A will be adsorbed on the surface of the liquid due to the random movement of its molecules and then will restrained by weak Van der Waals forces. The magnitude of the adsorption forces depends on the nature and condition of the gas and its molecules fluid, while the different magnitude of these interactions in each component constitutes and the basis of separation in liquid gas chromatography. Its simplest model absorption of gases from the aquatic environment is the theoretical model of the two layers proposed by Liss and Slater. According to the model, on its interface two immobile layers of liquid and gaseous phase are formed on either side of the liquid, thus creating a "resistance" to the dissolution of the gas in the liquid. (Fernández Y. B., Cartmell E., Soares A., McAdam E., Vale P., Darche-Dugaret C., Jefferson B., 2015). The mathematical expression of the model is as follows:

$$J= J_g= J_l = kg\ (C_g- C_{sg}) = k_l\ (C_{sl}- C_l)$$

Where J_g and J_l the mass flows of the component on either side of the gas and liquid interface phase, c_g, C_l the concentrations of the component in the gas and liquid phase respectively c_{sg}, C_{sl} are concentrations of the component at the interface of the gas and liquid phases respectively and k_g, k_l k the velocities for the mass transfer of the component to the gaseous and liquid phases, respectively. The amount of component gas A to be dissolved in the liquid phase depends on thermodynamic factors. In equilibrium this amount is described by one distribution coefficient, called Henry, H_A constant: (Van't Riet K., 1979)

$$H_A = \frac{P_A}{X_A}$$

where P_A is the partial pressure of the component in the gas phase and x_A is its molar fraction component in the liquid phase.

As a result, we have the component distribution constant, K:

$$K = \frac{C_l}{C_g} = \frac{\frac{P_A}{H_A}}{\frac{P_A}{P}} = \frac{P}{H_A}$$

At the interface, Henry's law turns into concentrations:

$$H_A = \frac{C_{sg}}{C_{sl}}$$

The last relation gives us the possibility of transforming his mathematical relation model by Liss and Slater: (Faust S. D., Aly O. M., 1998)

$$J = K_g (c_g - H_A C_l) = K_l (C_g / H_A - C_l)$$

$$\text{with } Kg = \frac{1}{k_g} + \frac{H_A}{k_l} \text{ and } Kl = \frac{1}{k_l} + \frac{1}{H_A k_g}$$

From the above relations we observe that Henry's constant, HA, is sensitive, in the most volatile physicochemical parameter of the system, temperature. It is expected that as the temperature increases, its solubility will decrease component A. However, in several physical and chemical systems, due to the complexity the reactions of the components and the many parameters involved, there are several exclusions. Thus, systems with the opposite have been observed behavior in the effect of temperature and nonlinear behavior in solubility.

Surface adsorption is followed by absorption into the main mass of the liquid. The main force for this dissolution is diffusion. Liquid phase diffusion is an activated process in which the diffusion coefficient changes exponentially with temperature, according to the equation: (Lee L.H., 1991)

$$D_A = D_0 \exp(-Ea / RT)$$

Where E_α the diffusion activation energy and D_0 a term that depends little on the temperature. Its price E_α can be determined by various methods. In gas-liquid chromatography, surface diffusion takes place at the gas-liquid interface, while in liquid chromatography at the liquid-solid interface.

SURFACE PHENOMENA

The extensive and most powerful network of Van der Waals and London links that is being formed in liquids, in relation to gases, is responsible for the appearance of the characteristics their properties. One of them has to do with the different configuration of its molecules surface of the liquid. This is because surface molecules are attractive forces only from the main mass of the liquid, resulting in the curvature of its surface. This condition results in the liquid surface being in a state increased energy, because in surface molecules, the component of forces, in unlike the molecules of the main mass, it is non-zero. If a force F transfers the surface film at a distance dx, in such a way that the surface energy to minimized, the surface area of the liquid will change by dA and the energy will be varied by γ dA, where γ= F/2l is the surface tension on both sides on

both sides of the surface film, with units Nm^{-1}. More specifically, the surface tendency is one force per unit length of surface film, referred to in interface two phases. Shows the force exerted by and on the main mass of the liquid, at surface film of the liquid molecules, as this is subject to a compensatory force tending to reduce its surface. (Rotenberg Y., Boruvka L., Neuman A.W.,1982)

The surface tension of solutions is different from that of pure substances. Depending on the type of solute, its concentration on its surface solution may be greater than the main mass of the solution (positive surface concentration), lower (negative surface concentration) or itself. The Surfactants, as their name suggests, exhibit their action on the surface of a medium or on the interface of two phases. The driving force that is responsible for this action is the minimum energy that the surface acquires from its presence surfactant. The degree to which the surfactant is concentrated on the surface, but also the structures it will form there, depend on the nature of the two phases of the medium, but also by the chemical formula of the surfactant. Because of these of restrictions there is no specific type of surfactant, but the choice will depend on the application to be used. A direct consequence of the above is the reduction of the surface tension of the solution, to some extent, in which does not show a decrease in surface tension. (Vargaftik N.B., Volkov B.N., Voljal L.D., (1983).

Water – gas	72-73
Gas- 10% NaOH	78
Gas-aqueous solution of surfactant	40-50
Hydrocarbon -water	28-30
Aromatic hydrocarbon- water	20-30
Hydrocarbon- aqueous solution of surfactant	1-10

Table 2: Indicative values of surface voltages of different types of solutions, $mN\ m^{-1}$

One method of measuring surface tension is Wilhelm's method, which was discovered in 1863 and is simple to use. (Wu N., Dai J., Micale F. J., 1999) This method refers to a plate immersed in the liquid and in which a force of gravity is exerted, as much as needed to pull out the plate, calculating this force with a precision balance. Due to the surface tension a surface film is formed on the plate by the molecules of the liquid, which pushes the liquid down.

So, in the balance we will have:

$$W_{tot} = W_{plate} + P\ \gamma\ \cos\theta$$

where Wtot the total weight that the plate receives from the yoke to come out of the liquid, Wplate the weight of the plate, P the perimeter of the plate, γ the surface tension and θ the angle forms the interface of the liquid with the surface of the plate. Thus, if the angle is known contact, we can measure the surface tension.

When a substance is deposited on the surface of solution, we have its increase of its surface concentration. This surface concentration on the one hand affects the surface properties of the solution, on the other hand, does not determine its further course substance in solution. In a specific physical state of the solution, we have the adsorption of a certain amount of the substance, which is determined by the species and the number of interactions with the solution molecules. So, when the restraint of molecules is made with weak forces (Van der Waals) we have natural adsorption, which is fast, reversible and occurs when the activation action, Eα, of the reaction adsorption does not exceed the limit barrier of 40kJ / mole or 10kcal / mole. Because of reversibility, if we increase the temperature or decrease the pressure, will be observed the phenomenon of desorption. In contrast, chemical adsorption is not reversible, it is stronger, from a few kcal / moles to 100 kcal / mole, and due to forces like these of chemical bonds. The adsorption effect has been applied in several variations of chromatography for mixture separations. To study its phenomenon adsorption various theories and corresponding equations have been proposed from time to time, with several assumptions (Langmuir, Freundlich, Temkin, B.E.T.). However due to the complexity of the phenomenon, each theory finds application in specific processes. The phenomenon of adsorption in solutions is very sensitive to its change temperature. This is because the surface tension decreases with increasing temperature and becomes minimum at the critical temperature, Tc, during which intermolecular forces of the solution tend to zero. The most representative relationship that shows the dependence of surface tension on temperature is:

$$\gamma = \left(\frac{Mx}{\rho}\right)^{\frac{2}{3}} = K(Tc - T - 6)$$

where ρ is the density of the solution, T is the temperature, M is the molecular weight of the liquid and x the degree of coupling of the liquid. (Dee G. T., Sauer B. B., (1991)

GAS CHROMATOGRAPHY

The gas chromatography was discovered by researchers Archer John Martin and Richard Laurence Millington Synge, (prize of Nobel 1952) to 1941. For 10 years later, gas chromatography used experimentally in 1955, when the first machine used from chemicals. The idea behind its discovery was that since the diffusion coefficients are higher in arias than liquids, then the equilibria concerning the distribution of substances between stationary and mobile phase will be faster in gases and for this reason the separation will be, if gas is used as the mobile phase.

(http://delloyd.50megs.com/moreinfo/gaschrom.html).

As we tell previously, the separation of the components of a mixture is based on gas chromatography in the different distribution of components between the two phases.

To achieve this certain separation, one way is to used tubular column made of: glass, stainless steel, ceramic material etc. These columns are empty inside and have a suitable material built-in in their inner walls which is the stationary phase in this case. This material in the form of a thin coating can be solid, liquid, porous polymer, or adsorbent material. The most common construction materials in the static phase are: polysiloxane due to stability, durability and reusable and polyethylene due to very good resolution. (MAS Medical & Scientific Eq. Co. Gas Chromatography). There are two columns in gas chromatography which are separated about the natural characteristic, the packed column and capillary column. The characteristics of these columns are:

Packed Column:

- The most common construction material is stainless steel.
- The inner diameter is about 2mm to 4mm.
- The length is from 1 to 4 m
- The main use of packed column is to gaseous samples
- Dilatation of peaks due to diffusion in zones.
- Short column length due to drop of carrier gas
- The theoretical plates are between 1000 and 4000 N/m

Capillary column:

- The capillary column is the most used.
- The inner diameter is from 0,1 to 0,53 mm.
- The length is from 10 to 105 m
- They have acute peaks.
- There is excellent separate of peaks.
- The theoretical plates are between 3000 and 7000 N/m.

ADVANTAGES AND DISADVANTAGES

The gas chromatography has plenty advantages and disadvantages. Some of advantages of gas chromatography we can mentioned are that a relatively simple technique, it is easily configurable in the conditions we desire to do the experiment, is a fast technique, reliable, quite widespread and with wide range of substance analysis and can be automated. On the other hand, there are plenty disadvantages. The disadvantages include any construction defects and gas leaks. The costs of the gas chromatograph are huge, and the gases cannot be recycled. The sample must be in the gaseous phase, so liquid and solid samples must be evaporated with this implying the relatively high operating temperature of the chromatograph which makes it difficult to analyze heat sensitive substances such as proteins. The main boost of the right separation of these substances is the thermo-programmed control of the column, changing it temperature of the column during the experiment, after adsorption and Its speed depends on the temperature. But lowering the temperature causes a reduction in the separation speed thus leading to long times analysis, with all that entails. So, the quantities which required to make the analysis must be very small and the accuracy and sensitivity of this method is quite high, even if we have ppb concentrations. (Hollingsworth B., 2004).

An important aid for the separation of the substances is the programmed control of the column, changing the temperature of the column during the experiment since its adsorption and velocity depend on the temperature. However, the decrease in temperature causes a reduction in the separation rate, thus leading to long analysis times, the electronic pressure control can change the flow rate of the carrier gas, reducing the analysis rate without changing the separation of the substances. (Tranchida P. q., Sciarrone D., Dugo P., Mondello L., 2012).

APPLICATIONS OF GAS CHROMATOGRAPHY

The uses of gas chromatography are and are separate complex mixtures, analyzes and details of what we use, create factors cross-section and adsorption isotherms. Some of the applications of gas chromatography are in the next table:

SECTOR	***APPLICATIONS***
Petroleum products	Fuel analysis, Hydrocarbon analysis and derivatives analysis of fuel additives.
Environmental analysis	Determination of pesticides, determination of by products from disinfection processes, determination polychlorinated biphenyls (PCBs), atmospheric air analysis and determination components responsible for infection, dioxin analysis and related compounds
Pharmaceutical	Drug purity check, check for residues from solvents in medicinal products.
Toxicology	Determination of the amount of alcohol in the blood, determination anabolic steroids, control foe the presence of dangerous substances in many sources.
Food	Food quality control for by-products alcoholic fermentation, coffee analysis, fat analysis, analysis of food additives and oil, analysis of volatile substances in food packaging, food improvement analysis.

Table 3: Applications of gas chromatography.

TYPES OF GAS CHROMATOGRAPHY

There are two types of gas chromatography, the gas- liquid chromatography (GLC) and the gas- solid chromatography (GSC). The first chromatography has more applications to analyze components in chemistry in contrast to gas-solid chromatography. The gas-

liquid chromatography is common like gas chromatography and we use gas mobile phase with liquid stationary phase. The gas-liquid chromatography is widely used in qualitative and quantitative analysis, especially for the detection, identification, and identification of organic substances in complex samples, as well as the determination of various physicochemical quantities.

The main principle for the application of this technique is the volatility chromatographic substances in column temperature. In the case of non- volatility substances, they are converted to volatiles derivatives when they react with the suitable reagents. Some of the implementations of GLC are the analysis of oil spill, biologic samples, foods, pesticides, insecticide, ethereal oil, steroid hormones, drugs, fertilizers, plastics, alcohol beverages, synthetic detergents, tests to control its contamination for the study of the movement of gas masses in Meteorology, for the exploring the planet's atmosphere. When the samples are confusingly, the gas- liquid chromatography is combined with chromatographic techniques such as thin layer chromatography, spectrophotometry, mass spectrometry. (McMurry,2001).

