UNIVERISIDAD POLITÉCNICA DE CARTAGENA
ESCUELA TÉCNICA SUPERIOR DE INGENIERÍA AGRONÓMICA

PROYECTO FIN DE CARRERA:

“EFFECT OF UV LIGHT IRRADIATION ON FULLERENES IN ENVIRONMENTAL MATRICES”

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ABSTRACT

Fullerenes are carbon-based nanomaterials that are receiving increasing interest due to their application in novel technologies. They are spherical or ellipsoidal molecules that consist of sp2 hybridized carbon. Fullerenes can be also functionalized by the attachment of chemical groups to their surface or the inclusion of atoms within their structure. The resulting fullerene derivatives usually preserve the same physical and chemical properties of the pristine fullerene. These novel materials have great potential application and their production is expected to increase in the next future. However, little is known about their environmental fate and toxicity and few studies focused on these topics.

This project focuses on ultraviolet light effect in low concentrations of fullerenes, in the top layer of sandy soil and in ultrapure water. Environmental matrices were amended with fullerenes and exposed to light. The samples were then extracted and analyzed with HPLC-UV.

Results point a clear degradation effect in soil and water, especially in the short term of the incubation. In soil (1.8mm thick), the degradation rate was the same when either UV-A, B or C were applied suggesting a role of the matrix in the process.

In water the losses of fullerenes seems to be related with the energy of the light applied and, the results obtained in low concentrations agrees with those from other works referenced. In water the degradation seems directly produced by UV-light, and UV-C shown the strongest effect.

RESUMEN

Los fullerenos son nanomateriales basados en carbono que están recibiendo un interés creciente debido a su aplicación en las nuevas tecnologías. Son moléculas esféricas o elipsoidales que constan de hibridación sp2 en sus carbonos. Los fullerenos pueden ser funcionalizados también por la unión de grupos químicos a su superficie o la inclusión de átomos dentro de su estructura. Los derivados resultantes de los fullerenos, generalmente conservan las mismas propiedades físicas y químicas del fullereno precursor. Estos nuevos materiales presentan un amplio potencial de aplicación y se espera que su producción aumente en el futuro próximo. Sin embargo, poco se sabe acerca de su destino ambiental y su toxicidad y pocos estudios tratan estos temas.

Este proyecto se centra en el efecto de la luz ultravioleta en los fullerenos a bajas concentraciones, en la capa superior de suelo arenoso y en el agua ultrapura. Estas matrices medio ambientales fueron modificadas con fullerenos y expuestas a la luz UV. Las muestras se extrajeron y analizaron con HPLC-UV.

Los resultados indican un efecto claro en la degradación del suelo y el agua, especialmente en las incubaciones a corto plazo. En el suelo (1,8 mm de espesor), la velocidad de degradación fue la misma tanto en UV-A, B o C, lo que sugiere un papel de la matriz en el proceso.

En el agua, las pérdidas de los fullerenos parecen estar relacionadas con la energía de la luz aplicada y los resultados obtenidos a baja concentración están de acuerdo con los de otras publicaciones referenciadas. En el agua la degradación parece producida directamente por la luz UV, donde UV-C muestra el efecto más fuerte.

1 OBJECT

The aim of this project is the evaluation of the effect of UV light irradiation on fullerenes in environmental matrices. Environmental fates of carbon-based nanoparticles are water and soil [1] [2] [3]. In water, fullerenes are also transported by different ways, such as rivers or rainwater, and they can experiment homo-aggregation and hetero-aggregation reactions with other molecules [4] [5]. In soil, fullerenes are accumulated and/or adsorbed to the matrix [6] [7] [8].

2 INTRODUCTION

2.1 GENERALITIES

Fullerenes are carbon based nanomaterials which present exceptional physical and chemical properties. They have great potential for novel applications and employment, e.g. in medicines, seeds, solar panels [9], personal care products [10] or in medical applications, such as imaging probes, optics and drug carriers [11] [12]. Engineered nanoparticles are manufactured in rapidly increasing quantities these days [13]. It is estimated that by 2015, about 7 million people will be employed in this sector [14].

There is a growing concern about the human health and environmental effects of fullerenes. Wastewater discharge, manufacturing factories, storm water runoff, erosion from composites, and wet deposition from the atmosphere are likely routes of release of fullerenes into the aqueous environment [15].

In addition, it should be noted that fullerenes are also formed during natural processes such as volcanic eruptions and forest fires and can be produced unintentionally by human activity (e.g. soot and diesel exhaust).

An essential aspect of environmental risk assessment is the study of the behavior and occurrence of fullerenes in natural environmental samples at low concentrations, the environmental concentrations are supposed to be relatively low (0,003 ng/L in surface water and 4 – 310 ng/L in treated wastewater) [16].

Fullerenes degradation and transformation in the matrix can be biotic and abiotic.

Several large national and international research programs highlight the risk assessment of engineered nanoparticles, e.g. by the OPPTS (Office of Prevention, Pesticicles and Toxic Substances), EPA (Environmental Protection Agency) and OECD (Organization for Economic Cooperation and Development). Nanoparticles researches are a priority line in the 7th Framework Programme (FP7), European Union [17].

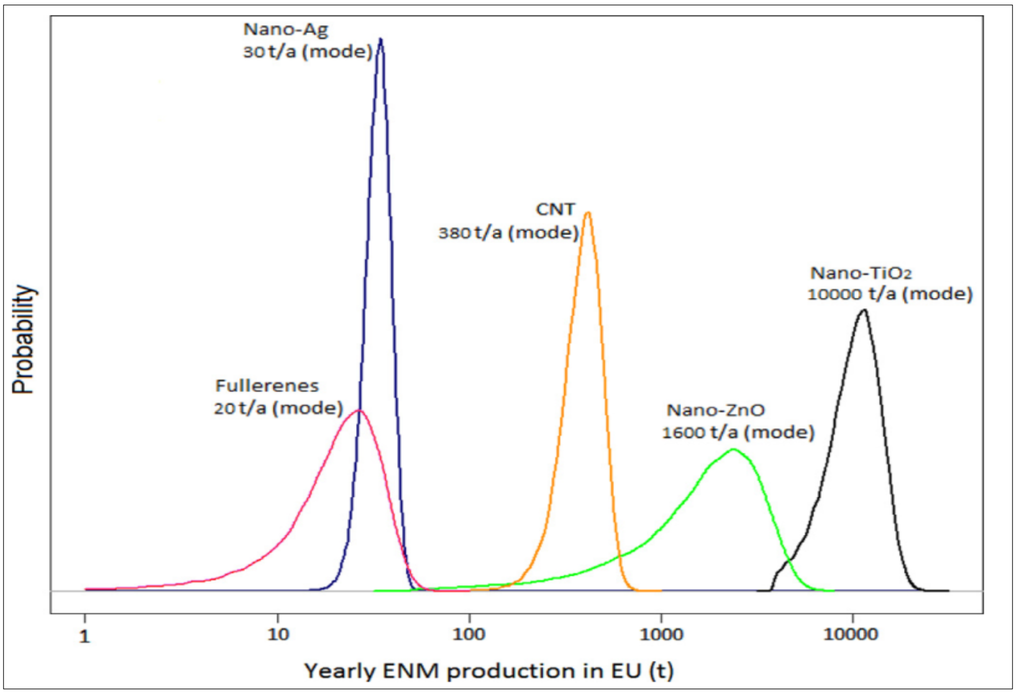


Figure 1. Yearly ENM production distribution and mode values in the EU in 2012 [18] [19].

European fullerenes production is expected up to 20t as shown in the figure 1 [18]. The raw data of ENM (engineered nanomaterials) production or use, given by the producers, were classified into groups according to their degree of belief, which formally represent the strength of various estimations.

Until 2011 fullerenes and other carbon-based nanomaterials (e.g. carbon-nanotubes) were ranked as the second among all the nanomaterials used in consumer products available [20] [21].

2.2 FULLERENES

Fullerenes are allotrope of carbon. Unlike other forms of carbon: graphite, grapheme, and diamonds, fullerenes have a highly symmetric molecular shape similar to a closed cage, with hexagons and pentagons of carbon atoms. Fullerenes are discrete molecules consisting of a defined number of carbon atoms [22]. Fullerenes properties, such as color when dissolved in solvents, depend directly on the number of atoms of carbon [23].

Fullerenes origin could be natural (e.g. widlfires, volcanic, etc) or industrial (laser vaporized and supersonic expansion, electric arch unload in inert atmosphere, fullerenes synthesis during combustions [24].

Fullerenes fulfill Euler's theorem, which connects the number of vertices (carbon atoms), edges (covalent bonds which join carbon atoms) and faces (hexagons and pentagons formed). $V - E + F = 2$

The smallest icosahedron is exclusively pentagonal, which has 12 faces and 20 vertices, C_{20} molecule (which is not stable).

Fullerenes can be deduced from geometrical considerations. It can calculate the smallest fullerene (C_{20}), but not the biggest. The number of atoms of the fullerene molecules is calculated from C_{20} fullerene (icosahedron pentagonal = 20 atoms) and the number of hexagonal faces (m), the equation can be defined by:

number of atoms = $20 + 2m$

e.g. C_{60}, 60 Carbon atoms = $20 + 2$ (20 hexagonal faces)

The hexagonal rings may be considered equivalent to benzene, but pentagonal rings are not as favorable for receiving electrons (due to the double bonds); therefore, there is not a total relocation of the electrons and the fullerene structure is not completely superaromatic but behaves more like an electron deficient alkene and reacts readily with nucleophiles such as halogens. The C60 and C70 fullerenes can capture up to 6 electrons in successive reversible reduction reactions.

Fullerenes are really stable molecules, each carbon atom is linked to other three, hybridation state sp2.The most stable fullerenes are C60 and C70 because of energetic reasons [25].

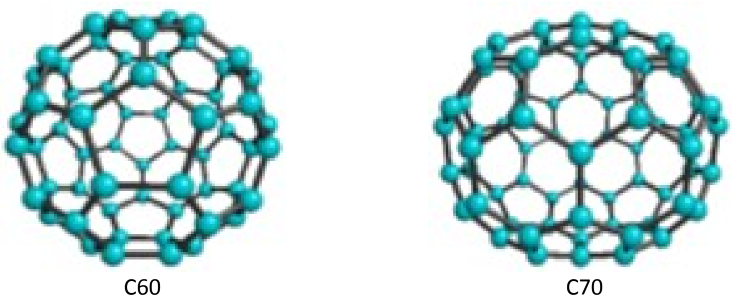


Figure 2. Schematic representation of the molecular structures of C60 and C70 fullerenes [26].

