

Contamination of endemic plants of Amazonia by ubiquitous pollution: Persistent organic pollutants (POPs) determination by GC-MS/MS

Stefano Falcinelli¹, Martino Giorgini², Bartolomeo Sebastiani³

¹ University of Perugia, Department of Civil and Environmental Engineering, Perugia, 06125, Italy

² "Vis Medicatrix Naturae s.r.l." – 50034 Marradi (FI), Italy

³ University of Perugia, Department of Chemistry, Biology and Biotechnology, Perugia, 06123, Italy



VIS MEDICATRIX NATURAE
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We report on the results of an analytical investigation about the contamination on three official plants of Amazonian rain forest due to Persistent Organic Pollutants (POPs), like organochlorine pesticides and polychlorinated biphenyls (PCBs).

POPs : "Persistent Organic Pollutants"

They are largely widespread in the world:

- Pesticides organochlorinated
- Polichlorobiphenils (PCB)

High stability

Volatility

Substance	Persistence Index
Chlordan	> 12
DDT	> 10
Dieldrin	> 8
Aldrin	> 8
Heptachlor	> 9

Bioaccumulation

Apoparity

"Grass Hopper effect"

- Evaporation at high temperatures
- Transportation towards cold areas
- Condensation and precipitation

Bioaccumulation

POPs are apolar and lipophile molecules

They accumulate in the adipose tissues and they are slowly metabolized

Organochlorinated pesticides

DDT and metabolites (DDE, DDD)

Structure

Substance	LD50 (mg/kg)	LD50 (mg/kg)	LD50 (mg/kg)
DDT	100	100	100
DDE	40	40	40
DDD	10	10	10

Uses

Pesticide in agriculture
Against insect vectors of diseases

Effects

Acts at the level of the nervous system
Inhibits reproduction in birds
Moderately toxic in humans

The analyzed plants - "balsamina" (Impatiens Balsamina), "mullaca" (Physalis Angulata) and "graviola" (Annona Muricata) - come from the Amazonian forest of the Peru where they grow spontaneously. All the three plants are well known for their numerous therapeutic properties and commonly they are used in the traditional medicine against several diseases like cancer, fever, diabetic, etc.

Official plants analyzed

Graviola	Mullaca	Balsamina
Family: Annonaceae Genus: Annona Species: Muricata	Family: Solanaceae Genus: Physalis Species: Physalis Angulata	Family: Balsaminaceae Genus: Impatiens Species: Impatiens Balsamina
Acetogenins: Anti-cancer Antibiotic, parasiticide, Diabetic, diarrhoea, etc.	Physalis B + D: Anti-cancer and Antibiotic Narcotic effect, Anti-inflammatory & diuretic	Peptides: Ib-AMP, Ib-AMP Fungicide and antimicrobial Therapeutic

Used Instrument

GC 3800

SATURN 2200 MS/MS

by Varian Inc.

Analytical procedure

Samples preparation: *ISTISAN 99/23 and 99/28 Reports*

- From 1 kg of dried leaves of each plant
- Selection of 100 g of plants material by quarantine technique
- Mechanical trituration by ULTRA TURRAX T25
- Weighing of 5,519 g of "balsamina", 9,028 g of "mullaca" and 7,532 g of "graviola"
- Soxhlet extraction during 24 h with hexane:acetone (1:1, v/v)

Samples purification: *ISTISAN 99/28 Reports*

- Solvent evaporation until small volume (2-3 ml) by Rotavapor (30°C)
- purification by chromatography on celite column eluted with n-hexane

Analytical procedure

Analysis GC-MS/MS:

- The eluate from the chromatographic column purification are concentrated by Rotavapor and completely dried by N₂ flux at 30°C
- Injection of 1 µL of purified sample dissolved in 25 µL of isoctane
- Operative conditions of GC:
 - Capillary column CP-SIL8 (30m x 0.25mm and film thickness 0.25µm);
 - Thermal program: 150°C for 4 min, 150-200°C with 6°C/min and after with 200°C for 5 min;
 - Injector: 250°C - "splitless mode";
 - Transfer line: 240°C.