On the other hand, in the gas-solid chromatography we use gas mobile phase with solid stationary phase. In GSC the separation is due to adsorption or molecular block of the components of the mixture in the static phase. The applications of chromatography gas-solid are limited due to the almost permanent retention of active or polar molecules and the intense appearance of a tail at the elution peaks. For this reason, this technique is not often used except in cases of separation of some gases of small relative molecular mass. Solid gas chromatography is based on the adsorption of gaseous compounds on solid surfaces.

The distribution coefficients are generally much higher than those of gas-liquid chromatography. Therefore gas-solid chromatography is useful for separating substances that are not retained in gas-liquid columns such as air components hydrogen sulfide carbon monoxide, carbon dioxide and noble gases. (Skoog, D. A. Holler, FJ. and Nieman T.A. 2002).

The basic organology in gas-liquid chromatography or gas chromatography is:

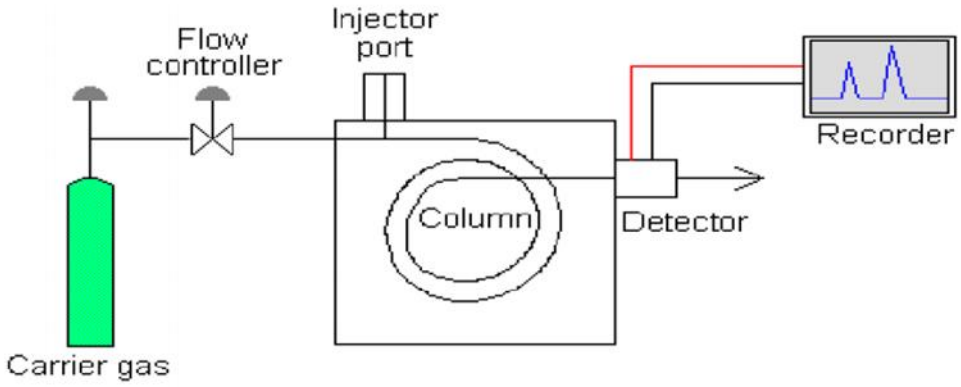


Image 2: The organology in gas-liquid chromatography.

INSTRUMENTS OF GAS CHROMATOGRAPHY

The gas chromatography has discovered such as analytical technic the last forty years. This certain technic is very simple, and we used it for analysis volatile substances which are in foods or drugs and gas products etc. The main instrument used is gas chromatography is the gas chromatograph (GC). The gas chromatograph, regardless of model, year or brand production consists of the following five main parts:

- carrier gas
- sample input chamber
- oven and capillary column
- detector
- recorder

CARRIER GAS

To be right the analysis of samples, it is necessary the existence of gases with different properties. These specific gases are:

a) The carrier inert gas, which serves to drag and push the sample from its insertion chamber into the capillary in order to the separation takes place. The carrier gas (usually N2, He, H2, Ar) from the high-pressure cylinder, through flow regulators, is led to the column. The sample is inserted with a micro-syringe into the sample inlet valve at the top of the column. The sample components are entrained by the carrier gas along the column and separated. The fractions are then detected in the detector and the detection signals are recorded by a logger. In some cases, there is then a device, where the various fractions are collected and a flowmeter to control the flow rate of the carrier gas. In addition, as carrier gas we can use any super-pure gas which can be differentiated in the detector, from the various components of the mixture. The carrier gas must be inert and free of impurities. It should also not contain oxygen, because it oxidizes the static phase and this means destruction of the column, especially when it is capillary, and the

amount of static phase is minimal. Traces of moisture also deactivate the static phase, for this carrier gas must be free of moisture. The choice of carrier gas depends mainly on the type of detector used. (Papadogiannis, IN, Samanidou, Thessaloniki 2001)

b) Fuel gas, which is required only when using a detector flame ionization. The flame is created by supplying H2 and synthetic air. Various gases such as acetylene, natural gas, propane, propylene, and hydrogen can be used as a fuel for cutting and welding, heat treatment, as power and energy for transportation and combined with oxygen, for various industrial processes. (H. Müller, 2005)

c)The makeup gas (carrier gas) in the gas chromatography process which is used to clean its appliances gas chromatograph (e.g., deletion of excess sample from inlet chamber). (Peter Froelich, President Peak Media, Franklin, MA, 2014)

SAMPLE INPUT CAMBER

The sample is inserted in chromatograph with the help of a glass syringe through the septum (silicone rubber) where immediately evaporates. The import quantity depends on the column type and the desired separation, usually 1-10 μl.

OVEN AND CAPILLARY COLUMN

First, the oven is used to maintain the column temperature at desired levels which are formed through programming on the instrument. During this programming we can define the initial, the final temperature, duration, and rate change. The temperature programming depends on the mixture under analysis and helps to separate substances of different volatility. (Aristotle University of Thessaloniki, Aikaterini Karamanoli, Thessaloniki 2014).

The heart of the chromatograph is the column. There are two types of columns: packed columns and capillary columns. The column consists of an elongated tube, usually in the form of a coil or U, to occupy as little space as possible, made of stainless steel, copper, aluminum, glass or plastic, 1-2 m long for the paid columns, up to several hundred meters for capillaries, internal diameter of the order of mm in the analytical columns, several tens of cm in the preparatory columns.

DETECTOR

A chromatography detector is a device used in Gas chromatography (GC) to detect components of the mixture being eluted off the chromatography column. The detector must have many of advantages such as sensitivity, selectivity, operating temperature up to 400°C, reliability, ease of use, stability and reproducibility and Linear response to analysts, which should cover a wide range concentration area. (Mericas rafail, 2013).

There are many detectors which can use in gas chromatography such as Flame Ionization Detector (FID), Thermal Conductivity Detector (TCD), Photo ionization detector (PID), Nitrogen/phosphorus detector, electron capture detector.

As we observe from the table above, the detectors can be them classified into eclectic and wide-ranging, as well as whether they count mass or concentration. Another feature is whether or not they destroy it sample. In recent years, many gas chromatographs have been connected to spectrometers thus increasing its substance analysis capabilities as well as its applications gas chromatography. (Grob R. L., Barry E. F. 2004).

We should also mention that for any substance detected with one a specific detector should have a response factor calculated. He calculated experimentally under specific conditions which must be the same and in the experiment. The response rate of each substance is related to the height or area of each and with the amount of template introduced into the chromatograph. A Gaussian-shaped vertex is created for each substance detected on a fixed baseline. The height of the top or its area, is the basis for quantitative analysis of the sample. The characteristics of the top to be specified to do the quantification is the sensitivity of the detector, the minimum traceability limit, linear operating range and dynamic operating range. (McNair H. M., Miller J. M., (1997).

Sensitivity

Sensitivity is defined as the ratio of the detector signal to its concentration substance or to the mass of the substance, depending on the type of detector. For a detector that based on the concentration of the substance, the selectivity is defined by the formula:

$$S = \frac{AFc}{W} = \frac{E}{C}$$

where S denotes the sensitivity, A the area of the peak in units of mV min or Ampere min, Fc the corrected flow of the substance in units of ml min^{-1}, W is the mass of the substance in mg, E peak height in mV or Ampere, C the concentration of the substance in unit's mg ml^{-1}. So, the resulting units of sensitivity are mV ml mg $^{-1}$ or Ampere ml mg^{-1}.

For a detector based on the mass measurement of the substance the sensitivity is defined

by type:

$$S = \frac{A}{W} = \frac{E}{M}$$

where E is the peak height in Ampere or mV and M is the mass flow of the substance in mg sec^{-1}. Thus, the resulting units of selectivity are Ampere sec mg-1 or Coulomb mg^{-1}. As shown by the units, the comparisons between the two above types of detectors, is difficult. (Duarte R. M.B.O., Duarte A. C., (2014) and Trojanowicz M., (2011).

Linear operating range

The range of linear operation of a detector is a feature for each detector and shows us the concentration range of the substance in which the relationship its with the detector signal is linear. This is between the minimum limit traceability and the upper limit of the linear operation of the detector. A lot more accurate graph is of sensitivity to the concentration of the substance. According to the A.S.T.M. (American Society for Testing and Materials), its upper limit linear mode is the point in the graphic that displays 95% of the maximum sensitivity. The I.U.P.A.C. (International Union of Pure and Applied Chemistry) has clarify the minimum traceability limit, D, with the formula:

$$D = \frac{2N}{S}$$

With N the noise level of the detector.

From the above we have the formula that gives us the area of linear operation of the detector:

$$\text{Εύρος γραμμικής λειτουργίας} = \frac{\textit{ελάχιστο όριο ανιχνευσιμότητας}}{\textit{άνω όριο γραμμικής λειτουργίας}}$$

From this ex shows that the linear operating range is a net number, and we want him to be as big as possible. Finally, it should not be done confusion of the upper limit of the linear function, showing the linear relation of the signal with the concentration, with the dynamic range of operation showing the maximum concentration of the substance that can be measured or recorded. (Spangenberg B., (2015).

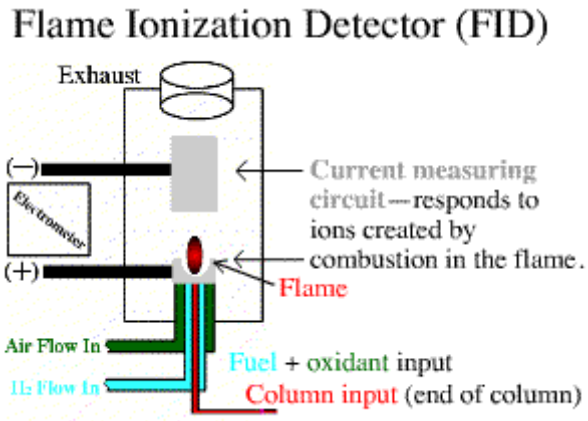
Response time

The detector response time is the time the detector needs to electrically change the composition of the carrier gas. This time affects the height and width of the top and even accordingly, but not its area top. In the present work, the detectors used in the gas chromatographs was the flame photometer and the thermal conductivity detector. In the following subchapters reference is made to these two detectors. (Spangenberg B., (2015).

Flame Ionization Detector

The Flame Ionization Detector (FID)is the most common detector used in gas chromatography. A Flame Ionization detector (FID) requires a carbon-hydrogen bond. The principle of operation is based on the change in conductivity that is caused by the ionization of an organic compound when burned in a flame hydrogen - air. During the combustion of organic compounds, the carbon is burning and products CO_2, H_2O, cation, and anion and also electronics. The ions are collected on a pair of polarized electrodes inside in the detector, the current generated is amplified and recorded. The electrons produced during the combustion of C also depend on the oxidative state of C burning. For this reason, compounds even though they have the same number of coals give a different signal. This method has many benefits and drawbacks. The FID is sensitive to, and capable of detecting, compounds that contain carbon atoms (C), which accounts for almost all organic compounds. However, the FID is not sensitive to carbon

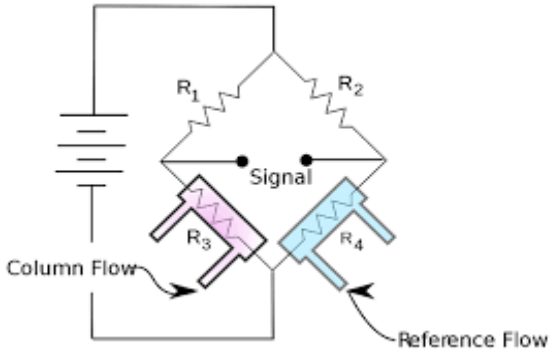
atoms with a double bond to oxygen, such as in carbonyl groups and carboxyl groups (CO, CO2, HCHO, HCOOH, CS2, CCl4, etc.) (Charles A. Burgett, Douglas H. SmithH, BryanBente).



Picture 3: Flame Ionization Detector.

Thermal Conductivity Detector

The Thermal Conductivity Detector is one of the first detectors used in gas chromatography, this detector is often referred to as the katharometer. The Thermal Conductivity Detector works by having two parallel tubes both containing gas and heating coils. The gases are examined by comparing the heat loss rate from the heating coils into the gas. Normally one tube holds a reference gas and the sample to be tested is passed through the other. Using this principle, a TCD senses the changes in the thermal conductivity of the column effluent and compares it to a reference flow of carrier gas. Most compounds have a thermal conductivity much less than that of the common carrier gases of hydrogen or helium. Therefore, when an analyte elutes from the column, the thermal conductivity of the effluent is reduced, and a detectable signal is produced. (D. McMinn, in Encyclopedia of Separation Science, 2000).

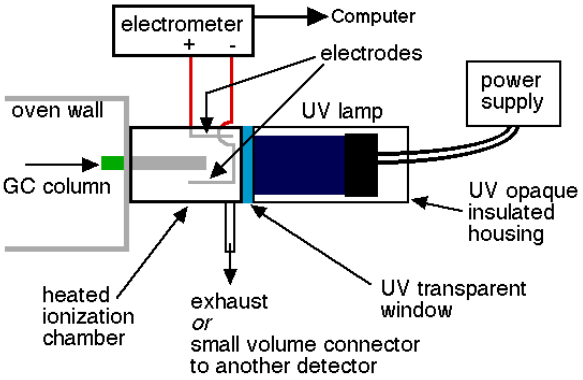


Picture 4: Thermal Conductivity Detector.