Multilamellar fullerenes consist of several layers, where each fullerene is enclosed in a larger structure; these multiwall carbon molecules have been named intuitively as "nanonions".

Endofullerenos are obtained by placing an atom or molecule within the internal cavity of the fullerene.

Heterofullerenos are obtained by replacing one or more carbon atoms from the structure.

Fullerenes have a very poor solubility in polar solvents such as water but can be dissolved in nonpolar (organic) solvents. Also for increasing their solubility, they can be chemically modified to get functionalized fullerenes, which preserve physical and chemical properties from pristine fullerenes. Artificial nanomaterials are useful due to their huge superficial area (mass relation) as chemical reactions catalysts [27].

Fullerenes have been widely functionalized or polymerized. It can be seen in figure 3.

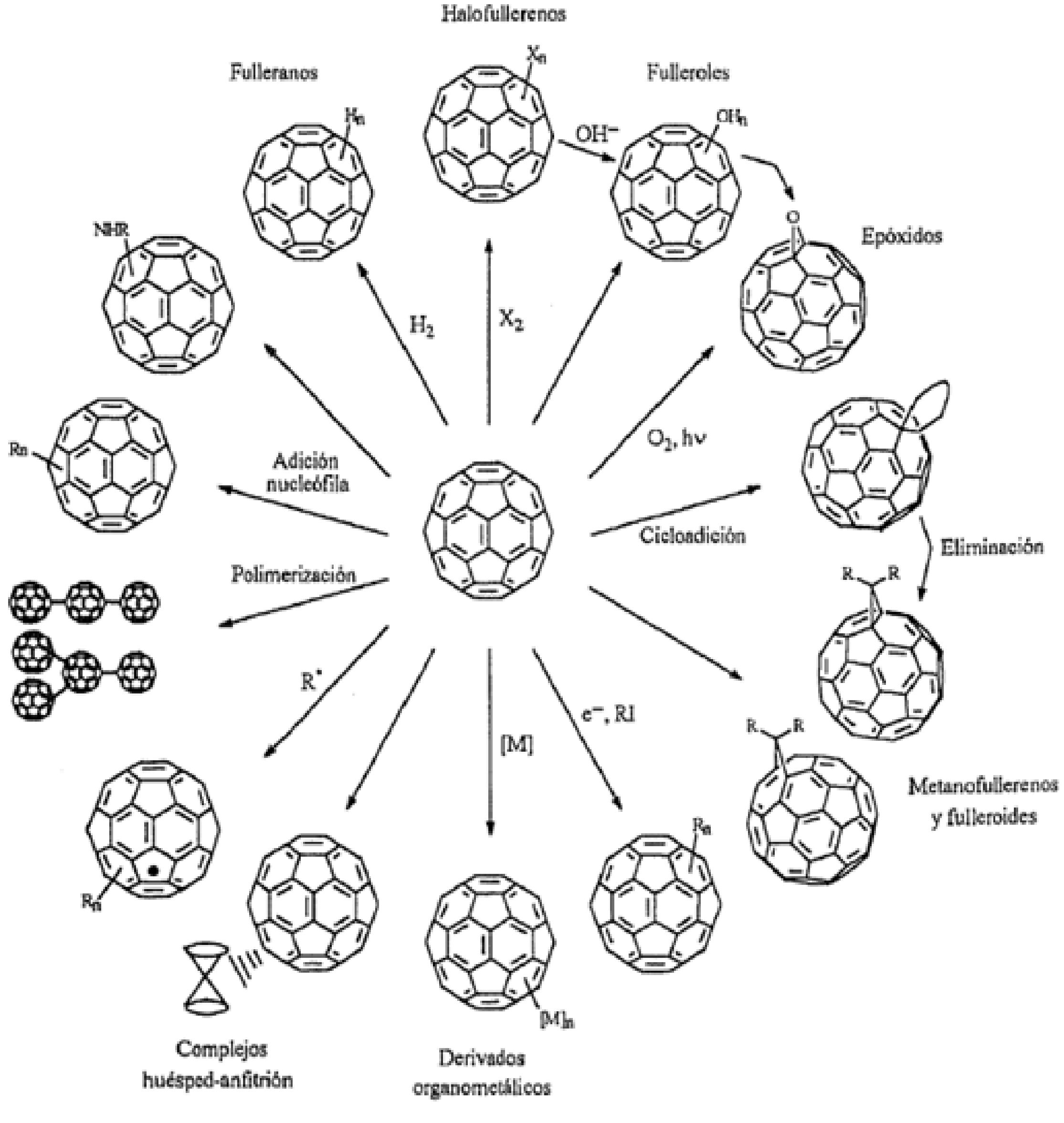


Figure 3. Fullerenes chemistry reactivity [28].

2.2.1 FULLERENE C_{60}

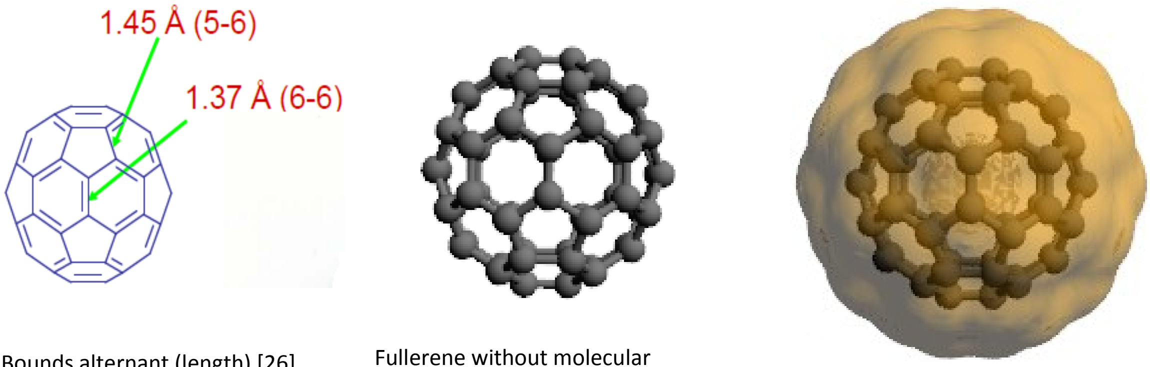
C60 fullerene molecules consist of 20 hexagons and 12 pentagons (connected by double exocyclic bounds as a sphere) and have diameter of 0.72 nm [22]. C60 fullerene are composed by rings interconnected in a football-like nano-sized structure, similar to the Richard Buckminster Fuller geodesic domes, after which the fullerenes are also called buckyballs or buckminsterfullerene [29]. In table one some of the properties of the C60 fullerenes are reported.

Sublimation	>500ºC
Color	Black (solid state) Violet solution (solvent: toluene) Yellow solution (solvent: water)
Structure	Icosahedron truncated
Bounds alternant (length)	1.37 Å (6-6) 1.45 Å (5-6)
Quantic emission efficiency	Low
Quantic generation efficiency	High (almost 1)
Sensitivity singlet	100%
Electron acceptation	High
Formation heat	10,16 kcal/mol per carbon atom
Solubility	Nonpolar: High (toluene 2.8 g/L) Polar: Low (water 8ng/L)
Charge	Highly electronegative
Absortion UV spectra	High
Absortion visible spectra	Moderate at 430 nm, provide the violet color.

Table 1. C60 fullerene properties.

Fullerene C60 stability is justified by isolated pentagon rule which only allows the formation of those fullerenes whose pentagonal rings are surrounded by hexagons.

When C60 fullerenes are exposed to intense ultraviolet light, such as a laser, molecules are polymerized and bounds between the different spheres are formed [30].



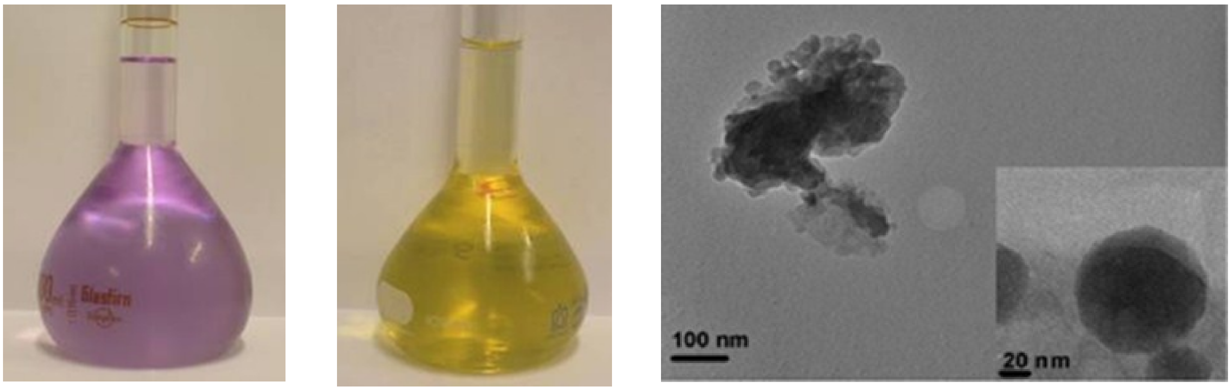
Bounds alternant (length) [26].

Fullerene without molecular superficial cover [25].

Fullerene with molecular superficial cover [25].

Figure 4. C60.

With relatively mild chemical treatments, such as evaporation of the nonpolar phase, some C60 become water stable in this yellow solution (nC60). Although chemical analysis shows the presence of C60, light scattering and electron microscopy confirm that the material is present as colloidal aggregates that contain between 100 and 1,000 fullerene molecules (figure 5).



C60 in toluene.

nC60 in water.

Comparison of cluster (nC60) and fullerene (Tol/C60) [31].

Figure 5. C60 solutions.

Colloid formation is not the only process that increases the aqueous stability of fullerenes. Once released into the environment, the fullerenes in the outer cluster of nC60 particles may undergo extensive chemical transformation that increases their individual water solubility. When the nC60 particles break up and/or erode over time (particularly owing to mechanical erosion), the internal fullerite crystal structure will be exposed. Larger C60 aggregates are expected to erode more slowly because of their smaller specific surface accessible to water [32]. The erosion of fullerene particles may enable them to decrease in size over time with concurrent release of polyhydroxylated fullerenes from their outer cluster. Water solubility of these fullerols can be >50mg L^{-1}.

