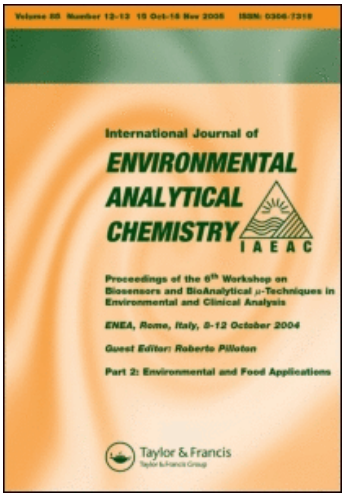

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

An Evidence for Nitrophenols Contamination in Antarctic Fresh-Water and Snow. Simultaneous Determination of Nitrophenols and Nitroarenes at ng/L Levels

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To cite this Article Vanni, Adriano , Pellegrino, Vincenzo , Gamberini, Roberta and Calabria, Adelaide(2001) 'An Evidence for Nitrophenols Contamination in Antarctic Fresh-Water and Snow. Simultaneous Determination of Nitrophenols and Nitroarenes at ng/L Levels', International Journal of Environmental Analytical Chemistry, 79: 4, 349 — 365
To link to this Article: DOI: 10.1080/03067310108044394
URL: http://dx.doi.org/10.1080/03067310108044394

Intern. J. Environ. Anal. Chem., Vol. 79(4), pp. 349-365
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AN EVIDENCE FOR NITROPHENOLS CONTAMINATION IN ANTARCTIC FRESH-WATER AND SNOW. SIMULTANEOUS DETERMINATION OF NITROPHENOLS AND NITROARENES AT ng/L LEVELS

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(Received 10 January 2000; In final form 12 May 2000)

A selective method for simultaneous extraction and analysis of nitroarenes and nitrophenols at ng/L levels from water samples is presented. Discontinue Liquid-Liquid extractions followed by a pre-concentration step (Enrichment Factor =1000) were performed to separate and extract sixteen nitrophenols (NP) and nitroarenes (NA) from aqueous matrices, before a RP-HPLC and a FID-HRGC analyses, respectively. Limits of detection were from 5 to 30 ng/L for NP and from 0.1 to 1 μg/L for NA. The procedure was employed to investigate the supposed presence of predicted analytes in Antarctic water samples (lake water and snow) collected during the last Italian expeditions of Antarctica Research National Project (PNRA). An evidence for 4-nitrophenol and 2-nitrophenol trace levels pollution, carried out by this method, was confirmed by LC-DAD-MS analysis. Some hypotheses concerning the sources of such a contamination are discussed.

Keywords: Pollution; nitrophenols; nitroarenes; Antarctic environment; lake water; snow

INTRODUCTION

Nitroarenes and nitrophenols represent an important group of pollutants because of their large use in various industrial processes with a subsequent massive discharge in wastewaters and athmosphere. Both of them are widely employed as intermediates in the chemical industry for preparation of dyes, perfumes, synthetic resins, explosives, pesticides, drugs and so on[1,2,3]. Moreover, nitrophe-

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nols (NP) are used as wood preservatives, herbicides or insecticides. Some are degradation products of other pesticides, like 4-nitrophenol, a well-known metabolite of Parathion[4]. Nitroarenes (NA) are suspected carcinogen agents; moreover, they may be oxidative products of benzene, a Group 1 carcinogen[5]. Phenol and its nitro- and chloro-derivatives are able to induce damage of cellular proteins and uncoupling of oxidative phosphorilation; they mainly affect the central nervous system, kidney function and the circulatory system leading to a severe poisoning even in low concentrations [6]. Because of their toxicity, many of these substances are listed by the United States Environmental Protection Agency (US.E.P.A.) and the European Community (E.C.) as priority pollutants and are controlled by legislation to strict admissible concentrations in drinking and bathing water [7,8].