While flame ionization detector (FID) can provide very good resolution, TCD is a good general-purpose detector for initial investigations with an unknown sample, as it responds to all compounds, thanks to the fact that all compounds, organic and inorganic, have a different thermal conductivity from helium. The TCD is also used in the analysis of permanent and inorganic gases (for example argon, oxygen, nitrogen, carbon dioxide, carbon monoxide, sulfur dioxide) because it responds to all these substances unlike the FID, which cannot detect compounds which do not contain carbon-hydrogen bonds (A. Uyanik, in Encyclopedia of Separation Science, 2000).

Photo Ionization Detector

The photo ionization detector (PID) contains compounds (aromatic rings, alkynes, and alkenes) which absorb photons via an ultraviolet lamp which are in the ionization chamber of photo ionization detector. So, the ions which created, collected at electrodes and final the ions give a signal. This detector used for analysis of benzene, toluene, ethylbenzene, and xylenes. (Chris Swyngedouw*, Robert Lessard). This detector has many benefits such as high sensitivity, quick response (< 3s), high precision at very low levels, one detector can detect broad range of gases and modern type (3D) is not affected by temperature and humidity changes. However, apart from benefits there is a basic disadvantage that is the low selectivity for most compounds. (Driscoll, J.N., and J.B. Clarici, 1976).

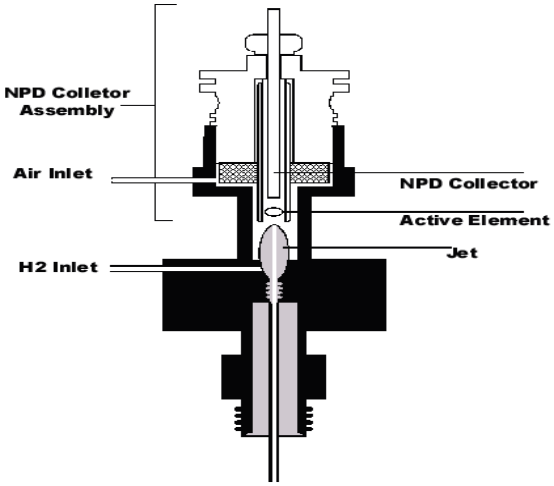


Picture 5: Photo ionization detector.

Nitrogen /Phosphorus Detector

The nitrogen/ phosphorus detector is like a flame ionization detector and passes sample and carrier through a hydrogen/ air plasma. This detector is very sensitive to nitrogen and phosphorus compounds but is not so sensitive like electron capture detector. The low hydrogen/air ratio cannot sustain a flame, minimizing hydrocarbon ionization, while the alkali ions on the bead surface facilitate ionization of nitrogen- or phosphorous-organic compounds. The output current is proportional to the number of ions collected. Nitrogen and phosphorus-containing compounds such as pesticides

cause the bead to emissions, which are then collected on a collector to produce a current. (Driscoll, J.N., and J.B. Clarici, 1976).



Picture 6: Nitrogen/ Phosphorus Detector

Recorder

This is the final stage of gas chromatography. In this stage the analog signal given by the detector is converted to digital, using a suitable card, and recorded with a suitable one software on a computer unit.

WATER ENVIROMENT

Water on our planet occupies a large area and is found in various places natural systems and forms. Indicatively to mention the water of the sea, the rivers, lakes, water in clouds, water vapor, droplets, ice, drinking water etc. Also, because it is a good solvent for many substances and they can do that to grow several organisms, there are several types of water with different properties and functions. Another physical parameter that affects the properties of water is pressure and temperature conditions. Apart from these reasons is its usefulness water to humans as well as in industrial treatments, which make water environment a very interesting system to study, especially in recent years with environmental pollution. Sources of water pollution are industrial waste, airborne substances, ship - generated waste, city waste and small-scale pollution caused by humans due to non-environmental consciousness. Pollutants that reach the aquatic environment as waste are classified in three categories:

A) Natural pollutants: Solids, colloids

B) Chemical pollutants: These are divided into organic (carbohydrates, fats, oils, proteins, hydrocarbons, pesticides, etc.) and inorganic (various chemical compounds, toxic compounds, dissolved gases, heavy metals, etc.)

C) Radioactive pollutants: Coming from factories that manage radioactive substances.

Water with a salt content of less than 1 g / L is classified as sweet, while if contains a larger amount as salty. The hardness of water is divided into total, transient and permanent. The total hardness is due to the total salts found dissolved in water and mainly in the magnesium (Mg) and calcium (Ca) ion compounds with anions such as carbonates, sulfates, and chlorine. The transient hardness is due to carbonates that precipitate after heating water. The permanent hardness due to other salts. The units of measurement are the German grades (d0), the French degrees (f0) and calcium salt content (mg Ca / L).

Drinking water can come from either groundwater or surface water, the which is then processed. Its quality is controlled based on international specifications. These specifications set certain limits on parameters such as color, h odor, taste, temperature, pH (6.5-8.5), conductivity (2,500 Ohm / cm), residual chlorine, dissolved oxygen, salt content, compounds of nitrogen (pollution level indicator), in toxic metals (eg As, Cd, CN, Cr, Hg), in insecticides, microbial load and its hardness (15-30 f °).

SEA WATER

The oceans cover about 71% of acreage of the land and are the major butt of water. Seawater is a complex mixture of 96.5 percent water, 2.5 percent salts, and smaller amounts of other substances, including dissolved inorganic and organic materials, particulates, and a few atmospheric gases. The six most abundant ions of seawater are chloride (Cl^-), sodium (Na^+), sulfate (SO_4^{2-}), magnesium (Mg^{2+}), calcium (Ca^{2+}), and potassium (K^+). By weight, these ions make up about 99 percent of all sea salts. In the case of the open oceans, the ocean is generally in a steady state. Based on a study, the chemical elements were classified based on their length of stay in the sea water. The residence times were calculated by dividing the concentration of this component in seawater by its annual rate of addition through rivers. The data were divided into three groups according to their length of stay: small (100-1000 years), medium (1000-1000000 years) and large (>1000000 years). The elements with a short time are Al, Cr, Fe, Pb and Th, and the elements with large stay years are Li, B, Na, Mg, S, Cl, K, Br, Rb, Sr, I, La, Ra and U. The elements with a short stay time are very active, they move away quickly from the seawater, while the elements with a long residence time have little activity in the sea water. (G. Nelson Eby,2004).

THE MAIN PATHWAYS OF WATER POLLUTION

TYPES OF POLLUTION	SOURCE	EFFECT

Cu, Pb, Zn, Cd, Hg, Co, Cr, Ag, As, CN	Chemical industries, metallurgy	Accumulation of the food chain
Phenol, ammonia, detergents, paper fibers	Chemical industries, food industries, pharmaceutical industries, and paper mill	Lack of oxygen, toxic products (ammonia and phenol), eutrophication phenomena and reduction of ecological diversity
Heavy metals, gas, organic compounds and anorganic compounds	landfills	Groundwater pollution
Pesticides, insecticides, fertilizers	Agricultural activities	Increase nitrogen ions, cancer
Nitrogen, phosphorus, bacteria, fungi	Livestock activities, slaughterhouses	Pollution of groundwater and surface water
Oxides of S and N	Acid rain	Disaster of forests
Radioactivity in water	Nuclear power plants	genetic alterations, accumulation of the food chain
Suspended particles, mineral compounds, acid waste	mining activities	air and groundwater pollution, soil subsidence
hot water	Energy stations, industries	killing fish eggs, reducing oxygen, increasing the metabolic rate of organisms
Salts	penetration of the sea	destruction of coastal aquifers

Table 4: The sources and effects of water pollution

- *Land sources*

Responsible for 75% of marine pollution, land-based sources feed on industrial and agricultural wastes such as fertilizers carried by rivers, pesticides, and herbicides, but also effluents that are poured directly into the body of the sea. They are distinguished in: (GESAMP (IMO/FAO/UNESCO-IOC/UNIDO/WMO/IAEA/UN/UNEP/UNDP Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection), 2009).

-Household wastewater is the wastewater that comes from the activities of the residents' areas and is connected to its functions. Such as laundries, patisseries, homes etc. They consist mainly of water and dissolved organic or inorganic compounds. They have characteristic odor due to their anaerobic breakdown by microbes and non-microbes. Organic materials are soaps, fats, feces, papers, urine, etc. and inorganics are eternity and nitrogenous compounds etc. Understand that the amount of these effluents depends on how densely populated this area is.

-Municipal waste, such as paper, glass, plastics, rubber, vegetable materials, textiles, etc. The man believed in the vastness of the ocean, so he thought he could load them. No garbage. The situation is deteriorating accordingly and with the increase of the world population. As the population grows, so do the rejections. In modern culture,

however the process of recycling materials that can be reused has become necessary. In this way, much of the waste that would end up in the environment is used and again. For example, recycling paper not only does not end up as waste in environment, but in this way also helps to reduce the overexploitation of forests. Many of these wastes contain small amounts of other harmful substances such as oils, mineral oils, detergents which are ultimately misused and recycled packaging, do not end up in the environment.

-Nutrients. As is well known, phosphorus and nitrogen salts end up in water, cause eutrophication and is due to the growth of phytoplankton. In the process eventually lead to anoxic conditions in the body of water dramatic effects on the aquatic ecosystem. These salts come mainly from agriculture waste, herbicides, and fertilizers, but also from household and municipal detergents rich in phosphorus. (Evangelos M. Apostolidis, 2012) (Theodoros Georgiadis, Ioannis Zioias, Lydia Ignatiadou, Georgios Papatheodorou, and others, 2004).

-Waste and floating solids. It is estimated that about 6 tons of rubbish end up in sea every year. Plastic waste and bags are the most dangerous because outside. Due to their long lifespan, many mammals and turtles die each year, either by swallowing them, or trapping them. In the stomach of dead mammals, turtles and fish plastic waste or small piles of nylon bags have been found.

-Underwater mining and exploitation of the seabed. Both cases are clear risk to the marine ecosystem. They cause leaks as for example in submarines oil pipelines, from mining facilities, accidents such as explosions, ship collisions on platforms, dismantling and disposal of useless structures and platforms in oceans.

-Coastal zones. Urbanization around coastal zones, but also development industrial units cause great pressure in these sensitive areas. Other activities such as tourism development, agricultural holding, pipelines, etc. burden coastal ecosystems with large amounts of pollutants in the marine ecosystem.

-Industrial waste is the waste produced during the processing and use of substances which do not concern the service of staff, but procedures such as for example chemicals paper processing. They are distinguished in biological, paper processing waste, food and textiles and non-biological wastes rich in acids, bases, chlorine, cyanide, metals, salts, hydrocarbons, and phosphates. Most industrial waste is toxic and are among the hazardous wastes such as hospital waste.

-Inorganic wastes, whether suspended or dissolved and containing heavy metals.

-Organic wastes and are divided into water soluble (APLs) and non-water soluble (NAPLs). In water-soluble, they are those that have water as a solvent and are pharmaceutical products industry, pesticides, solvents, and paints, while non-water-soluble wastes such as lubricants, petroleum products, oil paints, etc. Due to the lowest specialist weight in relation to water, the oils float on the surface and diffuse horizontally, while heavier DNAPLs diffuse vertically into the water body and thus pollute deep underground aquifers.

-Waste in the form of viscous liquids, sludge, and solids, such as refinery waste and cleaning of oil tankers.

-Mining waste, resulting from the extraction of mineral resources in coal mines and mines. During the extraction, its protective mantle is revealed soil and thus pollutants reach aquifers more easily. Note that there are mining activities that reach depths below its surface groundwater. This waste, when not hazardous, can be used as building materials.

-Waste from agricultural-livestock activity, is that which is carried away and reaches to groundwater, after filtration of water from permeable soils. During filtration, nitrates ions easily reach from the unsaturated zone to the underground aquifer. Many of the waters used in irrigation, return to the groundwater aquifer, and are enriched in Ca 2+ ions, Mg 2+, Na 2+, NO3 -, SO4 2-, Cl -, HCO3 -. Pesticides, such as herbicides, insecticides, pesticides are known to be a very important source groundwater and surface water pollutants. Problems such as eutrophication which can lead ports to necrosis, but also degrade coastal areas and points due to the presence of nitrogenous and phosphate compounds, products mainly of agricultural activity. Another source of pollution - Nitrogenous compounds and pathogens. Microorganisms are the drainage of cemetery waters. Of course, it is worth noting and the contribution to water pollution, the throwing of salt into roads to melt ice, because during runoff, these waters with an increased load of Na and Cl end up in surface waters and underground aquifers. (R.A. Duce, P.S. Liss, J.T. Merrill, E.L. Atlas, P. Buat-Menard, B.B. Hicks, J.M. Millertl, J.M. Prospero, R. Arimoto, 1 T.M. Church, W. Ellis, 1 J.N. Galloway, L. Hansen, T.D. Jickells, A.H. Knap, K.H. Reinhardt, B. Schneider, l! A. Soudine, J.J. Tokos, S. Tsunogai, R. Wollast, TM and M. Zhou, Global Biochemical Cycles, 1991).