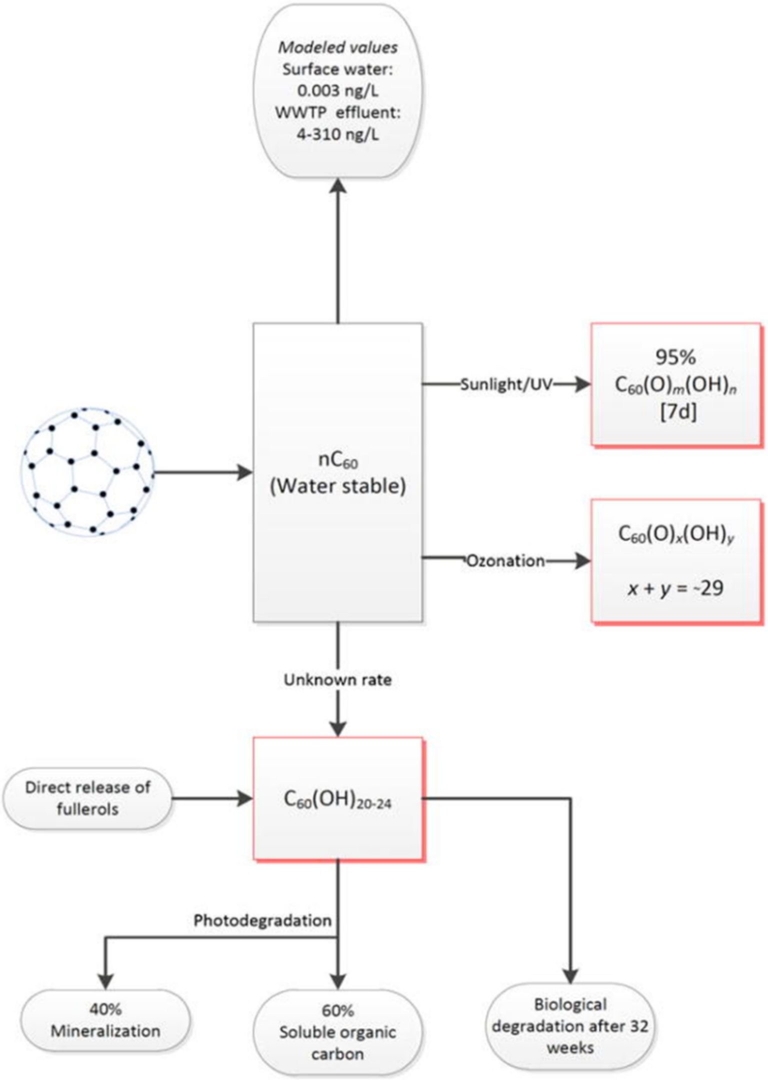


Figure 6. Transformation of fullerene C60 under environmentally relevant conditions [32].

C60 and C70 molecules are efficiently converted to the triplet state after being irradiated with UV light, consequently, excited fullerene quickly become triplet oxygen ($^3O_2$) in singlet oxygen ($^1O_2$). This fact suggests that fullerenes have great potential to produce photodynamic damage to biological systems. Modified fullerenes may be cytotoxic when they are irradiated by UV light; e.g. singlet oxygen interaction with cells or direct interaction of the fullerene in the excited state and the cells [33].

However, formation of radical oxygen species (ROS) has emerged as the principal cause of the toxicity of fullerenes [32] [34].

2.3 UV LIGHT

The sun emits a wide range of solar radiation, referred to as the electromagnetic spectrum. This radiation includes visible light, infrared (IR), radio, microwaves and ultraviolet light (UV). Each type of electromagnetic radiation refers to a band of specific wavelengths and frequencies. The shorter the wavelength the higher the frequency and consequently the more energetic. Hence shorter wavelength light is more energetic and tends to be the most degrading.

The visible and UV portion of this spectrum is reported in figure 7 where the different types of UV bands are shown [35]. These are classed according to the draft standard ISO-DIS-21348.

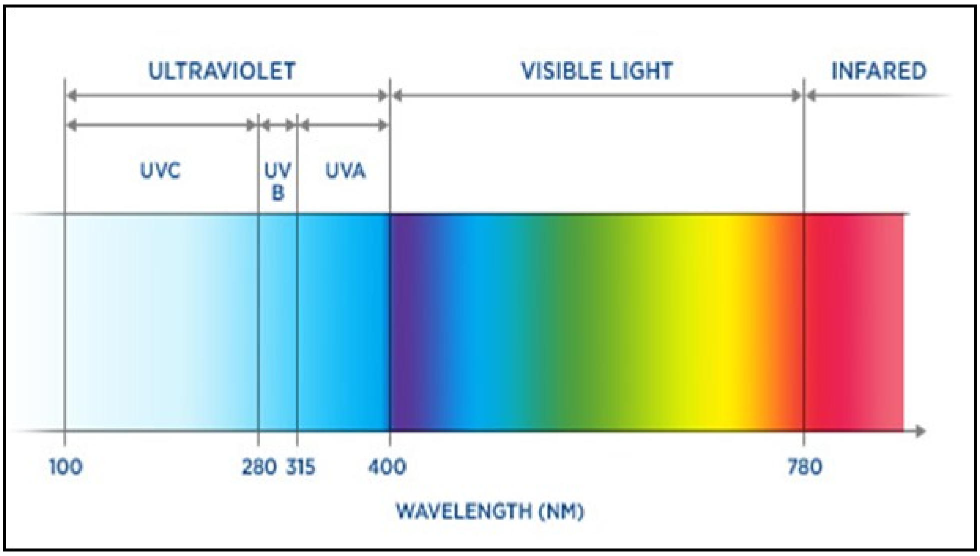


Figure 7. The visible and UV portion of light spectrum [35] [36].

Ultraviolet light (UV) is the portion of the spectrum of light between 100nm and 400nm. This spectrum can be divided in three bands designated, from the less to the most energetic, as UV-A (315-400nm), UV-B (280-315nm), and UV-C (100-280nm).

98.7% of the UV radiation emitted by the sun is blocked by the earth ozone layer and upper atmosphere. The majority of radiation reaching the earth surface is UV-A (95% of UV-A reaches the Earth surface) and a very small amount of UV-B (5%). This is demonstrated in the diagram below which shows the filtering effect of the earth atmosphere.

Ozone levels at various altitudes act as a shield for the different ultraviolet radiation. In general, all UV-C is blocked by oxygen (from 100–200 nm) or by ozone (200–280 nm) in the atmosphere. The ozone layer then blocks most UV-B. Opposite, UV-A is hardly affected by ozone and most of it reaches the surface.

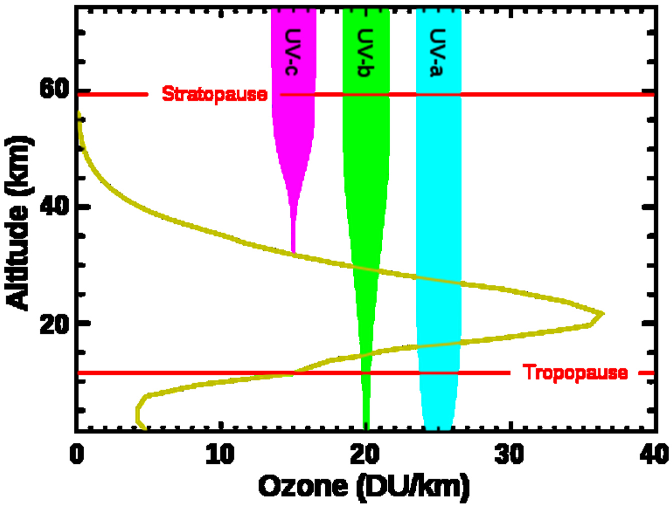


Figure 8. Ozone density profile for the northern hemisphere [37].

Figure 8 illustrates how far into the atmosphere each of these three types of UV radiation penetrates. In the figure UV-C (violet) is entirely screened out by ozone around 35 km altitude. On the other hand, most UV-A (blue) reaches the surface. Ozone screens out most UV-B (green), but some reaches the surface. C60 strongly absorbs the ultraviolet spectrum and moderately the visible band.

3 MATERIALS AND METHODS

3.1MATERIALS

3.1.1 COMPOUNDS

Compound	**Abbreviation**	**Purity**	**Producer**
C60 fullerene	C_{60}	>99.5%	Sigma-Aldrich (Steinheim, Germany)
C70 fullerene	C_{70}	>99%	Sigma-Aldrich (Steinheim, Germany)

Table 2. Fullerenes standards used in the present study.

3.1.2 REAGENTS AND CHEMICALS

Reagents and Chemicals	**Abbreviation**	**Purity**	**Producer**
Toluene	C_7H_8	>99.9%	Biosolve BV (Dieuze, France)
Ethanol	C_2H_6O	>99.9%	Biosolve BV (Dieuze, France)
Acetonitrile	CH_3CN	>99.9%	Biosolve BV (Dieuze, France)
Nitrogen	N_2	>99.9%	Supelco (Steinheim, Germany)
Magnesium perchlorate	$Mg(ClO_4)_2$	>98%	Sigma-Aldrich (Steinheim, Germany)

Table 3. Reagents and Chemicals used in the present study.

3.2 METHODS

3.2.1 UV MEASUREMENT

The output intensity of the ultraviolet emitted from a multi-wavelengths lamp, even when each tube that lamp has the same wattage rating, will vary with wavelength. In the case of the model 3UV-38 each of the three tubes is rated at 8 Watt current draw, yet note how the intensities vary:

- The longwave UV-A (365nm) ultraviolet intensity measured at 20 cm from the source is 22.2µW/cm^2.

- The mediumwave UV-B (302nm) ultraviolet intensity measured at 20 cm is 53.4µW/cm^2.

- The shortwave UV-C (254nm) ultraviolet intensity measured at 20 cm is 189µW/cm^2.

<u>Experiments Photochemical.</u>

The incident light intensity of each lamp at the set up was measured at 11mW/cm^2 using a UVX-25 radiometer (Ocean Optics USB 4000).