The good stability and low vapour pressure of nitrophenols together with their polarity, and the semi-volatile nature of nitrobenzenes, are risk factors for their worldwide dispersion through atmospheric precipitations. In largely populated agricultural or industrial countries nitrophenols were found in rain and snow samples, river water and sediments[9,10], whereas nitrobenzenes and their chloro-substitutes were documented in water, soil, sediments and even in fish samples, at relatively high concentrations[11].

The Antarctic Continent, very far from human productions, biologically poor and particularly isolated by the Circumpolar Atmospheric Circulation, should be an uncontaminated territory, with few traces of organic compounds of biogenic origin, almost exclusively due to coastal and sea wildlife. Unfortunately, traces or ultra-traces of anthropogenic organic substances, such as PCB, chlorinated pesticides, PAH and other pollutants have already been described in different antarctic samples by many authors[12–14].

However, very little is known about nitro-monoaromatics in the antarctic environment, despite their worldwide diffusion. The aim of our study, submitted to a major bio-geochemical and physical monitoring Italian project called Antarctica Research National Project (PNRA), is assessing the presence of NA or NP in snow and lake-water samples collected during last years' Italian expeditions to Antarctica in the area of the Italian National Base of Terra Nova Bay, (Ross Sea-Victoria Land, Figure 1). In order to screen a wide range of nitroaromatic pollutants, our research involved some of the most diffuse and EPA-listed NA and NP. In this paper, we describe a sensible and selective method for determination of sixteen nitroarenes and nitrophenols (Table I) from water, particulate lake suspension, and melted snow samples. To achieve a grade of sensibility compatible with trace or ultra-trace levels of these analytes in antarctic samples, selective liquid-liquid extractions were employed to gain suitable enrichment factors before analysis. Nitroarenes were first analyzed for a screening study by

GC-FID, then followed by GC-MS confirmation; nitrophenols, on the other hand, were analyzed by HPLC with UV-Vis detection and then submitted to a confirmation by DAD-MS detection.