-Thermal pollution, refers to the water that returns after its use for cooling of nuclear power plants, oil refineries, etc. With the increase in temperature most plant and animal organisms suffer from apoptosis. Temperature changes and has as a result the change of physiognomy with the predominance of thermophilic species. Of course, this problem becomes more serious if we consider that. As the temperature rises, the solubility of oxygen decreases, but it also causes thermal water layering.

Solid waste that reaches the sea, when in large quantities, at the bottom can cover up and eventually kill the organisms that live there causing extra pollution problem with the killing of these organisms. Also processing residues minerals can change the granulometric composition of bottom sediments.

The main pollutants of the seas are petroleum hydrocarbons, suspended particles, heavy metals and chemicals, nutrients, heat, organic waste, salts, and radioactive materials. Pollutants, particularly chemists, can be distinguished from their toxicity. Toxicity of a substance is called its concentration in ppm for which 50% of the organisms exposed to it are killed over a specified period.

PETROLEUM

ACCIDENTS AND MEASUREMENTS

Accidents that have come from oil tankers, oil production platforms or oil pipelines have caused many and sometimes very large oil spills. These leaks are the most obvious, visible, and dramatic causes of acute oil pollution of the marine environment. However, the largest oil spill ever created was caused by the Iraqis. They deliberately released about 240 million gallons (about 800,000 tons) of crude oil in the Persian Gulf during the Gulf War in 1991 in English. According to NRC estimates, oil tanker accidents and oil production platforms account for 10% of the annual total amount of oil entering the marine environment. As reported by INTRERTANKO tankers, they carry almost 40% of the sea trade in the world. In 2001, 57% of the oil consumed in the world was transferred from the sea. The Oil Spill Intelligence Report in their annual statistical report in 1999 states that this year, 32 million gallons had been leaked to water and soil, to 257 pollution incidents. Of these events only 11 were the leaks from tankers, representing about 6.6 million gallons or about one fifth of the total volume leaked. The largest volume of oil leaked was from accidents in oil pipelines or installations. In the 1990s according to ITPOF statistics, 358 oil spills of 7 tons or more were counted, corresponding to 1,133,000 tons of lost oil. 73% of this amount was injected into just 10 cases. In the 1990s, 179 oil spills were counted. In January 2014, a small tanker sank south of China, which was loaded with about 3,000 tons of bitumen. There were four medium-sized oil spills from this incident. The total amount of oil spillage in the environment recorded in 2014 was about 4,000 tons. The vast majority (>700 tons) can be attributed to the accident in January in the South China Sea. This amount is still much lower than the average over the previous decades and is in line with the trend over the last four years. As far as the Mediterranean is concerned, UNEP figures estimate that some 700,000 tons of petroleum products are discarded annually, of which a 10% / 20% share results in the Greek seas (mainly in the Aegean), because of the key position of our country. In the Aegean area an annual quantity of crude oil is increased by more than 65 million tons (mainly from the Black Sea), which weighs 0.01% / 0.06% on marine ecosystems. (V. Meligkounaki, 2015).So, the seriousness and the extent of the adverse effects from the oil spills that occur in the marine environment varies depending on the amount of oil its composition, the presence of different micro-mechanisms capable of metabolizing hydrocarbons, location and weather conditions prevailing at the time of the accident. (Fingas M (2012).

Picture 7: Petroleum Spillage

Frequent oil spills into the environment have increased society's sensitivity to environmental issues and have led to the search for ways to deal with the oil spills that are being created. According to insurance company statistics, 80% of oil tanker accidents that cause oil spills at sea are the result of human error (HCMR 2012):

- poor maneuvering,

- neglect of maintenance,

- insufficient control of systems,

- lack of communication between crew members,

- fatigue,

- insufficient response to a minor incident resulting in escalation to a major accident.

Statistically, more than 700 accidents since 1974 are due to:

• 34% in ship grounding on shore

• 28% in collision between ships

• 13% Structural damage to oil tankers

• 9% leakage during the loading and unloading process

• 9% in explosion or fire on the ship

• 7% in other causes and causes that have not become known

Collisions are generally due to incorrect maneuvers, in particular poor visibility and / or a busy maritime traffic area.

Grounding is also often due to incorrect maneuvers, which are often exacerbated by strong winds, currents and provocative bad weather. The Sea Empress stranding at the entrance to Milford Haven, Wales (UK), is one such example. But equipment failures are a more common cause of grounding than collisions. The sinking of the Braer tanker in the Shetland Islands, Scotland (Great Britain), caused by incoming seawater in a fuel tank, causing engine failure, is a classic example of technical failure (HCMR 2012).

The most recent incident, which negatively affected the quality of the marine environment in Greece and reached the coast of Athens, was the oil spill from the sinking of the tanker Agia Zoni II on September 10, 2017. After a possible influx of water, Agia Zoni II boat , which was anchored in the sea area southwest of Atalanta, west of the port of Piraeus, and had sailed the previous day from the Aspropyrgos refineries with 2200 tons of fuel oil and 370 tons of marine oil, sank releasing a large amount of it into the sea. The oil spill that formed was not limited to the area of the accident but spread to the area of Lagonisi. All seawater samples were analyzed for TPH, which was considered to be the sum of n-alkanes ranging from n-C10 to n-C40. The method used was suitable for the determination of hydrocarbons in drinking water and seawater, using a gas chromatography ionization ion detector (GC / FID). A SHIMADZU GC-2010 PLUS model with PTV importer, AOC 20i automatic sampler

model was used for GC analysis. The hydrocarbons were separated from the aqueous phase using liquid-liquid extraction with n-hexane (ULTRA residue from JT Baker) extraction solvent. The organic layer was removed using a microparticle and passed through a forisil column as an additional purification step to remove polar compounds prior to GC separation using a DB-1 MS column, 15 m, id 0.53 mm, df 0.15 μm (US Silica Company). Significant fluctuations in hydrocarbon concentrations were found approximately one year after the leak (October 23 and 24, 2018). This can be attributed to the fact that the Saronic Gulf is subject to various sources of pollution such as the industrial zone of the Gulf of Elefsina, the port of Piraeus and the intense maritime traffic that affect the marine environment. While significant fluctuations in hydrocarbon concentrations one year after the sinking of the Agia Zoni II oil tanker can be attributed to other sources of pollution, maritime transport and high anthropogenic background of the study area, mechanical shoreline clearance and natural reservoir Environmental legislation, combined with the type of fuel spilled, the Mediterranean climate, natural depletion and natural weather conditions, can reduce the impact of oil spills on the aquatic environment (HCMR 2012).

Another study was done in the port of Agios Nikolas. Concentration of hydrocarbons was recorded in the sediments of Agios Nikolas, sampled in 2012. In all examined sediment samples the concentrations of aliphatic hydrocarbons were particularly large (> ~ 3000 μg g-1). These concentrations are similar to those measured in polluted coastal areas and show a high burden of petroleum products. The total concentrations of Polycyclic Aromatic Hydrocarbons PAH are particularly high in all collected sediment samples (6885-10559 ng g -1) and so they are characterized as extremely polluted. The origin of PAHs is pyrolytic (from incomplete combustion of oil, biomass and carbon) and this is confirmed by the cross correlation of "standard" ratios of PAH concentrations such as (HCMR 2012) :

• fluorescent / / fluorescent + pyrene]

• anthracene / [phenanthrene + anthracene]

• indene (1,2,3-cd) pyrene / [indene (1,2,3-cd) pyrene + benzo (ghi) perylene]

• benzo (a) anthracene / [benzo (a) anthracene + gold]

In high concentrations (520-781 ng g-1) benzene (a) pyrene (BaP), which is considered to be the most dangerous by PAHs with proven carcinogenic properties. These concentrations can cause irreversible damage to benthic marine organisms. For comparison, the usual concentrations of benzo (a) pyrene (BaP) in surface sediments from the Greek seas range from 0-5 ng g -1 in open sea and from 3-50 ng g -1 in coastal areas, while in extreme polluted systems such as the Gulf of Eleusis have been priced up and 270 ng g-1. The organochlorinated compounds measured in the sediments of the Saint Nikolaou include polychlorinated biphenyls (PCBs, 13 congeners), p, p'-DDT and the major metabolites of p, p'-DDD and p, p'-DDE, the hexachlorobenzene (HCB) and trans-nonachlor. The results of the measurements are given in Table 10. The compounds hexachlorobenzene and trans-nonachlor were detected in all sediment samples in very small quantities which do not pose a problem. Concentrations of DDTs (total DDT and its major metabolites DDD and DDE) ranged from 16-29 ng g-1.

Similar prices in the Saronic Gulf have been measured in the wider area of Psyttalia. In the Gulf of Elefsina values up to 10 ng g-1 have been measured, while in other parts of the Saronic Gulf concentrations less than 3 ng g-1 have been measured. The contents of polychlorinated biphenyls (PCBs, total of 13 congeners measured) ranged between 20 ng g-1 and 42 ng g-1. These prices are considered large and are similar to those measured in contaminated marine sediments such as those of the Gulf of Eleusis and Thessaloniki and of the area around Psyttalia. In the rest of the Saronic Gulf they have been counted PCBs concentrations less than 5 ng g-1. The results of the assessment of the degree of carcinogenic toxicity of sediments of Agios Nikolaos it is obvious that the TEQ toxicity values at all sampling sites are extremely high far exceeding the corresponding natural background. The content of the analyzed samples in dangerous urban heavy metals such as arsenic, cadmium, chromium, copper, mercury, nickel, lead and zinc is high. In fact, the measured concentrations of en due to metals are capable of causing irreversible damage to the benthic biocommunities. In almost all cases the concentrations of heavy metals in the surface sediments of Prolimenas are larger than their counterparts' sediments of Agios Nikolaos (HCMR 2012) .

The contents of the analyzed samples are both aliphatic and Polycyclic aromatic hydrocarbons (PAHs) indicate a high degree of pollution from petroleum and pyrolysis products. The measured high concentrations of dimethyl naphthalene and carcinogenic benzo (a) pyrene as well as the extremely high values of the carcinogenic index toxicity (TEQ) at all sampling sites (especially those Prolimena) characterize the examined sedimentological material as biologically sufficient dangerous. The concentrations of organochlorinated compounds in the sediments of sub examination of areas of Agios Nikolaos and Prolimenas are increased compared with their counterpart sediments in unpolluted areas Saronic Gulf. The test for the release of hydrocarbons from the sediments of Agios Nikolaos showed that in a possible (i) excavation of the bottom in front of the existing Agios Nikolaos cruise ship service wall or (ii) Discharge of dredges to another sea area will be limited release of aliphatic hydrocarbons but not pyrolytic PAHs in water column. However, in the latter case, the high contents of PAH in the sediment can cause adverse effects on benthic organizations. Respectively, the heavy metal release test showed that in aqueous column there will be release and enrichment of metals such as Pb, Fe, Ni and Zn. The Microtox® SPT bioassay showed that the surface sedimentary material in the area of Agios Nikolaos is toxic. In case this is discharged to other marine area, the likelihood of toxic effects on marine organisms are in the range of 25-30%. It follows from the above that dredging works in the study area can be carried out without taking precautionary measures as long as they do not are expected to impose an additional burden on the already degraded ecosystem of the Port of Piraeus. The release of organic pollutants (mainly aliphatic hydrocarbons) during dredging will be limited, while the most harmful organic substances, such as polyunsaturated aromatic hydrocarbons, do not have the ability to escape into the water column. (HCMR 2012).

CATEGORIES OF HYDROCARBONS

Hydrocarbons are a large group of compounds that, as their name states, consist only of atoms of carbon and hydrogen. Their large number and the need for a meaningful study of the properties and methods of manufacturing forced the chemists to classify them in groups either based on the form of the carbonate chain or the way the carbon atoms connect with each other. Hydrocarbons can be subdivided into the following types of groups:

(A)acyclic alkanes (paraffins)

(B)Circular alkanes (naphthenes)

(C)Alkanes (olefins)

(D)Alkanes

(E)Aromatic

Due to its complex composition of primarily aliphatic and aromatic hydrocarbons, the measurement of petroleum from environmental samples is reported as total petroleum hydrocarbons (TPHs); thus, covering a wider range of hydrocarbons.