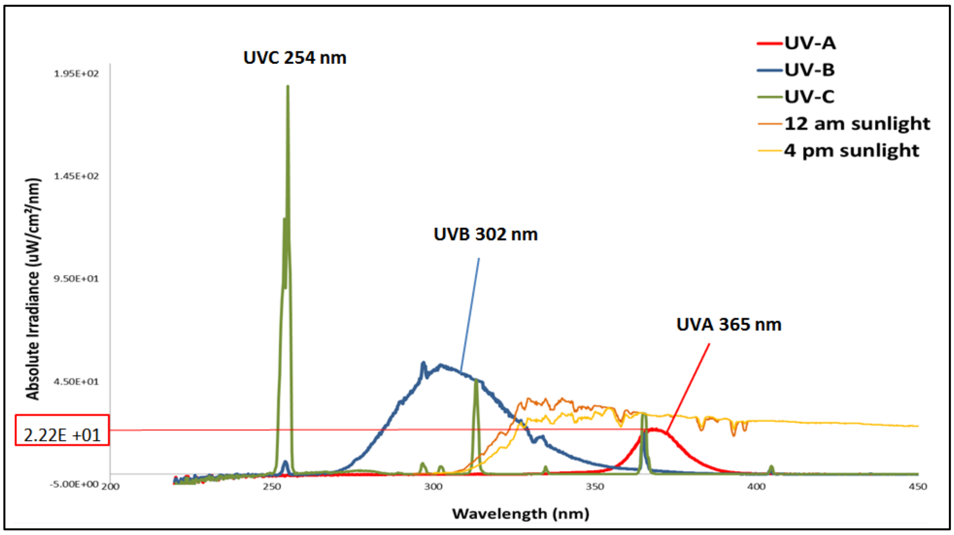


Figure 9. Absolute irradiance measurement.

In the figure 9, the UV-A lamp irradiance peak at 365nm is 22.2 µW h/cm^2 s. Also, sunlight irradiation is higher than lamp irradiation due to the moment chosen to test the sunlight, which was really sunny.

$$1\,\frac{\mathrm{kW\,h}}{m^2\ \mathrm{d}} = 1.157\,\frac{\mathrm{\mu W\,h}}{cm^2\ s}$$

$$\frac{22.2\,\frac{\mathrm{\mu W\,h}}{cm^2\ \mathrm{s}}}{1.157\frac{\mathrm{\mu W\,h}}{cm^2\ \mathrm{s}}} \bullet 1\,\frac{\mathrm{\mu W\,h}}{m^2\ \mathrm{d}} = 19.18\ \frac{\mathrm{kW\,h}}{m^2\ \mathrm{d}}$$

Absolute Irradiance from the lamp= 19.18 kW h/m^2 d

Amsterdam Absolute Irradiation Media = 2.92 kW h/ m^2 d

19,180 Wh/m^{2}d is the result from the lamps used during the experiment; it is much bigger than the media irradiation data from Amsterdam, shown below. The difference between results is because the lamps were at 20cm from the UVX-25 radiometer (Ocean Optics USB 4000). The closer the light is to the radiometer, the bigger irradiance it is.

Month	***H_h***	***H_{opt}***	***H(90)***	***I_{opt}***	***T_{24h}***	***N_{DD}***
Jan	695	1280	1380	69	3.3	433
Feb	1270	1950	1870	60	4.5	387
Mar	2510	3320	2740	49	6.3	333
Apr	4410	5180	3550	37	9.4	211
May	5200	5340	3060	22	13.2	106
Jun	5520	5400	2850	15	15.8	48
Jul	5130	5100	2820	18	18.0	9
Aug	4210	4620	2940	31	18.3	24
Sep	3020	3740	2870	44	15.6	117
Oct	1700	2500	2290	57	11.8	251
Nov	810	1370	1410	66	7.4	387
Dec	521	1010	1130	71	3.9	472
Year	**2920**	3410	2410	37	10.6	2778

H_h: Irradiation on horizontal plane (Wh/m^2/day)
H_{opt}: Irradiation on optimally inclined plane (Wh/m^2/day)
H(90): Irradiation on plane at angle: 90deg. (Wh/m^2/day)
I_{opt}: Optimal inclination (deg.)
T_{24h}: 24 hour average of temperature (°C)
N_{DD}: Number of heating degree-days (-)

Table 4. Amsterdam irradiance data [38].

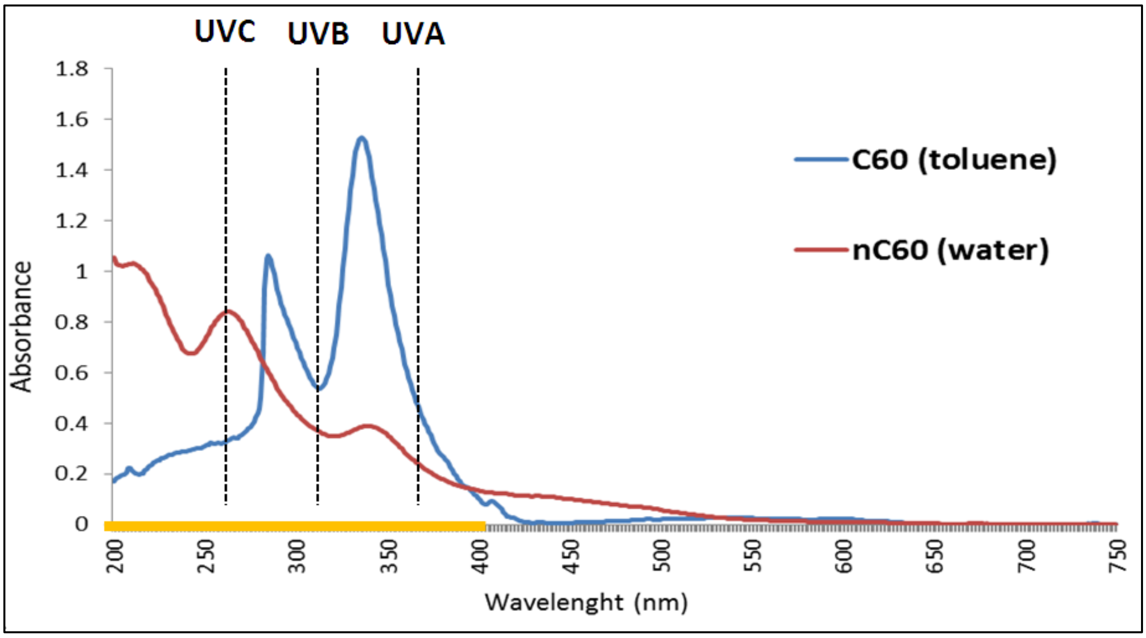


Figure 10. UV-vis absorption spectra of C60 and nC60 [39].

C60 fullerene absorbs light in the ultraviolet and visible spectrum in toluene (C60) as well as in water (nC60), as shown in figure 10.

nC60 (red line) absorbs UV spectrum with a peak around 260nm, higher than the one dissolved in toluene, and decrease in the UV-B, UV-A and visible bands. It presents a small peak at 350nm lower than toluene.

C60 in toluene (Tol/C60) absorbs in the UV spectrum, with two peaks at 286 and 332nm, in the visible band it has the typical weak band.

In the maximum points, transformation processes may be expected [20].

During HPLC-UV analysis with toluene as a main mobile phase component, detection is set at 332nm instead of 286nm. The latter wavelength (286nm) was not applied in the measurement owning to the toluene absorbance in the same range that resulted in a greater base line noise [40] [41].

3.2.2 HPLC-UV DETECTION

The separation of fullerenes in liquid chromatography necessitates the use of a nonpolar mobile phase able to dissolve and elute the compounds in a relatively short time. In the present study toluene was applied as eluent in the mobile phase in combination with a specific stationary phase composed of pyrenyl-propyl functionalized silica (Buckyprep) that enhances the retention of fullerenes as a result of the large ligand that can interact with the aromatic structure of the fullerenes.

The method consisted in a gradient elution starting with 75% toluene and 25% acetonitrile, after 6 minutes the gradual conversion to 100% toluene to allow a faster elution of the more nonpolar compounds. With this setting the elution of the fullerenes C60 and C70 was obtained within 13 minutes while the whole method lasted 20 minutes to allow the system to equilibrate prior to the next analysis. The order of elution is correlated with the size of the cage, C60 (minute 7.5) elutes earlier than C70 (minute 12.4).

When fullerenes are dissolved in organic solvents such as toluene, spectrophotometric detection is a powerful tool for their analysis owing to the strong absorption of these compounds in the UV range. As reported Bouchard and Ma [34], the wavelength selected for the detection during the chromatographic runs were 332 nm for C60 and C70 fullerenes, despite the fact that the maximum absorbance for all the compounds was recorded at 286 nm. As shown in the figure 11 ”HPLC-UV chromatogram of fullerenes” apart from the C60 (retention time 7.5 min) and C70 (retention time 12.4) peaks, appeared another unknown peak at 4 minutes, Carboni et al. [41] reported it as a functionalized C60 fullerene.

UV spectra of the fullerenes were obtained analyzing the stock solution of the single compounds in quartz cuvettes with an Olis DW-2000 spectrophotometer equipped with Olis SpectraWorks software. Liquid chromatography was performed with a Shimadzu Prominence system equipped with a diode array detector. The wavelength monitored for UV detection was 332 nm. The data were collected with the LCsolution software. The separation was achieved with a Cosmosil Buckyprep column. External calibration curves were obtained analyzing 8 levels of standard solutions in toluene at concentrations of fullerene C60, 500µg L-^{1}, 250 µg L^{-1}, 125 µg L^{-1}, 62.5 µg L^{-1}, 31.3 µgL^{-1}, 15.6 µg L^{-1}, 7.8 µg L^{-1}, 3.9 µg L^{-1} and fix concentrations of fullerenes C70, 125 µg L^{-1} (Table 5). Quantification was based on chromatographic peak areas whereas limits of detection and quantification were assessed observing the signal to noise ratio (S/N) and considering limit of detention (LoD) as the concentration with S/N=3 and limit of quantitation (LoQ) as the concentration with S/N=10.