A portion of about 10 g for each vegetable sample was submitted to extraction procedure with hexane-acetone (1:1, v/v) solution by using soxhlet technique for 24h, following procedures by ISTISAN 99/28. The three extracts were analyzed by Gas Chromatography-Tandem Mass Spectrometry (GC-MS/MS) and Multi Reaction Monitoring (MRM) techniques (by using ion trap detector Varian Saturn 2200 system) after their purification by chromatography on celite column eluted with n-hexane (ISTISAN 99/25). The three extracts were analyzed by Gas Chromatography-Tandem Mass Spectrometry (GC-MS/MS) and Multi Reaction Monitoring (MRM) techniques (by using ion trap detector Varian Saturn 2200 system) after their purification by chromatography on celite column eluted with n-hexane (ISTISAN 99/25)

Results

GC-MS/MS profile of pesticides standard solution

Peak	Compound	m/z
1	Hex-Cl	284
2	Hexachlorobenzene	284
3	Hex-Cl	284
4	Hex-Cl	284
5	Hex-Cl	284
6	p,p-DDE	240
7	p,p-DDD	240
8	p,p-DDT	240
9	p,p-DDD	199
10	p,p-DDT	199
11	p,p-DDT	199

Quantitative analysis (pesticides)

GC-MS/MS profile of "GRAVIOLA"

MS/MS spectrum of Hexachlorobenzene

MS/MS spectrum of p,p-DDD and p,p-DDT

Chromatogram

Quantitative analysis (pesticides)

GC-MS/MS profile of "MULLACA"

MS/MS spectrum of p,p-DDE

MS/MS spectrum of p,p-DDD and p,p-DDT

Chromatogram

Quantitative analysis (pesticides)

GC-MS/MS profile of "BALSAMINA"

MS/MS spectrum of p,p-DDE

MS/MS spectrum of p,p-DDD and p,p-DDT

Chromatogram

Synoptic table of organochlorinated pesticides

By AMD ("Automated Methods Development")

Compounds	Molecular weight	Parent Ion Mass	Window	Waveform	CID (V)
α-Lindane	290.83	219 [M-Cl]	1	Resonant	0.75
β-Lindane	290.83	219 [M-Cl]	1	Resonant	0.75
γ-Lindane	290.83	219 [M-Cl]	1	Resonant	0.75
Hexachlorobenzene	284.78	284 [M-2]	1	Resonant	2.00
Heptachlor	373.32	272 [M-C ₂ H ₂ Cl]	1	Resonant	1.00
p,p-DDE	318.02	318 [M-2]	1	Resonant	2.00
p,p-DDD	318.02	318 [M-2]	1	Resonant	2.00
p,p-DDT	354.48	235 [M-C ₂ Cl ₂]	1	Resonant	1.50
p,p-DDD	328.04	235 [M-CH ₂ Cl]	1	Resonant	1.50
p,p-DDT	328.04	235 [M-CH ₂ Cl]	1	Resonant	1.50

Conclusions and future developments

- Presence of DDT and metabolites (DDE e DDD) in each plants samples
- Total concentration of pesticides in the range of ppb (µg/kg)
- Laws limits of pesticides for aromatic herbs: 1 mg/kg
- The herbs are able to be used in herbal preparations
- The presence of contaminants is not due to a direct use of pesticides
- Ubiquitary pollution
- Measurements of samples taken at different times of the year
Extending the analysis over a larger sample of plants

The obtained results show the presence of DDT and its metabolites, like DDD e DDE in the three analyzed samples and of hexachlorobenzene only in the "graviola" (0.041 µg/kg). The total POPs quantities are in the concentration range of ppb, varying from 0.349 and 0.614 µg/kg respectively for "mullaca" and "graviola", up to 2.328 µg/kg in the case of "balsamina". These concentration values are an indication of a plants contamination in trace due to the organochlorinated pesticides which come from an ubiquitous pollution and not from a direct use of these substances in the place of origin of the samples. Finally, concerning the PCBs determination, the analysis GC-MS/MS excludes their presence in all the three plants investigated: the concentration values are in this case lower than the detection limits of the method (0.003-0.013 µg/kg).