AROMATIC HYDROCARBONS

Aromatic hydrocarbons are a category hydrocarbon that differ significantly from other aliphatic hydrocarbons (alkanes, alkenes, alkynes) in both structure and chemical properties. They have a special structure and present special chemical properties. The alkanes, alkenes and alkynes having carbon chains are referred to as aliphatic hydrocarbons, while aromatic hydrocarbons have a circular structure. The term Aromatic is not related to the smell of these compounds (few have a pleasant perfume) but characterizes a set of characteristic properties that differentiate them from aliphatic compounds. Also, aromatic hydrocarbons can be Monocyclic (Monocyclic Aromatic Hydrocarbons, MAH) or Polycyclic (Poly-Aromatic Hydrocarbons, PAH). (J. S. Warner,1976)

The simplest aromatic hydrocarbon is benzene (C6H6). Benzene is a circular molecule consisting of six carbon atoms forming a six-membered ring. The carbon atoms are interconnected by three single and three double bonds. THE actual structure of benzene, as well as of all aromatic compounds, is described by coordination structures. All carbon in the ring is equivalent. But when in benzene is a substituent, the ring carbons are no longer equivalent. Benzene, although it has double bonds C = C, is not an alkene. That is, it does not give double bond addition reactions such as alkenes. On the contrary, it gives reactions substitution by which a hydrogen atom of benzene is substituted by surrogate. (J. S. Warner,1976)

POLYCYCLIC AROMATIC HYDROCARBONS (PAHS)

Polycyclic aromatic hydrocarbons (PAH or PNA for short) are organic chemicals containing carbon and hydrogen. They are compounds with two or more aromatic rings, joined together so that some carbon atoms belong to two or more rings. Such a structure also characterized as a system of welded rings. The rings can be arranged in a straight line or form angles or form a cluster. The most characteristic compound in this category is benzo (a) pyrene (C20H12), which has been studied and analyzed more than any other and often has demonstrated as a carcinogenic compound. PAHs are divided into low molecular weight compounds (naphthalene, fluorine, phenanthrene and anthracene) containing 2-3 aromatic rings and in high molecular weight (gold, coronene) with 4-7 aromatic rings. They are associations insoluble in water, high boiling point and vapor pressure. In addition, as far as the PAHs are of particular importance, despite their low concentrations, because are considered dangerous toxic components. PAHs are compounds of man-made and natural origin. Along with many derivatives come mainly from the incomplete combustion of organic matter (eg combustion of minerals fuel) or by heating many organic compounds to high temperatures. This results in them appearing in high concentrations in the residues oil distillation, in coal tar and in coke production furnaces. In addition, PAHs are also found in the aluminum industry on Friday graphic anode electrodes. In addition, PAHs are blamed as the main cause of the development of various types cancer and other forms of genetic mutations in humans. The diet is one extremely important source of infection, while for non-smokers it is also the main source exposure. Food can be contaminated from all the aforementioned sources emissions such as industrial pollution (gaseous pollutants, etc.), various food treatments (eg smoked foods or poor cooking practices at home) but also from natural causes (such as forest fires or volcanic eruptions). (J. S. Warner,1976).

METHODS FOR DETERMINING PAHS IN OIL

The determination of PAHs in oil is carried out with the help of three, mainly, methods. Initially, FT-IR infrared spectroscopy can be applied with Fourier transform. It is a spectroscopy technique, which is based in the reflective scattering of monochromatic radiation by matter molecules. It concerns, that is, the exchange of energy between a molecule and a photon. It is a method, which relies on the detection of functional groups such as nitriles, olefin conjugates and aromatic compounds. In general, FT-IR infrared spectroscopy gives us information about the skeleton of a molecule (eg C-C, C = C, etc.). In addition, either gases can be applied to determine PAHs in oil or liquid chromatography with suitable detectors. In the liquid method chromatography separation can be done either by normal or by reverse phase. Their determination is mainly done by fluorescence detector (HPLC-UVD). On the other hand, it is used in gas chromatography flame ionization detector (GC-FID) or coupled to ionization mass spectrometer from electron collision (GC-EI-MS)

INFRARED SPECTRUM REGIONS IN CHARACTERISTIC GROUPS HYDROCARBONS.

In terms of petroleum products, the most common groups of hydrocarbons are are found are olefins, naphthenes, aromatics, paraffins, isoparaffins etc. The absorption shown by the above organic groups in different regions of the infrared spectrum are a function of species and number of the bonds contained in the molecules of their compounds. Listed below detailed table with the frequencies and intensities of some groups of organic hydrocarbons.

Δεσμός	Είδος ένωσης	Εύρος συχνοτήτων	Ένταση
C-H	*Αλκάνια*	2850-2970	Μέτρια
		1340-1470	Μέτρια
C-H	Αλκένια	3010-3095	Μέτρια
		675 995	Μέτρια
C-H	Αλκίνια	3300	Ισχυρή
C-H	Αρωματικοί δακτύλιοι	3010-3100	Ισχυρή
		690-900	Ισχυρή
O-H	Μονομερείς αλκοόλες, φαινόλες	3590-3650	Μέτρια
	Αλκοόλες, φαινόλες με δεσμούς υδρογόνου	3200-3600	Ισχυρή
	Μονομερή καρβοξυλικά οξέα	3500-3650	Μέτρια
	Καρβοξυλικά οξέα με δεσμούς υδρογόνου	2500-2700	Ισχυρή
N-H	Αμίνες, αμίδια	3300-3500	Ισχυρή
C=C	*Αλκένια*	1610-1680	Μέτρια
C=C	Αρωματικοί δακτύλιοι	1500-1600	Ασθενής
C≡C	Αλκίνια	2100-2260	Μέτρια
C=N	Νιτρίλια	2210-2280	Ισχυρή
C-O	Αλκοόλες, αιθέρες, καρβοξυλικά οξέα, εστέρες	1050-1300	Ισχυρή
C=O	Αλδεϋδες, κετόνες, καρβοξυλικά οξέα, εστέρες	1690-1760	Ισχυρή
NO_2	Νιτροενώσεις	1500-1570	Ισχυρή
		1300-1370	Ισχυρή

THE OIL HYDROCARBONS

The Petroleum hydrocarbons are composed of many chemical compounds, which are found in crude oil but also in other fossil fuels, such as natural gas, coal, and peat. Hydrocarbons present in oil are predominantly alkanes or paraffins, cycloalkanes or naphthalene or cycloparaffins or alkyclic hydrocarbons (Naphthenes, Cycloparaffins) and aromatic compounds, and alkenes or olefins and rarest alcinia are rarely found. The alkanes are directly or branched hydrocarbons (Braced or Chained) which are practically insoluble in water because of the simple nature of their molecules and the inability to create hydrogen connections. They have a general molecular formula CnH2n + 2. Cyclic alkanes (naphthenes) contain one (single-naphthene) or more (very naphthene) saturated rings. They may have one or more (branched) paraffins joined to the ring. Naphthenic rings of the oil components include either six or five carbon atoms. Their general type is: CnH2n for mononaphthene, CnH2n-2 for di-naphthenes, etc.

Alkenes may be branched, straight or circular. Crude oil and products derived from it by distillation generally do not contain olefins. Products obtained by processes such as thermal or catalytic cracked hydrocarbons may contain large quantities of olefins. Their general type is: CnH2n for mono-olefins, CnH2n-2 for di-olefins or naphthenic, etc. Alkanes with a general type of CnH2n-2 usually do not exist in petroleum hydrocarbons. Aromatic hydrocarbons shall be those containing at least one benzene ring. They may have one or more paraffins or naphthene joined to the ring. Molecules containing a benzol ring are called single-aromatic hydrocarbons, these with two aromatic ring rings and so on. Their general type is: CnH2n-6 for monoaromatic without alkyl substitution, etc.

Total petroleum hydrocarbons - TPH

Total petroleum hydrocarbons (TPH) are used for the description a large family of several hundred chemical compounds, which initially come from crude oil. TPH, is a sample of chemicals, cm all of which are mainly composed of carbon and hydrogen atoms, ie hydrocarbons. Scientists have divided TPHs into groups that act similarly on land or water, calling them petroleum hydrocarbon classes. Each class contains many separate chemical compounds. Some compounds that classified in TPH are hexane, aircraft fuels, mineral oils, gasoline, toluene, xylene, naphthalene, and fluorine as well as other petroleum products and fuel components. However, samples from TPH may be present only some or a sample of these chemicals. The term TPH refers to the amount of hydrocarbons that can be measured in an environmental sample with a specific method of analysis and through it the percentage of pollution from the mixture of each petroleum products. Therefore, the concentration of TPH in a soil cannot be used immediately for risk assessment of human health. The same TPH concentration may represent a completely different composition oil and therefore different health risks and environment (Irwin R.J., 1997). Necessary, therefore, is considered to be done correctly correlation between TPH concentration and the risk arising from their presence, which depends on the soil in question, the time and the specific oil that caused the pollution.

HOW OIL SPILL AFFECTS THE ECOSYSTEM

Regarding the effects of oil spills on the local ecosystems, it should be stressed that the creation of oil spills on the surface of the sea, inter alia, significantly impedes the exchange of gases between the atmosphere and the surface of the sea. This reduces the amount of dissolved oxygen in water, causing effects on living organisms. This decrease with the concomitant increase in sea temperature helps to further develop micro-organisms, which consume the remaining oxygen, significantly affecting the balance of the local ecosystem. At the same time, the presence of petroleum inhibits the penetration of the rays of the sun at sea, resulting in a reduction in the photosynthetic capacity of the aquatic plants, leading to the reduction of the existing chlorophyll and the inhibition of the photosynthesis of the underwater plants. After several days, a long process of biodegradation of oil is started, depending on temperature (25 ° C the most

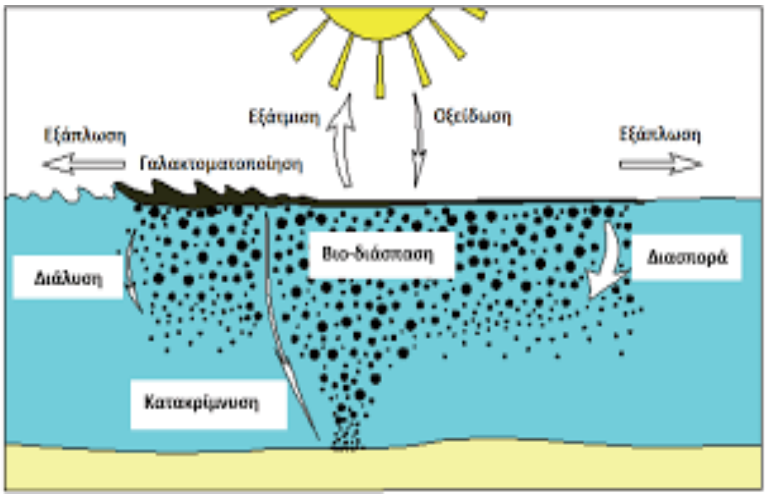
favorable price), the availability of nutrients and oxygen (for bacterial growth) and the type of oil. Finally, many oil droplets end up in marine sediments resulting in the disturbance of benthic bio-societies as well as the health of fish. For the above reasons, except in cases of special weather conditions, immediate and effective measures are necessary to protect marine ecosystems from the negative effects of oil spillage.

TOXICITY AND MICROBIAL BREAKDOWN

The level of toxicity varies among the different groups of compounds that the petroleum hydrocarbons consist of. Aromatic hydrocarbons appear to be more toxic and resilient to microbial degradation when compared to alkanes, isoalkanes, cycloalkanes, and the unsaturated aliphatic hydrocarbons. Toxicity of water pollutants is mostly influenced from their degree of solubility. Moreover, microbial degradation of hydrocarbons is depended on many factors such as level of dissolve oxygen, Ph, and microbial populations and the environment. The rate of susceptibility to microbial degradation differs from one class to another and bacteria can degrade aliphatic, alicyclic, and aromatic hydrocarbons of low to moderate weights at a higher rate when the environmental conditions are very ideal. The typical decreasing order of biodegradation is given as follows: n-alkanes >branched alkanes > low-molecular-weight aromatics > cyclic alkanes > high-molecular-weights aromatics. Aromatics hydrocarbons are generally less biodegradable than the corresponding aliphatic hydrocarbons. (A.O. Adeniji, O.O. Okoh, and A.I. Okoh, 2017).

MINERAL OIL BEHAVIOR

As oil flows into the sea, a series of processes are taking place, causing physical and chemical changes in oil and which directly affect its toxicity. Collectively, these changes are reported as changing (weathering). The main alloying processes are the spread, evaporation, natural dispersion, emulsifying, dissolving, photochemical oxidation, precipitation, biodegradation Spread: One of the most important processes in the early stages of leakage. First the oil spreads like a solid (related) spot, and after a few hours it dissolves and forms narrow strips parallel to the wind direction. The spread velocity depends on the thickness of the spot, the properties of oil, the state of the sea, etc. The transition from one phase to another becomes slower for a large volume of oil. It has been observed that about 12 hours after the oil spills have been created, oil can be spread over more than 5 square miles [5 sq km].



Picture 8: Procedure for the rescue of oil in a water environment.

•Evaporation: Is the process of transferring part of the oil mass from the stain to the atmosphere. The speed and degree of evaporation depends on the volatility of the oil. The evaporation rate increases with the spread, the wind, the wave of the sea, etc. Within about 24 hours most of the crude oil species have 25-30% of their lighter components. Evaporation causes an increase in density (and viscosity) which lasts a week. The emulsifying and coagulation process, which reduces the area of the spot and the evaporation rate, is followed.