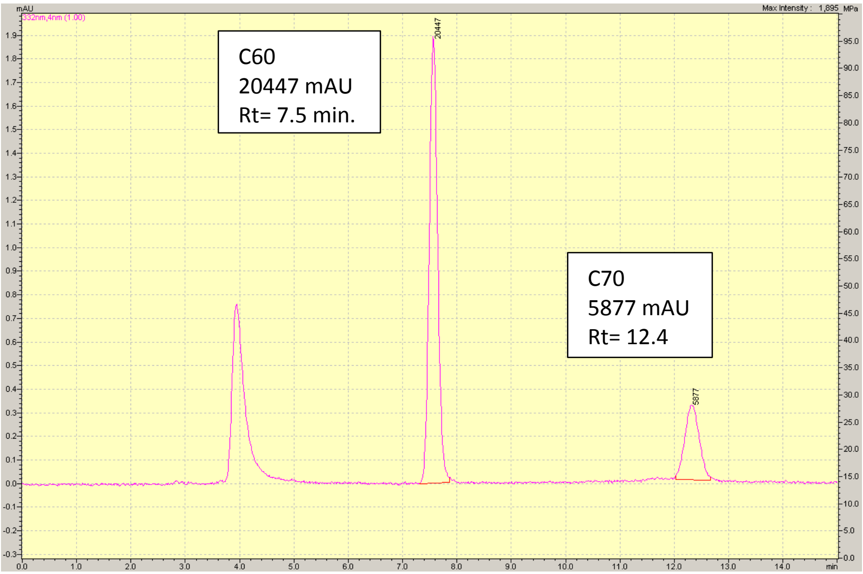


Figure 11. HPLC-UV chromatogram of C60 and C70 fullerenes.

The quantitation of fullerenes can be made through the analysis of the HPLC-UV peak areas in the chromatograms. Area ratio and concentration ratio from the eight calibration levels were used to get the equation of the calibration line, to relate them (Figure 12). Internal Standard concentration (C70) was necessary to transform the concentration rate (C60/C70), got in the equation, in C60 concentration (Table 5).

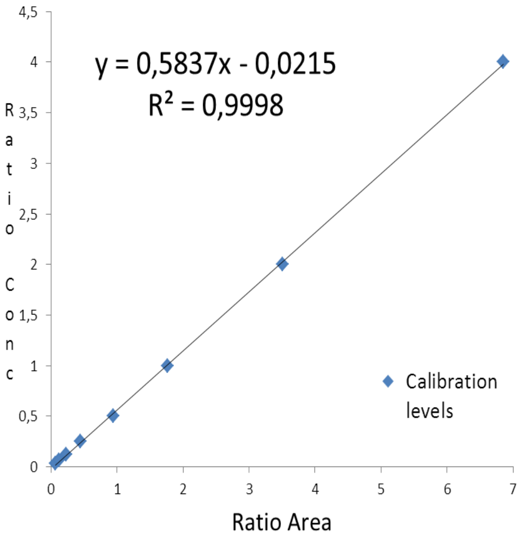


Figure 12. Calibration curve and equation for C60.

	C60 Area mAU	C70 Area mAU	Ratio Area	C60 µg L^{-1}	C70 µg L^{-1}	Ratio Concent
Lev_08	371	5521	0,0671	3.9	125	0,03125
Lev_07	652	5339	0,1221	7.8	125	0,0625
Lev_06	1262	5481	0,2302	15.6	125	0,125
Lev_05	2351	5214	0,4509	31.3	125	0,25
Lev_04	5058	5380	0,9401	62.5	125	0,5
Lev_03	9745	5510	1,7686	125	125	1
Lev_02	19033	5421	3,5109	250	125	2
Lev_01	35341	5155	6,8556	500	125	4

Table 5. Calibration levels data.

There are eight levels of calibration, C70 is always stable and C60 increases in concentration. Each analyte is represented by a unique peak area. These areas are used in a simple equation since the only unknown data in the system is C60 concentration in the samples.

4 ESSAYS

4.1 DEGRADATION IN SANDY SOIL MATRIX

4.1.1 EQUIPMENTS

Equipment	**Producer**	**Characteristics**
Oven	Glassinstruments B.V.	
Sieve	Twente	NEN 2560, 1.68 mm mesh
Analytical Balance	Mettler Toledo	AT200, Resolution 0.1mg
Petri Dish	Steriplan, Duran Group.	Diameter: 80mm
Vial	VWR International	40ml, 10ml, 5ml, 1ml 60ml amber glass
Sonic bath	Bransonic	Bransonic 12, 50 Hz
Filter	Whatman GF	prepleated paper filter 4 – 7 µm pore size
	Whatman GF	0.45 µm cellulose ester membrane syringe filter
Syringe	HSW	Norm-Ject, 20ml
Lamps	UVP, Co.	3UV-38, Ultraviolet 254nm, 302nm, 365nm
Laboratory support	Gibson.	Pipets, Erlenmeyer, …
Fume hood	AirFlow Control	
Orbital shaker	Gerhardt Laboshake orbital	
HPLC-UV	Shimadzu Scientific Instruments, Inc	Shimadzu Prominence system
Fridge	Siemens	iQ 500

Table 6. Equipment used in the present study in sandy soil.

4.1.2 PREPARATION OF THE STANDARDS IN TOLUENE

Stock solutions of the individual fullerenes C60 and C70 were prepared in toluene at a concentration of 19.8 µgC60·ml^{-1} and 5.0 µgC70·ml^{-1} according to the method described by Kolkman et al [42]. Toluene was analytical grade. The solutions were placed in the dark for twelve hours on an orbital shaker to achieve complete dissolution of the fullerenes. Diluted solutions for the individual fullerenes and their mixture were obtained by diluting aliquots from the individual stock solutions. The solutions were stored in a fridge at 4ºC in the dark. Before using them, the solutions were shaken by hand, and sonicated for 4 min.

4.1.3 SOIL SAMPLING, SOIL CHARACTERIZATION AND SAMPLE TREATMENT

Sandy soil was collected in Oude Schulpweg, Castricum, The Netherlands (52°32'39.6888''N, 4°39'5.6232''E). The sand was taken only from the first horizon of the soil, 10 – 15 cm, after moving aside the first cm of soil because it was full of stones and parts of shells. The sample was placed in an oven at 65ºC for a week in order to remove traces of water. The dried sample was sieved with a 1.68 mm mesh. Rejection is not taken into account. After being sieved, the sample was kept in the oven until the beginning of the tests.

In order to characterize the soil, 10g of sandy soil was added to 50 ml of ultrapure water, dilution 1:5, before to undergo shaking extraction overnight at 160 rpm with an orbital shaker. After that, the supernatant was transferred and filtered with 0.45 µm syringe filters. The pH of the filtered extract was measured with Consort C831 electrode. The final pH was 8.67. DOC and IC were determined using a TOC-V CPH. The total carbon estimated in the sample was 0.32% total Carbon and 0.19% as inorganic Carbon (= 1.57% $CaCO_3$). Left (0.32 - 0.19) = 0.13% organic Carbon. That is about 2X 0.13% = 0.26% soil organic matter in the sample.

4.1.4 SOIL INCUBATION

100g of the sandy soil used in the present experiment was spiked with the total volume of the Standard C60 fullerenes for a final concentration of… and let overnight to let the solvent evaporate…it was homogenized by stirring with a metal spoon. Then 300 g quarters left were slowly added in small portions and meanwhile the soil was homogenizing by stirring for 1 hour and left in the dark semi covered for 2 days to allow the toluene to evaporate.

The soil amended with C60 was then added to the petri dishes (10 g each). The lamps were located 20 cm up to the Petri dishes. Samples were placed in line under the lamp, to get the same irradiation in all of them at ambient temperature (25ºC). The different environments (UV-A, B and C) were separated; there was not irradiation mixture of the light in the samples.

Control samples were obtained by incubating samples in the same environment but preventing the exposure to light with the use of aluminium foil. Twelve samples were removed for analysis each time (three per each UV-A,B,C and three dark controls). Samples and control tubes were uncapped. Dark controls were loosely wrapped with aluminum foil to maintain the oxygen concentration but avoid the UV-light.

First experiment (Recovery test): fullerenes were spiked in concentration of 200 µgC60/kg sandy soil. Each sample of 4mm depth was exposed for zero, one, three and seven days under the UV-B lamp.

Second experiment: fullerenes were added in concentration of 100 µgC60/kg sandy soil, 1.8mm depth and exposed for zero, seven and twenty-eight days to UV-A, B, C.

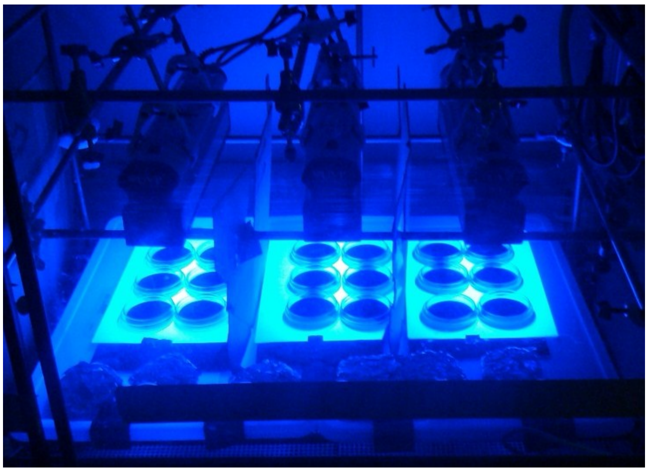


Figure 13. Soil incubation set up.

4.1.5 SONIC AND SHAKING EXTRACTION

Ten grams of soil from each petri dish were weighted and placed into a glass tube, 150µl of C70 standard (concentration 75µg/kg) was added as Internal Standard. Before adding clean toluene, the toluene from C70 standard was evaporated under the fume hood. Nine milliliters of toluene were added and the samples were placed open into an ultrasonic bath for minimum 15 minutes, the bath was kept at a temperature of 34ºC. Then, the tubes were closed with a glass stopper and shaking extraction was performed with an orbital shaker at 160 rpm for 90 minutes, (S-Sh). The toluene supernatant was filtered though a 4 -7 µm pore size prepleated paper filter into 60 ml amber glass vials (it is amber because this kind of vials does not let the UV light to go through and degrade the fullerenes). The filter was rinsed with 6 ml of toluene. Each sample was evaporated under a gentle nitrogen flow until approximately 5 ml. Nitrogen gas was used to concentrate the volume under anoxic condition. After the evaporation, the extracts were filtered with 0.45 µm regenerated cellulose syringe filters and concentrated to a final volume of 4 ml. All experiments were performed in triplicate and dark samples (control) were extracted with the same protocol.

4.1.6 RESULTS AND DISCUSSION

First experiment (Recovery test): Fullerenes were spiked in concentration of 200 µgC60/kg sandy soil. Each sample of 20g and 4mm depth was exposed for zero, one, three and seven days under the UV-B lamp. This experiment was able to test the set up, the spiking method and to quantify the degradation of the fullerenes in seven days. From this experiment onwards, samples were 10g (1.8mm depth) and 100 µgC60/kg sandy soil concentration instead of the initial conditions, owing to get a thinner top layer soil and more UV light exposition, from now on UV-A, B and C. Moreover, incubation length was increased up to 28 days.

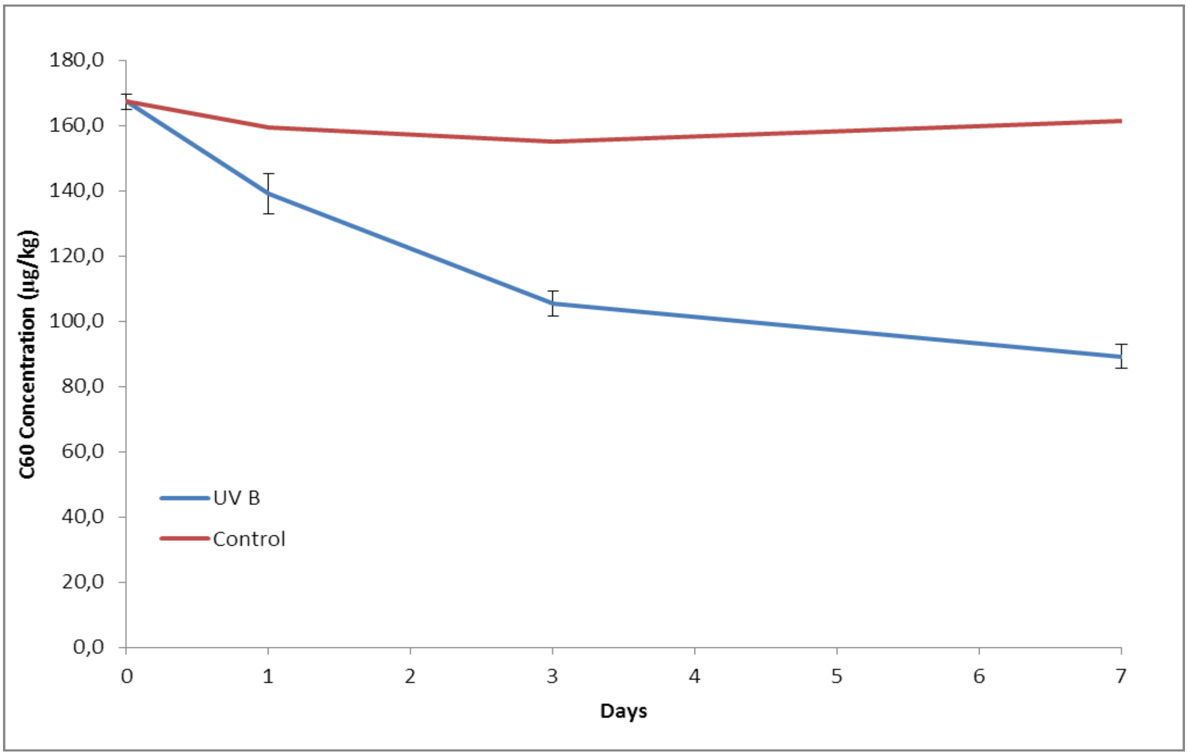


Figure 14. Sandy soil first experiment results (recovery test).

Second experiment: Fullerenes were added at the lower concentration of 100 µgC60/kg sandy soil, 1.8mm depth and exposed to light for zero, seven and twenty eight days to UV-A, B, C.

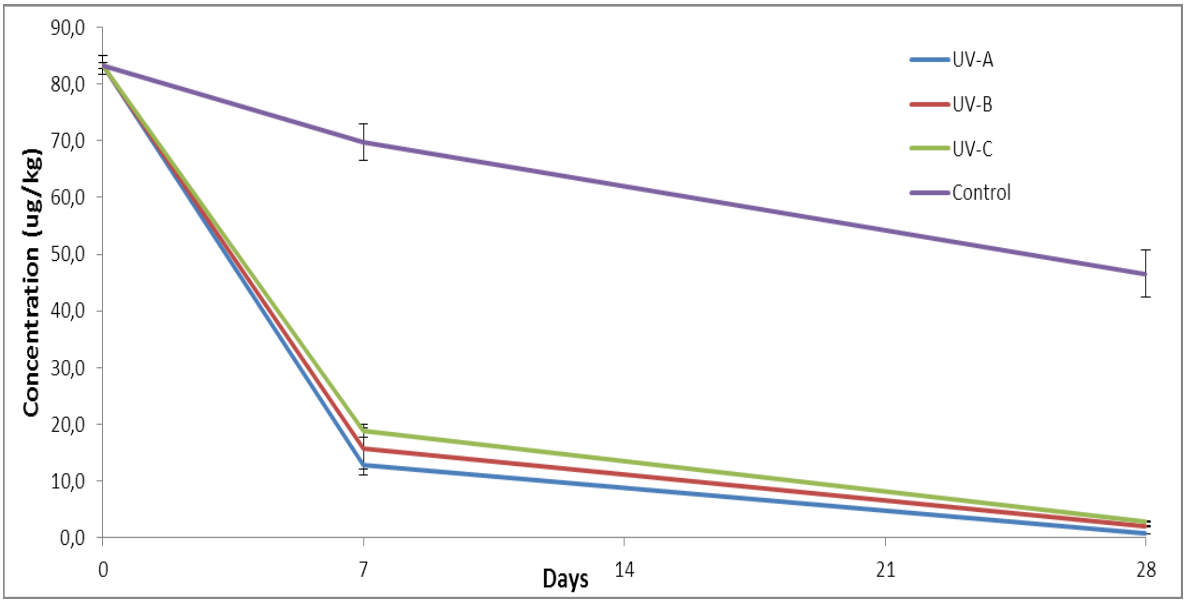


Figure 15. Sandy soil second experiment results.

In 4 mm thick sandy soil, fullerenes exposed to UV-B light for 7 days were degraded the 46.71% of the initial amount (167 µgC60/kg sandy soil). Fullerenes non-exposed to UV-light (dark control samples) were only degraded 3.6%; the difference between the initial amount of fullerenes and the amount seven days later was similar. Control samples were constant for the seven days incubation.

In the second experiment, 1.8 mm thick, in 7 days the 81.15% of initial fullerenes (83.3 µgC60/kg sandy soil) were degraded. Dark control samples were degraded for 7 days the 16.21%, and for 28 days the 44.06%, the degradation rate was similar during all the days in the incubation, The daily degradation rate for the first seven days was 2,32% and for the next 21 days, the daily rate was 1,57%.

Difference between degradation at second and first experiment was almost the double due to UV-light penetration in sandy soil. The degradation speed was clearly higher in the second experiment; it was probably due to the fact that, being the thickness of the soil smaller, the UV light could penetrate. Dark control samples from the second experiment were more degraded because fullerenes were more exposed at the top of the sample than in the first sample, where fullerenes were distributed in 4mm thick. This is probably due to oxidation of fullerenes and possible losses that are bigger when the amount of soil is lower. Loss in the control might be caused by oxidation redactions and long term absorption (fullerenes C60 could be seized during the incubation by different soil compounds). Furthermore, the soil was not sterilized and bacteria and microorganism may play a role in the degradation of fullerenes. This is likely to be enhanced by darkness since UV light such as UV-B is well known to damage the DNA.

As can be seen, fullerene C60 degradation rate is fast in the first 24 hours, from that point onwards it decreases in the second and third day. On the first experiment, the degradation of the initial amount of fullerene C60 for the first day was 16.17%, meanwhile, the degradation on the second and third day was 20.96%, and for the following 4 days was 9.58%. On the other hand, the sample of 1.8mm thick was exposed to UV-B light radiation for 28 days, and the final degradation was the 97.60%.

UV-A light degradation during seven days was the 84.63% of the fullerenes added and during 28 days the 99.04%. It means that in the last 21 days the C60 degradation was 14.41%.

UV-C light degradation was in seven days 77.31% and in 28 days 96.52%, which means in the last 21 days the C60 fullerenes degradation was 19.21%.

The different degradation of fullerene C60 by UV-A, B or C lights for 28 days was almost the total amount of the fullerene C60 added.

Losses during the process can cause error (standard deviation).

Fullerenes degradation in soil was not exclusively caused by the UV-light irradiation. UV irradiation could affect the matrix (soil temperature, organic matter, carbonates,…) and consequently could act in the fullerenes loss. It could explain the similarity degradation between UV-A, UV-B and UV-C.

4.2 DEGRADATION IN WATER MATRIX

To understand the environmental fate and model environmental concentrations, next to emission also physico-chemical properties and transformation pathways of fullerenes are of importance. The aqueous solubility of C60 in solvent-saturated water is approximately 8 ng/L [43]. However, fullerenes can form a stable aqueous suspension of crystalline aggregates of nC60 [44] [45].