•Natural dispersion: Waves and swirling on the surface of the sea create droplets of different sizes. The large drops are going back to the surface, where they are agglomerated with others and re-create a spot or spread by creating thin 'film'. The small drops are suspended with water and biodegradation and precipitation begin. The rate of physical dispersal and evaporation rate determine the lifetime of a spot. The oil emulsions are not stable, and the drops are agglomerated and returned to the surface forming again a stain. (GREENPEACE)

•Emulsifying: Many crude oil types tend to absorb water by forming oil-water emulsions. This increases the volume of pollutants by 3-4 times. The percentage of water in the emulsion can reach 75-`80%. As this percentage increases the color varies between black, orange and red. The speed of emulsifying depends on the state of the sea. (Easy at turbulent sea and difficult to calm). The process of water absorption is completed in a few hours. The whole process makes it difficult for the exhaust and the whole process of cleaning. When emulsions are particularly stable, they have dark brown color, contain 80% water, and usually called "Chocolate mouss" Dissolution: The dissolution of the oil in the water is small and only concerns light components. The heavy ingredients are practically insoluble, whereas aromatic hydrocarbons (benzene, toluene) are poorly soluble. (GREENPEACE)

•Photochemical oxidation: It is the effect of oxygen and solar radiation on the spot and depends on the thickness of 'film'. Under the influence of intense sunlight, minutes of film are split at 0,1% per day. Photochemical oxidation of thick layers can lead to the formation of high molecular weight compounds (e.g. tar slices) with long life times. (GREENPEACE)

•Sedimentation: It occurs in some heavy oil derivatives, which are submerged in the water. It is also caused by adhering particles of sand to oil. Temperature changes can cause transient immersion (and then re-emergence) of oil. Biodegradation: Seawater contains organisms that can use oil as a source of coal and energy. When conditions for organisms are not favorable, biodegradation can take decades. Biodegradation velocity depends on temperature, oxygen, and nutrients. It can only be done on the water-oil contact surface. The processes of spreading emulsifying and dissolving are important in the early life of the oil spill, while oxidation, precipitation and biodegradation are long processes. Regardless of all of this, the stain is still on the surface throughout its life. This movement can be predicted with some safety from the effects of the winds and surface currents present in the area. What is also known is that - according to international experience - the percentage of oil that can be recovered in an oil spill rarely exceeds 10-12% of the quantity spilled. This percentage even requires persistent, painful, and costly efforts. (GREENPEACE).

The processes of spreading, evaporating, dispersing, emulsifying, and dissolving are significant in the early stages and oxidation, precipitation and biodegradation are significant in later stages and essentially determine the fate of the polluters. What would you do? If it is to be emulsified or retracted, it shall be determined by one several factors such as the quantity and rate of leakage, the conditions prevailing in an environment such as temperature and gestation and of course from its physicochemical properties. (Itopf., (2011a).)

THE IMPACT OF MINERAL OILS ON WATER

The impact of oil spills on surface water is many and indeed not all recorded and fully understood. Regarding the effects of oil spills on the local ecosystems, it should be stressed that the creation of oil spills on the surface of the sea, inter alia, significantly impedes the exchange of gases between the atmosphere and the surface of the sea. This reduces the amount of dissolved oxygen in the water, causing suffocation to living organisms. This decrease with the concomitant increase in sea temperature helps to further develop micro-organisms, which consume the remaining oxygen, significantly affecting the balance of the local ecosystem. At the same time, the presence of oil inhibits the penetration of the rays of the sun at sea, resulting in a reduction in the photosynthetic capacity of the aquatic plants, leading to the reduction of the existing chlorophyll and the inhibition of the photosynthesis of marine plants. After some days, a long process of biodegradation of oil is started, depending on temperature (25oC the most favorable price), availability of nutrients and oxygen (for bacterial growth) and the type of oil. Finally, many oil droplets end up in marine sediments resulting in the disturbance of benthic bio-societies as well as the health of fish. For the above reasons, except in cases of special weather conditions, immediate and effective measures are necessary to protect marine ecosystems from the negative effects of oil spillage.

POLLUTION OF GROUNDWATER BY MINERAL OILS

Hydrocarbons shall be one of the most common pollutants recorded on spills and leaks or from the breaking of their underground transport and storage facilities. Because the solubility of these substances in the water is very small, when a large quantity of oil or gasoline moves deep down to the ground level, the wealth created will move floating in the underground water as shown in the picture. Hydrocarbons in the groundwater remain for decades with an unpleasant odor in water pumped from polluted water bodies. Most cases of accidental pollution incidents could be avoided by proper management of appropriate dams to avoid spills and rapid cleaning. Move the mineral oil to the groundwater. Petroleum hydrocarbons belong to the non-water liquids (non-aqueous phase liquids, NAPLs), which have a distinct liquid phase in the aquatic environment. The risks of NAPLs in groundwater are due to their stay below ground and the ability to pollute large volumes of water due to their limited removal. The movement of these substances into the soil depends on the quantity released on the ground, the physical properties of the soil and the structure of the soil through which they are moved. The release of liquid waste under the surface of the soil results in moving towards the deeper layers, towards the groundwater level of free water bodies. When the quantity is small, local problems are created and groundwater is affected by the deep filtration of rainwater. The extent of movement in the vertical and horizontal direction depends on the porous, permeability of the medium and the territorial moisture content. In the case of large quantities, there is faster penetration. In dry soils, links between the substances and the solid soil are created, and when there is water due to the hygroscopic strata, the links are not intense. The movement of NAPLs to groundwater is made to the direction of movement of the groundwater with the effect of the procedures:

(A) molecular diffusion and

(B) dispersal

Lighter-than-water substances are and move near the surface of the saturated zone, while the heaviest move vertically into the unsaturated and saturated zone. Because of the high density these substances are immersed in the bottom of the saturated zone and installed over the impenetrable substrate. Water insoluble substances do not mix and remain a particular phase. These substances shall be ventilated when they are under high pressure. Pollutants in the gaseous phase shall be moved with the flow into the unsaturated zone, while in the areas of smaller permeability the transfer is carried out by diffusion.

GC FOR IDENTIFICATION AND DETERMINATION OF OIL POLLUTANT IN THE ENVIRONMENT

For identification and determination of oils and petroleum products pollutants in the environment, the following structural features were used in GC and GC-MS: (B. Beskoski et all.,2012)

• typical shape of chromatograms (fingerprints) of petroleum products.

• peaks of n-alkanes (C6 through C40) in chromatograms.

• measurement of total petroleum hydrocarbons.

• shape of the UCM.

• stable carbon isotope ratio (δ13C) is also included in many cases.

• close-to-unity ratio between n-alkanes with even and odd numbers of carbon atoms.

• presence of some isoalkanes, including pristane and phytane and in some cases farnesane, trimethyl-C13, and norpristane isoprenoids.

• ratio between phytane and pristane and the closest C17H36 and C18H38 n-alkanes.

• presence of biomarkers (isoprenoids, steranes, triterpanes, etc.).

• predominance of methyl- and alkyl-substituted mono-, bi-, and polynuclear aromatic hydrocarbons over unsubstituted aromatic hydrocarbons.

• volatile hydrocarbons including BTEX (benzene, toluene, ethylbenzene, and three xylene isomers) and alkylated benzenes (C3- to C5-benzenes), volatile paraffins and isoparaffins, and naphthenes (mainly cyclopentane and cyclo-hexane compounds)

• distribution (profile) of polycyclic aromatic hydrocarbons (PAHs) and the petroleum specific alkylated (C1–C4) homologues of selected PAHs.

• typical profile of sulfur-containing aromatic hydrocarbons.

• determination of NSO heterocyclic hydrocarbons for oil spill identification using ratio between different hydrocarbon groups (group composition).

• specific ratio between the concentration of PAHs and the background

GAS CHROMATOGRAPHY ANALYSIS AND PETROLEUM

Gas chromatography is in routine use in environmental laboratories today to provide a detailed analysis of many individual organic compounds and compounds found in oils. Quantitation methods for TPHs are available usually through the modification of gas chromatography (GC) techniques based on the recommendations primarily from United States Environmental Protection Agency (USEPA) and International Standards Organization (ISO) (USEPA,1996 &ISO 9377-2:2000, 2000). In addition to providing quantitative measurements, can provide details of the components present at a contaminated site and thus indicate where the pollutions have come from. The characterizations of individual aliphatic and aromatic compounds in petroleum are

mainly based on gas chromatography/flame ionization detection (GC-FID) and gas chromatography/ mass-spectrometry (GC-MS) analysis.

The most used columns for the separation of petroleum hydrocarbons are 30km non-polar inert capillary columns such as DB-5, DB-5MS, HP-5 and HP-MS5, inside filmed with 5% phenyl-methylpolysiloxane. The total petroleum hydrocarbons (TPHs) are usually estimated by integrating the areas of the resolved and unresolved components of both fractions. For GC-based methods TPH is defined by anything extractable by a solvent or purge gas and detectable by GC-FID within a selected carbon range. Cryogenic cooling can also be employed to analyze the low molecular weight compounds. Detection limits for GC-FID TPH can be as low as 50 μg l−1 while individual analytes may be detected at approximately one order of magnitude lower. Oil and oil products can be readily identified from their GC traces during the early stages of an oil spill especially where the spilled oil is heavy and background hydrocarbon levels are low. A simple technique is to produce a chromatogram under standard conditions (column type, flow rate, temperature), which can be compared to the trace of a sample of material suspected to have been discharged. Certain fuels can be identified by characteristic reproducible chromatographic patterns. Hydrocarbon fuels give chromatograms with regularly spaced peaks (consecutive members of homologous series of compounds) while lubricating oils have fewer resolved peaks (M. Fingas, 2016).

GC-MS OPERATING PRINCIPLE

Regarding gas chromatography-mass spectrometry (GC-MS), the two systems are compatible with each other and therefore it is possible to pair them. The conditions prevailing in both systems are identical. More specifically, operate at temperatures of 200-300oC, are suitable for substances in the gas condition and require small sample quantities (μg or ng). The only downside is that the atmospheric pressure should decrease as the sample exits the GC at gap at 10^{-5}-10^{-7} Torr for entering MS. The coupling of the two should aims to reduce stress. In addition, to date the purity of the sample has been a problem in MS. This was because of the minimal amounts of impurities or derivatives of a compound can give many unexplained spectral lines resulting in make it difficult to interpret the spectrum. However, if the sample passes through the column separation of a gas chromatograph, then received on a completely pure condition. In addition, the quantities (10^{-4}-10^{-3}g) coming out after chromatography is in the gas phase is in ideal conditions. Then they go to the ward ionization of MS, provided that the carrier gas (usually He) is removed. The carrier gas is removed by filters (made of fused glass) or by membranes, while the most common method is with the effect of vacuum. In the next step, the carrier gas and the organic compound fraction are transmitted, after being separated from the GC, in a jet separator. With thus the organic compound (in gaseous state), which is heavier than He, passes in its entirety along the high gap (between the two nozzles) in ionization chamber. At the same time, the low molecular weight gas diffuses sideways, under the effect of vacuum. Since GC is directly connected to MS, the mass spectrum of each substance in the mixture is

recorded as it leaves separation column. In this way and with the help of electronic recording the chromatogram achieves accurate separation of the substances in a sample, while at the same time small quantities of impurities are detected and their certification.

HOW GC-MS WORKS?

GC-MS consists of a gas chromatograph and mass spectrometer. Once a sample is introduced into a gas chromatograph instrument, its various components are vaporized. Analyte properties such as volatility and polarity determine their affinity to the liquid stationary phase in the analytical columns. For instance, non-polar stationary liquid phase is better at retaining and, hence, separating non-polar analytes. The physical dimensions of the column also affect how long the analytes are retained in the column and eventually eluted.

Once the analytes are eluted from the analytical column, they are then ionized, fragmented, separated, and captured as a function or spectrum of their mass-to-charge ratios by the mass spectrometer. The analytes' identity is verified by comparing them to libraries of mass spectra of known compounds. The peak areas of the mass-to-charge functions also inform users of the relative quantity of the analytes.

GC-MS APPLICATIONS IN OIL

The major groups of compounds identified by the GC-MS assay are aromatic hydrocarbons and petroleum biomarkers. Its biomarkers such as normal alkanes, as well as other cyclics saturated hydrocarbons (eg rods, steranes, etc.) show a relative stability in environmental conditions. The aromatic ingredients, although a small fraction of the oil, are widely used in GC-MS assays. Thus, quantitative and are often applied qualitative analyzes, mainly for detection, identification and their identification. A necessary condition for the application of the technique is volatility of the chromatographic substance at column temperature. In the case of non-volatile substances, they are converted to volatile derivatives, after reaction with appropriate reagents. First of all, as far as the qualitative identification of the data is concerned: The compounds of a mixture are identified either on the basis of the reduced time t'R or with the reduced V'R holding volume, compared to the corresponding values on a known sample chromatogram observed with the same just experimental conditions. According to the spectra obtained from MS, one item states that exists only when at least one of the following techniques is satisfied:

1. Peaks corresponding to ions of at least two isotopes are observed element at the correct ratio of relative intensity.

2. Peaks are observed due to multiple charged ions in one second or one third of the masses of the major isotope. This condition is particularly strict when the peaks due to such multiple charged ions are present in fractional masses.

Then, as far as the quantitative analysis of the data is concerned: To use a chromatogram as a basis for quantitative analysis will should:

1. The signal recorded must be proportional to its concentration

2. The experimental conditions and especially the flow velocity of the carrier gas to are stable

3. The pen response of the recorder should be adjusted to the time detector response.

The area of a chromatographic peak under certain conditions is proportional of the amount of one component and is used to plot the curve reference. So for small t'R values, the vertices of the components of a chromatogram they are tall and narrow. The height of the peaks can be used instead area, but this reduces the accuracy. (Stenafanopoulos, 2017)

USEFUL FEATURES OF GC-MS FOR THE PETROCHEMICAL FIELD

GC-MS is a very sensitive technique to detect for compounds in petroleum products. Petrochemical companies have exploited this feature of GC-MS to assess the commercial value of oil reserves by characterizing the types of hydrocarbons and their relative concentrations without having to go through expensive drilling operations. This is made possible with modern-day GC-MS instruments that are sensitive enough to detect analytes in the microgram range.
The speed and through put at which GC-MS is able to provide analytical data also helps petrochemical companies maintain quality control throughout production. Close to 100 million barrels of petroleum products are generated daily, and it is important for companies to be able to detect the slightest abnormalities in huge volumes of products quickly and accurately.