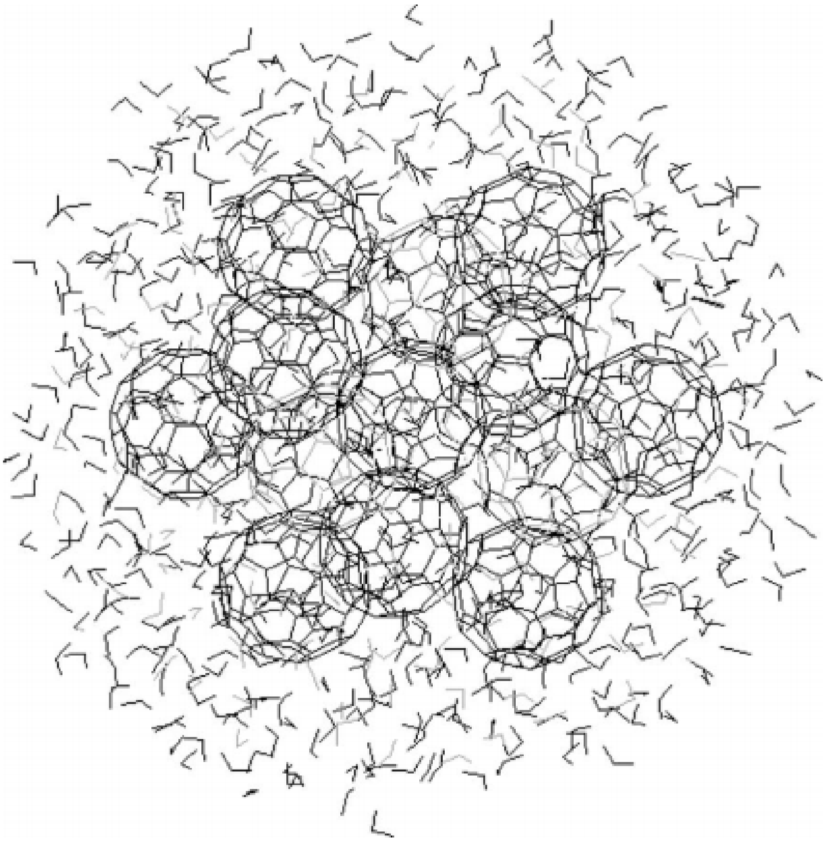


Figure 16. Water soluble aggregates (cluster) of nC60.[46]

After UV irradiation C60 is excited to a singlet state and subsequently to a triplet state, producing reactive oxygen species when returning to ground state [47] [48].

4.2.1 EQUIPMENTS

Equipment	**Producer**	**Characteristics**
Water Purity System	Elga	Purelab Ultra ANMK2 2l/min, Water Quality I^+,I
Fridge	Siemens	iQ 500
Analytical Balance	Mettler Toledo	AT200 Resolution 0.1mg
Vial	VWR International	40ml, 10ml, 5ml, 1ml
Sonic bath	Bransonic 12 ultrasonique bath	50 Hz
Injection bomb	Harvard	Apparatus 11 plus Injection speed: 0.25ml/min
Lamps	UVP Co.	Ultraviolet, 3UV-38 254nm, 302nm, 365nm
Laboratory support	Gibson,	Pipets, Erlenmeyers, …
Fume hood	AirFlow Control	
Orbital shaker	Gerhardt Laboshake orbital	
HPLC-UV	Shimadzu Scientific Instruments, Inc	Shimadzu Prominence system

Table 7. Equipment used in the present study in water.

4.2.2 PREPARATION OF THE STANDARDS IN WATER

Amounts of 97 ml of ultrapure water and 3 ml of ethanol were mixed in a 100 ml Erlenmeyer, and were placed in an ultrasonic bath for 15 min. The ultrasonic bath was kept at a constant temperature of 34 °C by means of a cooling circuit. Next, 2 ml from the combined C60 fullerenes stock (100 µg/L) solution was infused (at a flow rate of 25 ml/min) near the bottom of the vial under continuous sonication, which resulted in a milky emulsion. After 4 h of sonication the toluene was evaporated, and the resulting solution was yellow and clear. This protocol was repeated in order to get the nC70 solution (this solution was reddish). The resulting aqueous fullerene suspensions nC60 and nC70 were used to spike the different water samples in the present study.

Fullerenes have a low solubility in polar solvents, such as water, so fullerenes do not dissolve; if the stock solution of the fullerenes in toluene is directly added to the water, toluene will never mix with water and once toluene evaporates, fullerenes will precipitate without being in a water stable solution [27].

Free radicals from fullerenes dissolved in toluene take nonpolar molecules. When the stock solution in toluene is sonicated, fullerenes bonds with nonpolar molecules are broken and water molecules can replace them. Buckyball's radicals are then surrounded by water molecules (solvated). Ethanol favors radical reaction swaps (the radical exchange reaction). Ethanol is less polar than water and acts as an intermediate solvent between toluene and water. In this condition fullerenes can form colloidal aggregates that contain between 100 and 1,000 fullerene molecules.

Fullerenes clusters are more easily dissolved in water due to the high negative charge. When fullerenes are in agglomerate state cannot dissolve in toluene [49].

4.2.3 WATER SAMPLING, CHARACTERIZATION AND SAMPLE TREATMENT

Ultrapure water (UPW) was obtained by purifying demineralized water in an ELGA PURELAB Ultra ANMK2 system. Tap water was obtained from the town of Amsterdam (The Netherlands).

Water characteristic were electrical resistivity 18.2 MΩ-cm, quality I^+, temperature was 26.4ºC and TOC 001.

4.2.4 WATER INCUBATION

Ultrapure Water samples were spiked one by one with the C60 fullerene suspension, concentration n/C60 100 µg/L, and placed in the setting up under the UV-A(365 nm), UV-B (302nm) and UV-C (254nm) lamps. Samples were exposed for zero, one, three and seven days. Figure 17 shows the aqueous samples, concentration of 100 µg/L nC60, colorless. Samples and control tubes were without lids.

The set up was modified to place the lamps at 20cm from the samples. All the samples were located strictly in line in the central axis under the lamp to avoid shadows at ambient temperature (25ºC).

Each incubation was made in triplicate and with non-exposed to irradiation samples (dark controls).

Tubes used as dark controls were loosely wrapped with aluminum foil (to maintain the oxygen exposure but avoid the UV-light) and placed in a sample rack in the incubator. At specific time intervals, three irradiated samples from each batch (replicates) and three dark control samples were removed for analysis for a total of 12 samples each time.

Figure 17. Water Incubation set up.

Figure 18. Samples placed under the lamp.

4.2.5 LIQUID−LIQUID EXTRACTION

Liquid−liquid extraction was used to determine the concentration of the individual C60 fullerenes in the aqueous fullerene suspension. The Internal Standard nC70 was added to the samples, concentration n/C70 50.8 µg/L, to homogenize them, samples waited for 15 minutes. LLE was performed by adding to the 10 ml aqueous fullerene suspension (0.53 ml nC60 + 9.47 ml UPW) and the internal standard, 5 ml of toluene, and 1 ml of $Mg(ClO_4)_2$, 0.5M, to the vials [31] [50]. This solution was shaken at 160 rpm for 60 minutes to allow extraction of the fullerenes into the toluene phase. By difference of density between toluene and water, C60 fullerenes in toluene were separated from the water solution and kept apart in a vial.

4.2.6 RESULTS AND DISCUSSION

An “initial recovery test” was not necessary due to the existence of related bibliography [49] [50] [51].

This experiment was able to quantify the degradation of the fullerenes in ultrapure water. Fullerenes were added in concentration of 100 µgC60/L UPW, 10 ml volume and 3cm of water layer and exposed for zero, one, three and seven days to UV-A, UV-B and UV-C.

The concentration in the samples exposed to light decreased with the time. Samples degradation by UV-A for one day were the 2.21% of the initial concentration of fullerenes (95.0 µgC60/L UPW) for 3 days the 25.47% and for seven days the 38.95%.

After one day of incubation, samples were degraded by UV-B the 2.63% of the initial concentration (95.0 µgC60/L UPW) for three days the 24.53% and for seven days the 71.79%.

Samples degradation by UV-C for one day was the 10.0% of the initial concentration of fullerenes (95.0 µgC60/L UPW) for 3 days the 75.26% and for seven days the 86.95%.

Degradation rate by UV-A and UV-B radiation in the first 24 hours was similar and low in both cases (2.21 and 2.63% respectively). However, fullerene degradation speed by UV-C was higher, 10.0%. Until the third day UV-A and UV-B degradations were similar, degradations were much higher than in the first day though (23.26% and 21.90%). Fullerene C60 degradation rate under UV-C light was really fast until the thirst day, for 48 hours was degraded the 65.26% of the total amount. From the forth day fullerenes degradation speeds by UV-A and UV-B radiation were very different. Fullerenes degradation speed under the UV-A light decreased in the last four days, it was 13.48%, under the UV-C light degradation speed increased until 47.26%. Fullerenes degradation speed under UV-C radiation strongly decreased in the last forth days.

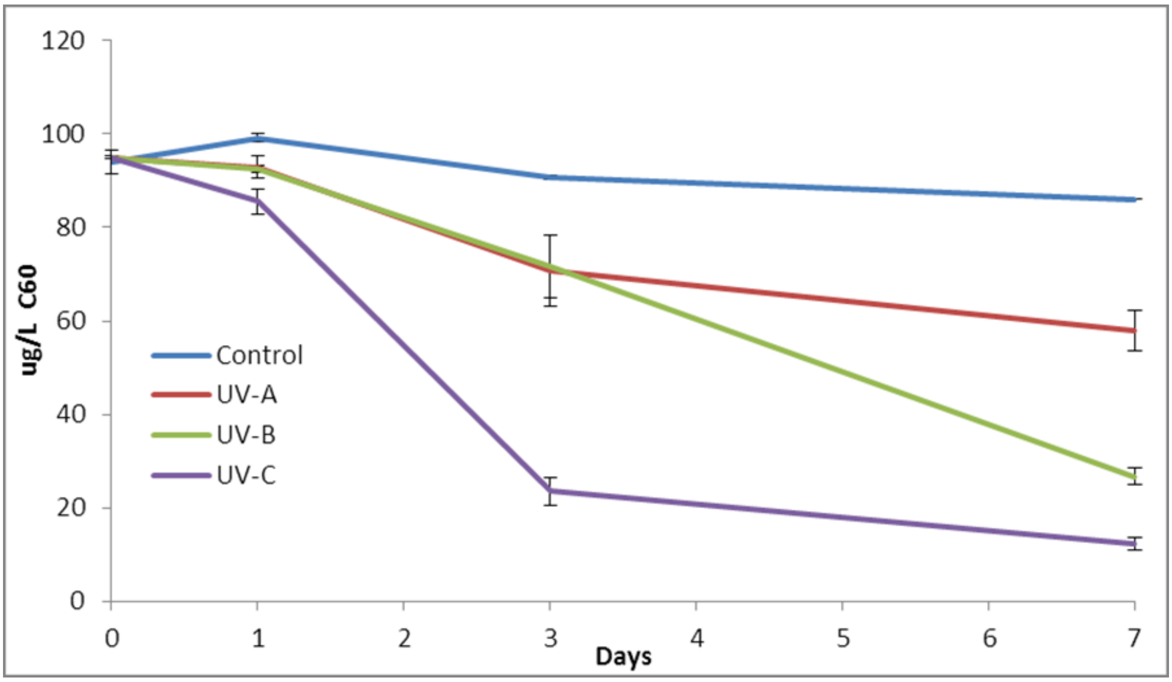


Figure 19. Results of the ultrapure water experiment.