Crude oil contains a complex mixture of hydrocarbons with a wide range in boiling points and molecular weights. Petroleum products derived from crude oil are highly purified, and GC-MS is used to detect contaminants, and determine if the refining process was effective. Many compounds found in crude oil may also have similar column retention times or mass-to-charge ratios. When mass-to-charge ratio spectra overlap, column retention times can be used to identify different compounds, and vice versa. The detection limit of GC-MS can be as sensitive as parts per billion, and some GC-MS models are able to provide analysis in as short as a few minutes. This enables petrochemical companies to perform high throughput quality control.

The ability to fine tune or optimize GC-MS for detection and analysis also helps in developing product formulations. For example, oil additives are often added to petroleum products to improve lubrication and prolong the lifetime of the combustion engine, and prevent equipment fouling. Other types of additives are also commonly added to reduce corrosion and fuel line freezing. By adjusting parameters in the gas chromatograph such as the liquid stationary phase and size of the analytical column, different analytes in petroleum products can be separated and eluted, then detected.

This allows companies to develop and improve proprietary recipes or formulations to suit the needs of their clients. As a highly reproducible technique, GC-MS is helpful for regulators establishing emission guidelines. It also helps companies comply with environmental standards. Burning petroleum contributes many pollutants into the atmosphere, including organic molecules and gases. Regulators are interested in quantifying pollutant levels in petrochemical products to safeguard the interests of society. GC-MS is a reliable method for companies to quantify the levels of toxic gases like carbon monoxide and sulfur oxide emitted from their products and comparing them to GC-MS standards set by regulators. Compounds in petroleum products can also affect living organisms with different levels of toxicity. In situations such as oil spills, GC-MS can determine the types and concentration of pollutants, inform intervention strategies for cleanup, and assess the adverse impact on marine life. For instance, the use of GC-MS has provided insights into how marine microbial populations were altered and the types of hydrocarbons that still lingered 15 years after the 1991 oil spill in the Saudi Arabian Gulf coast.

GC-MS is a powerful technique for identifying and analyzing molecules. Because of this, it has been widely adopted by the petrochemical industry throughout the entire production chain–from sourcing oil fields to formulating new products and complying with environmental standards. (Van't Riet K., 1979).

CASE STUDY- The application of GC to Organometallic compounds

The determination of organotins (TBT) in marine sediments and biota via gas chromatography–flame photometric detection and Mass spectrometry

The increasing worldwide use of organotin compounds, in conjunction with their known toxic potential, has given rise to increasing concern about the ecotoxicology of these substances over recent years.

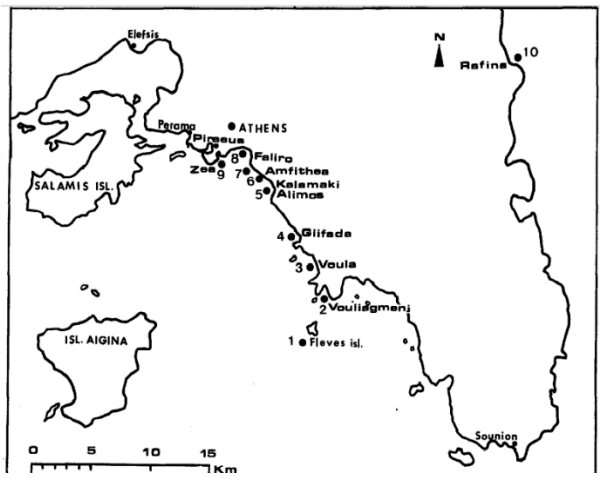
Organotins have been used as insecticides, bactericides and fungicides in the wood, paper and textile industries, and occur in other industrial processes such as disinfecting cooling water in industries and electrical generation power plants, and as heat and light stabilisers for PVC materials (Thompson et al., 1985). In coastal zones, however, the main source of organotin biocides arises from antifouling paints containing TBT, which have been in use for nearly 30 years. Many methods have been developed to estimate the quantities of these moieties in marine samples. However, Gas Chromatography has proved to be the most efficient.

In the 1972 Oslo Convention, organotin compounds were included in the 'Grey' List, after reports indicated increased toxicity towards humans. These substances were promoted to the 'Black' List in 1980 by the European Union and similarly were included

as a group in Annex I of the Protocol for the Protection of the Mediterranean Sea against Pollution from Land-Based Sources (UNEP, 1988) and in the ipriority list' of environmental contaminants prepared under the Toxic Substances Control Act (ToSCA) by the Environ-/ mental Protection Agency of the USA (US EPA / 1982).

The most commonly used trialkyltin biocides, tributyltin (TBT) and triphenyltin (TPhT), have been shown to be toxic also to non-target aquatic biota, especially since the first reports indicating shell chambering effects and high mortality rates of the Pacific oyster Crassostrea gigas. Bryan et al. (1986) provided evidence that the incidence of 'imposex', i.e. the induction of male sex characters in the female dogwhelk Nucella lapillus, rises in response to increasing TBT pollution, correlation well with declining populations in areas with high contamination levels.

In the study presented here, the sampling sites are, as resented below :



Sample locations and basic physical parameters of the sampling strategy.

Sampling stations	Open sea (1)	Vouliagmeni marina (2)	Voula marina (3)	Glyfada marina (4)	Alimos marina (5)	Amfithea marina (6)	Faliro marina (7)	Piraeus harbour (8)	Zea marina (9)	Aquaculture (10)
Berth sites yachts	–	115	500	1000	1500	160	200	60 (ships)	950	–
Maritime traffic*	–	50/d	75/d	260/d	300/d	80/d	40/d	140/d	200/d	–
Sampling depth	25 m	3.5 m	4 m	3 m	4 m	4 m	4.5 m	7 m	5.5 m	15 m
Sampling distance†	1 km	50 m	15 m	100 m	75 m	5 m	50 m	20 m	55 m	300 m
Grain size	Coarse	Fine	Fine	Fine	Fine	Fine	Fine	Fine	Fine	Coarse
Organic matter	Low	High	High	High	High	High	High	High	High	Slight
Water clarity	> 10 m Secci	< 1 m Secci	< 1 m Secci	< 1 m Secci	< 1 m Secci	< 1 m Secci	< 1 m Secci	< 0.5 m Secci	< 0.5 m Secci	~ 8 m Secci

*Distance from coast: distance from original coast line.
†Average of daily calls and departures measured during the summer period.

The western side of the Attica peninsula was selected, due to its high maritime activity and urban development at the coast. The marinas selected total over 4000 berth sites, and are operating at full capacity. The port of Piraeus has the largest passenger traffic in the Mediterranean, with an annual throughput of 12000000 passengers. Recent studies have measured as many as 140 ships entering and departing Piraeus Harbour

within a 24-h period. Within the area of the port, two dry-dock sites exist for ship repairs and maintenance, which handled 195 ships in 1994.

Methodology

Organotin compounds were extracted using the Microdigest model A301 open monomode focused microwave system, equipped with a TX32 programmer (Prolabo, France). The determination of the recovered species was carried out on a Shimadzu GC-14A gas chromatograph equipped with a split-splitless heated injector and a flame photometric detector (FPD) with a 610 nm bandpass filter at standard conditions. Analytical grade chemicals (Merck, Germany) and Milli-Q water were used throughout, unless otherwise stated. Artificial sea water was prepared by dissolving 32 g of NaC1, 14 g of MgSO4"7H20 and 0.2 g of sodium acetate in 1 1 of water. A 2% (w/v) aqueous solution of sodium tetraethylborate (NaBEt4) was prepared daily. The desulphurization reagent tetrabutylammonium sulphite (0.01 M) was prepared by saturating 100 ml of a 0.01M tetrabutylammonium hydrogen sulphate solution with 25g of sodium sulphite. The solution was rinsed three times with a fresh 20 ml portion of hexane to remove the remaining tetrabutylammonium hydrogen sulphate. Organotin standard stock solutions of mono-, di-, and tributyltin (MBT, DBT, TBT), and of mono-, di-, and triphenyltin (MPhT, DPhT, TPhT) were prepared daily by the dilution of stock solutions in methanol. Tripropyltin (TPrT) stock solution was also prepared in methanol. Multi-compound working solutions were prepared and diluted with water as required. Certified materials were obtained from the National Research Council of Canada (PACS-1), the European Union Reference Bureau (BCR-462) and the National Institute of Environmental Studies of Japan (NIES-11). Sediment samples were collected using a Teflon coated grab. Sampling depth in the marinas was on average about 3.5 m, and the distance from the original coastline varied from 5 to 100 m, as shown in Table 1. Most of the sediment recovered was discarded and only 5-10 g of sediment situated at a depth of 3-5 cm.

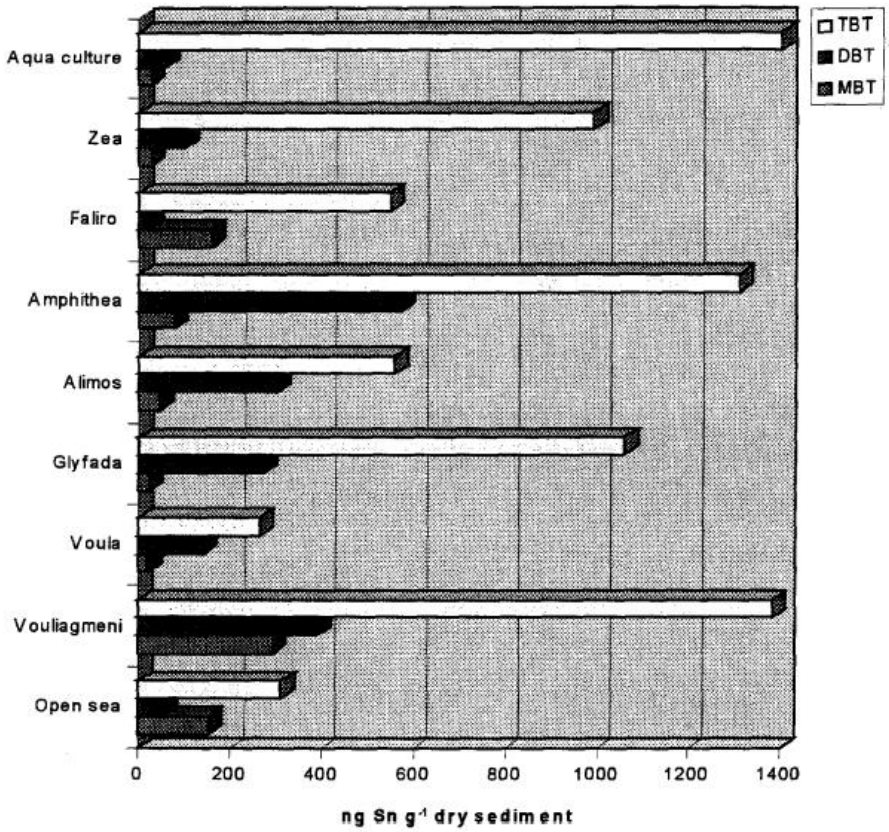
A sample of 2 g of sediment was placed in a borosilicate extraction tube containing 10 ml of 0.5 M acetic acid solution in methanol and 100 B1 of the tripropyltin solution, and exposed to a microwave field (Lalere et al., 1995). The optimum conditions for leaching were 70 W applied power and a 3 min reaction time. The resulting supernatant was transferred to a flask equipped with a narrow neck for the derivatization process. 50 ml of artificial sea water and 10 ml of acetate buffer were added to the supernatant and the pH adjusted to 5. Iso-octane (1 ml) and 100µl tetraethylborate (NaBEt4) were added successively, and stirred vigorously for 30 min. A sufficient quantity of water was then gently added to recover the organic phase inside the narrow neck. The organic phase was subjected to the desulphurization procedure, prior to GC-FPD analysis. Elemental sulphur was removed from the derivatized sediment samples by tetrabutylammonium sulphite. 300 µl of 2-propanol and 300 µl of tetrabutylammonium sulphite were added to 600µl of iso-octane extract and stirred for 3 min in the presence of excess sodium sulphite. The organic phase was rinsed by shaking with 5 ml of water and analysed by GC-FPD under the conditions described below :¨

Operating parameters for GC-FPD analysis.

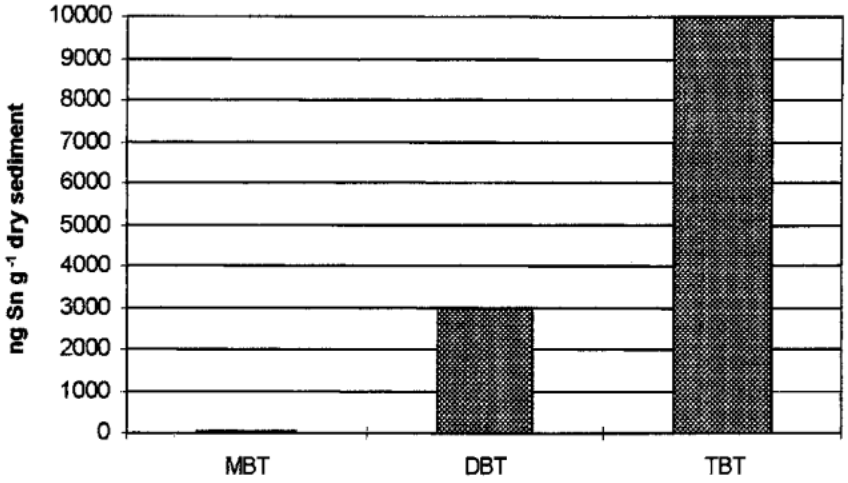
Apparatus	Chromatograph: Shimadzu GC-14A Integrator: Logiciel Borwin 1.0
Column	Chrompak CP-SIL-8CB, 25 m × 0.25 mm i.d., film thickness: 0.41 μm
Gas	N_2: 2.5 kg cm
Injection port	Splitless, 250°C, volume 1–2 μl
Oven program	Initial temperature 60°C, 1 min Ramp rate 25°C min^{-1} to 280°C Final temperature: 280°C, 3 min
Detection	Flame photometer with 610 nm filter Flame: air 0.8 kg cm, H_2 1 kg cm H_2 flow rate 60 ml min^{-1} Cavity temperature 280°C

Biological samples were captured by grabs and nets, kept on ice, and subsequently freeze-dried with analysis within 10 days of capture. Protein and/or lipid bound organotin moieties were solubilised with the use of tetramethylammonium hydroxide (TMAH). In addition, a clean-up procedure was used, using adsorption to alumina. 5 ml TMAH was added to 200 mg of homogenised tissue and exposed to a focused microwave field of 60 W applied power for 3 min. reaction time. The solution was diluted with 15 ml of water, taken to a pH of ca. 5 by the addition of concentrated acetic acid and buffered with 5 ml of buffer solution. 1 ml of the NaBEt4 solution and 1 ml of the extracting solvent were added, and the tube was shaken for 5 min. The emulsion was broken by submitting the extraction tube to the microwave field for 1min at 20 W. Thereafter, the organic phase was recovered, introduced onto a clean-up column filled with alumina and analysed by GC-FPD, under the conditions described above. Certified biological tissue (NIES-11) material was treated similarly to environmental samples. Throughout the analysis, spiking and internal standards were used. Fine sediment fractions have been used as reliable indicators of organotin contamination, since it appears they act as the final sink for organic contaminants and trace metals. Organotin levels in surface sediments from the marinas are presented below, and compared to levels in the open sea and an aquaculture site. Data from Piraeus Harbour are presented in the following figure, due to the much higher measured levels of organotins. In all marinas and the port of Piraeus, TBT was the predominant organotin moiety, levels always being higher than DBT and MBT (TBT > DBT > MBT)

Results



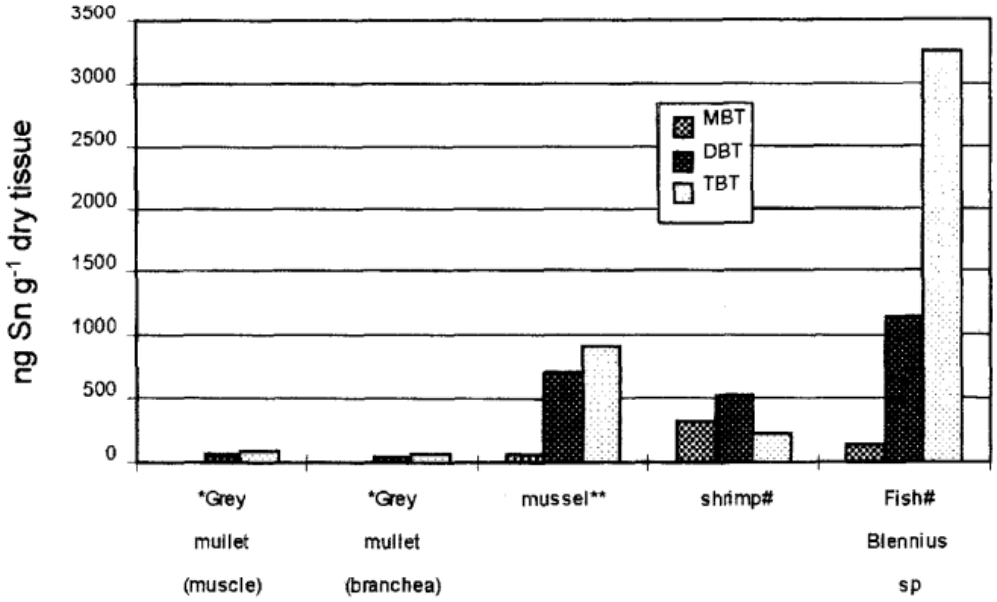
Concentrations of butyltin compounds in sediments originating from Greek coastal areas.



TBT and degradation product levels in surface sediments from Piraeus Harbour.

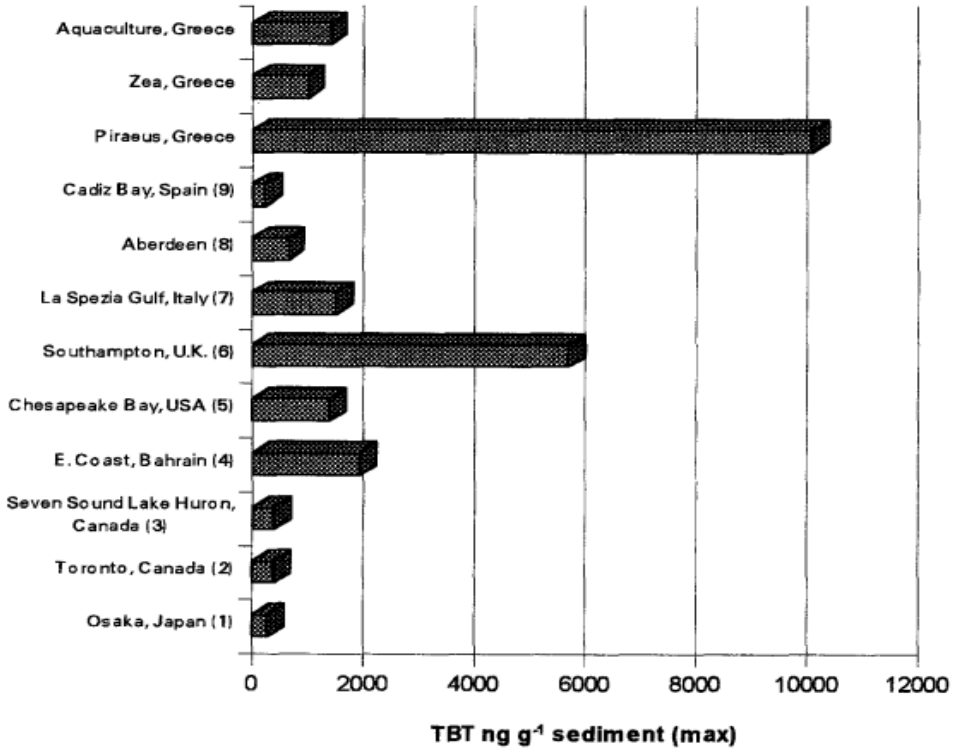
The main types of organotin compounds found in fish and shellfish studied here were the tri- di- and monobutyltin compounds. See below. Degradation products have proven

important to measure, after studies (Kuballa et al., 1995) showed that the genotoxic order of butyltins was different from the general toxic order, since dibutyltin was proven to be more genotoxic than tributyltin.

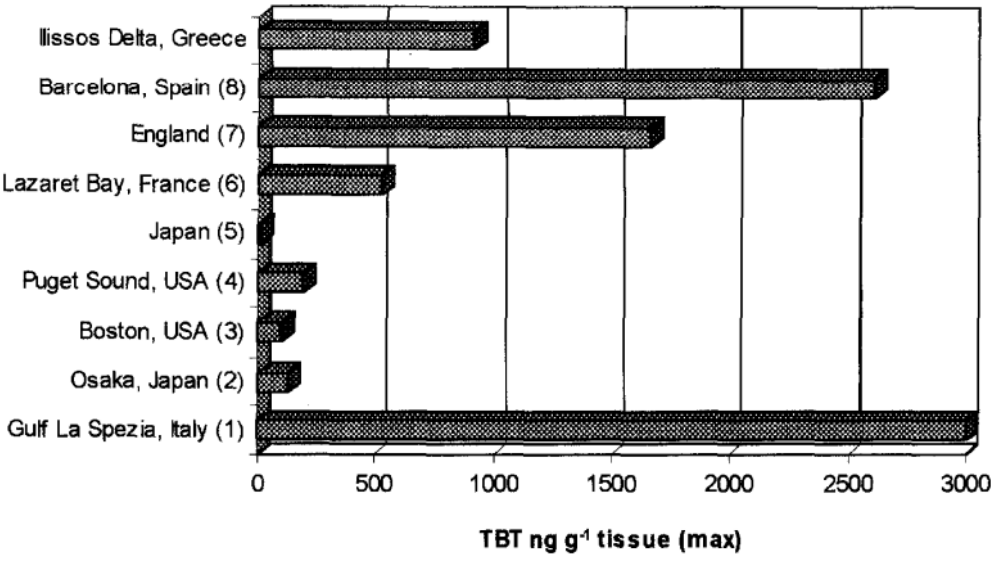


Organotin concentration levels in marine biological tissue. *Grey mullet captured in the open sea (pollution level estimate: low). **Mussel: (*Mytilus edulis*) from the Illissos Delta (pollution level estimate: high). # Shrimp: (*Leander* sp.) captured in Zea marina (pollution level estimate: high). # Fish: (*Blennius* sp.) captured in Zea marina (pollution level estimate: high).

The Figures below represents a comparison of the results from this study to a selection of values reported for sediments and bivalves in other locations. The criteria for selecting the values presented were the comparable sampling and analysis methodology; the similarity of sites; and the citation of studies performed in recent years that take into account many methodological pitfalls of the past. It is clear that the Greek sites studies here are significantly contaminated. It appears that the major source of organotin compounds in the areas studied here involves the use of antifouling paints. Leaching rates of over 50 μg TBT / cm^{-2} / day^{-1} are reported from wetted yacht/ship surfaces treated with such paints, indicating the problems arising from berthing and marine traffic within confined spaces. Additionally, all waste products are a potential source of pollution. Legislation has been introduced in the European Union banning the use of antifoulants containing tin on vessels below 25 m in length. However, as shown here, it is obvious that this is poorly implemented in many parts of the world. In countries where appropriate legislative measures have taken effect, there are reports indicating diminishing contamination levels.



Maximum concentrations of tributyltin (TBT) in sediments originating from several European and world coastal areas.



Maximum concentration levels of tributyltin (TBT) in bivalves originating from several European and world coastal areas.

It is clear that measures introducing effective waste management, the introduction of environmentally friendlier methods of surface preparation, and the reduction of waste toxicity through the introduction of tin-free antifouling paints, are imperative for such heavily polluted areas.

CONCLUSION

Gas chromatography is in routine use in environmental laboratories today to provide a detailed analysis of many individual organic compounds and compounds found in oils. Quantitation methods for TPHs are available usually through the modification of gas chromatography (GC) techniques based on the recommendations primarily from United States Environmental Protection Agency (USEPA) and International Standards Organization (ISO).Petroleum, like all fossil fuels, primarily consists of a complex mixture of molecules called *hydrocarbons* (molecules containing both hydrogen and carbon). When it comes out of the ground, it is known as *crude oil*, and it may have various gases, solids, and trace minerals mixed in with it. Through refinement processes, a variety of consumer products can be made from petroleum. The world's reliance on petroleum is expected to grow, despite widespread environmental, economic, and political consequences. The U.S. oil extraction industry continues to aggressively search for new oil deposits and lobby the federal government to open up restricted areas to drilling. The Arctic National Wildlife Refuge in Alaska has been on the oil industry agenda for several decades, creating a long-standing environmental controversy. Advances in oil well technology have allowed extraction in the deep ocean beyond the continental shelf, but these have not been enough to reverse the trend of declining production in the United States. Heavy metals are naturally occurring elements that have a high atomic weight and a density at least 5 times greater than that of water. Their multiple industrial, domestic, agricultural, medical, and technological applications have led to their wide distribution in the environment raising concerns over their potential effects on human health and the environment. Their toxicity depends on several factors including the dose, route of exposure, and chemical species, as well as the age, gender, genetics, and nutritional status of exposed individuals. Because of their high degree of toxicity, arsenic, cadmium, chromium, lead, and mercury rank among the priority metals that are of public health significance. These metallic elements are considered systemic toxicants that are known to induce multiple organ damage, even at lower levels of exposure. Metals shall not be assimilated, nor readily disposed of by the body and this results in selectively accumulating in certain tissues showing high concentrations. However, the pollution of soils with heavy metals is directly linked to Water pollution and therefore the reduction of aquatic organisms as Result of the accumulation of a large quantity of heavy metals in water Ecosystems.

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