In the present work, the losses of fullerenes were higher when the samples were incubated under UV-C. The samples under UV-A and UV-B showed a similar behavior in the first three days with comparable losses. However, after three days the losses of C60 in the UV-B irradiated samples were much higher than those in UV-A. The half of the degradation samples (50%) caused by UV-B, UV-C radiation happened during this experiment, however UV-A radiation did not degrade so much the samples.

This study demonstrates that nC60 in the aqueous phase undergoes photochemical transformation by short-wavelength UV irradiation in the presence of oxygen; as seen in figure 19, samples under the UV-C radiation (the most energetic) were clearly the most degraded. As Lee reported in an article, after 110h of UV irradiation nC60 still absorbs strongly [51]. All samples tended to a total degradation.

In the present study, recoveries of 90.63% were obtained in dark samples (control) after seven days incubation. Control samples losses were caused by the oxidation of the matrix (UPW). Losses during the process can cause error (standard deviation).

4.3 DEGRADATION IN NO MATRIX

4.3.1 NO MATRIX INCUBATION

In this experiment the effect of UV irradiation on fullerenes was tested without the presence of soil or water matrices. Fullerenes dissolved in toluene were directly spiked onto the Petri dishes. The solvent was let to evaporate and the incubation was achieved with a similar set up as described above. In order to prevent the losses of fullerenes from the Petri dish, the samples were covered with quartz domes (dome shaped). In these conditions, the pristine C60 in absence of any matrix was exposed to the UV light (UV light can penetrate quartz). However it is possible to expect that traces of toluene were still pre=sent on the surface of the C60.

Samples were exposed for zero, two hours, one day and seven days under the UV-A, B, C lamps.

Each incubation was developed in triplicate and with non-exposed to irradiation samples (dark controls). Petri dishes used as dark controls were closed with their glass lids and wrapped with aluminum foil to avoid the UV-light.

4.3.2 EXTRACTION

Five milliliters of clean toluene were added to every Petri dish and then transferred into a glass tube. The procedure was repeated two times for a final volume of 10 ml. The extracts were filtered with 0.45 µm regenerated cellulose syringe filters and moved to a 10 ml vial. All experiments were performed in triplicate and dark samples (control) were extracted with the same protocol as reference.

Figure 20. No matrix incubation set up.

Figure 21. Dome shaped lids over the samples.

4.3.3 RESULTS AND DISCUSSION

When the samples were exposed to UV-A light a 4.21% loss in the initial concentration (95.1 μgC60/L) was observed after 2 hours. After one day the loss was 42.90% and after seven days was 51.63%.

After two hours of incubation samples exposed to UV-B light were degraded in two hours the 18.40% of the initial concentration (95.1 μgC60/L), in one day the 26.92% and in seven days the 65.51%.

When samples were exposed to UV-C light a 15.56% loss in the initial concentration of fullerenes (95.1 μgC60/L) was observed after 2 hours. After one day the loss was 33.33% and after seven days was 50.58%.

After two hours of incubation UV-C seemed to have the biggest effect on C60, but after one day of incubation UV-A had the strongest effect. Eventually, the biggest effect after seven days seemed to be caused by UV-B. All irradiations present a similar trend.

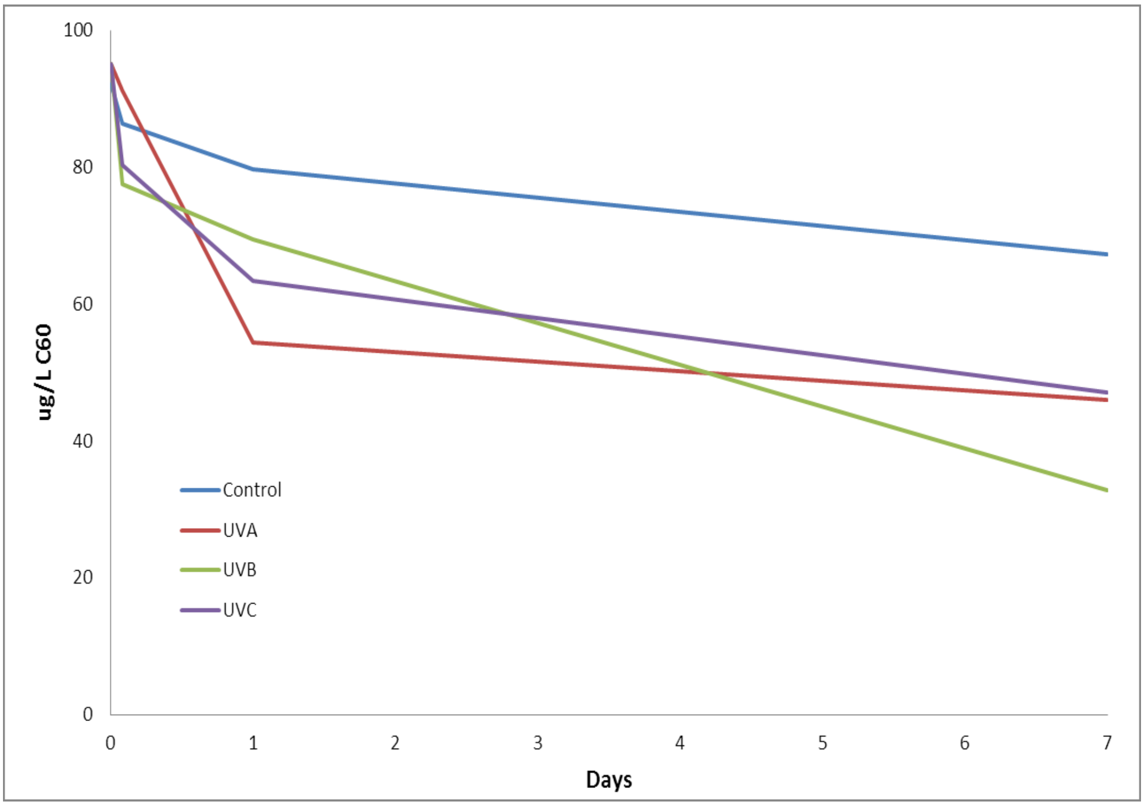


Figure 22. Results of the no matrix experiment.

C60 in the dark control samples were more degraded than those in soil control samples (27.04% and 16.21% respectively). However, irradiated no matrix samples by UV-A, B, C were less degraded than irradiated soil samples, the difference was 33,00% for UV-A, 15,64% for UV-B and 26,73% for UV-C.

UV irradiated no matrix samples results were lower than soil samples. No matrix control samples are higher than control soil samples. This could be due to oxidation which is more favorable when no matrix is present. Also, losses are more likely to happen since the fullerenes are not adsorbed to the soil and can be easily lost during the processing.

4.3.4. SOIL VS NO MATRIX COMPARATION

Dark controls in the no-matrix experiment showed larger losses than the dark controls in soil incubation. Specifically, the losses of C60 in the no-matrix controls were 10,83% more than those of the soil controls. On the other hand, the losses of C60 in the no-matrix samples were lower than those of soil samples at all the wavelength tested and respectively 33% more for UV-A, 15,64% for UV-B and 26,73% for UV-C.

There is an effect of the irradiated soil matrix in the degradation of fullerenes, as shown in the table 8. The degradation rate is the same for all the UV-A, B and C (it does not happen in water). Thus, the degradation in soil is not only because of a direct effect on the C60.

Probably, UV-light causes changes in the organic matter that in turn has an effect on the C60. If the UV-light was directly degrading the C60 we would expect a behavior like the one in water where UV-C has a stronger effect than UV-A.

SAMPLES	**% Degradation**			
	2 hours	**1 day**	**7 days**	**28 days**
CONTROL Soil 4 mm thick			3.60	
CONTROL Soil 1,8 mm thick			16.21	44.06
CONTROL No matrix	6.30	13.57	27.04	
UV-A Soil 1,8 mm thick			84.63	99.04
UV-A No matrix	4.21	42.90	51.63	
UV-B Soil 4 mm thick		16.17	46.71	
UV-B Soil 1,8 mm thick			81.15	97.60
UV-B No matrix	18.40	26.92	65.51	
UV-C Soil 1,8 mm thick			77.31	96.52
UV-C No matrix	15.56	33.33	50.58	

Table 8. Comparative results from soil and no matrix results.

5 CONCLUSIONS

Effect of UV light irradiation on fullerenes in the environment focus on the UV-A and UV-B electromagnetic bands, these two are the part of the sunlight which is able to go through the ozone layer to the surface of the Earth. Fullerenes degradation by UV lights depends on the radiation intensity of the source and the distance with the samples. Fullerene C60 degradation is mainly developed for the first seven days of exposition in the top layer surface of the soil (UV-A 84.63%, UV-B 81.15%, UV-C 77.31%), and in water (UV-A 38.25%, UV-B 71.79%, UV-C 86.95%), relatively fast degradation - half-life: 2-4 days. These results confirm a clear effect of UV light in the different environmental matrices [50].

Putative transformed products identification was not the target of the present work. The detection method HPLC-UV is not able to identify them, it is a quantify method. Fullerenes extraction was carried out by toluene. In the present work the HPLC-UV analysis didn’t provide a clear evidence of the formation of transformation products (e.g. chromatographic peaks at different retention times). For such analysis a more specific HPLC-MS analysis would be needed.

C60 degradation in soil samples seems to be influenced by the matrix, i.e. the UV-light may cause modification in the soil matrix that in turn causes the losses of C60. This phenomenon may explain why the same rate of degradation is observed when very different UV light (UV-A, B C) are applied.

In water, nC60 degradation seems a direct effect of UV light, where the most energetic radiation (UV-C) has the strongest effect. The average size of the clusters and their concentration gradually decreased as the exposition time rises [51] [52].

C60 degradation in no matrix samples is a direct effect of UV light. Fullerenes in these conditions are transformed by oxidation reactions. Irradiated no matrix samples by UV-A, B, C were less degraded than irradiated soil samples, the difference was 33,00% for UV-A, 15,64% for UV-B and 26,73% for UV-C. Also, losses are more likely to happen since the fullerenes are not adsorbed to the soil.

The present researching project contributes new details of irradiated fullerenes in top sandy soil and in water, where it shows that the obtained results agree with the referenced works.

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