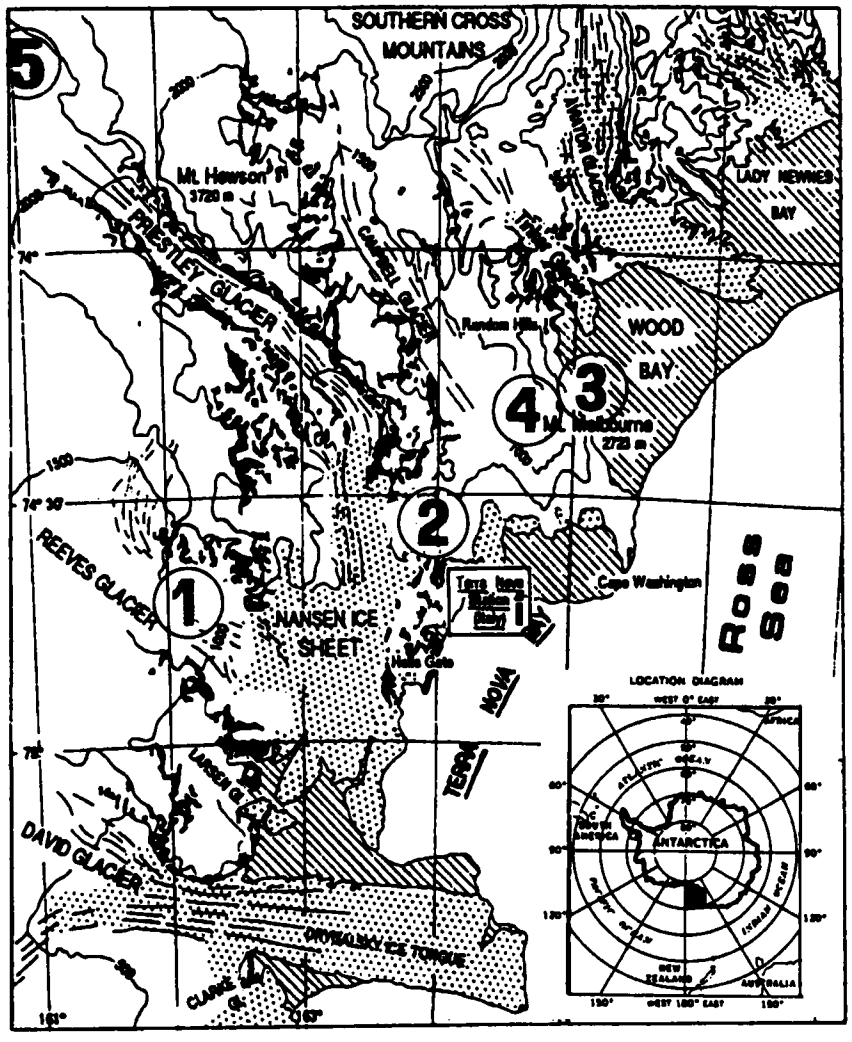

FIGURE 1 Geographic localisation of Terranova Bay, PNRA Antarctic Italian Base and of Sampling Stations. 1 Tarn Flat Lake(Lat. 74°58'S-Long. 162°31'E) 2 Carezza Lake (Lat. 74°43'S – Long. 164°01'E) 3 Edmonson Point (Lat. 74°19'S-Long. 165°04'E) 4 Mount Melbourne (74°31'-74°23'Lat. S; 164°46'-164°42'Long. E) 5 Rennick Glacier (73°23'-72°53'Lat. S; 160°15'-159°53'Long. E)

TABLE I Extraction recovery and limits of operative detection (LOD) for Standard Mixture Nitrophenols + Nitroarenes. Analytical conditions of Table II-III

Analyte (Nitrophenols)	*Recovery%*				R_t	*LOD ng/L*
	50ng/L	*(RSD%)*	*500ng/L*	*(RSD%)*		
2,4-Dinitrophenol	74.4%	(12.8%)	79.3%	(10.6%)	3'04±0.07	5
3,4-Dinitrophenol	66.3%	(11.0%)	70.2%	(9.5%)	3'24±0.10	5
2,5- Dinitrophenol	75.0%	(17.1%)	74.8%	(10.4%)	3'64±0.19	30
4-Nitrophenol	43.5%	(10.3%)	51.9%	(8.8%)	4'16±0.30	5
2-Methyl-4,6-Dinitrophenol	46.6%	(19.6%)	52.5%	(11.2%)	5'00±0.23	10
2,4,6-Trinitrophenol	38.5%	(21.7%)	49.8%	(14.5%)	5'95±0.34	20
2-Nitrophenol	89.6%	(16.3%)	91.2%	(7.7%)	7'88±0.60	20
3-Methyl-4-Nitrophenol	82.4%	(17.7%)	88.7%	(8.4%)	8'72±0.67	10
2-secButil-4,6-Dinitrophenol	87.9%	(7.3%)	92.1%	(7.1%)	19'48±0.88	10
Phenol[a]	63.3%	(13.5%)	65.6%	(9.4%)	4'00±0.09	30
3-Nitrophenol[a]	54.5%	(18.4%)	66.7%	(10.5%)	7'28±0.23	20
Analyte (Nitroarenes)	*Recovery%*				R_t	*LOD ng/L*
	1 µg/L	*(RSD%)*	*10µg/L*	*(RSD%)*		
Nitrobenzene	62.5%	(3.2%)	64.3%	(4.0%)	9'57±0.02	100
1-Chloro-2-Nitrobenzene	80.0%	(4.1%)	79.8%	(2.3%)	11'76±0.01	500
3,4-Dichloronitrobenzene	79.0%	(5.2%)	82.6%	(4.9%)	13'35±0.02	100
2,3-Dichloronitrobenzene	50.4%	(2.3%)	60.1%	(2.6%)	13'46±0.01	100
1,3-Dinitrobenzene	86.5%	(3.2%)	87.9%	(1.8%)	13'97±0.02	1000

Average values refer to five independent trials (n=5). Signal / Noise ratio= 2,5

a. For phenol and 3-nitrophenol determination was carried out at λ= 280 nm.

EXPERIMENTAL

Sampling

Lake-water samples were taken from some Lake Stations near the Italian Base at Terra Nova Bay: Carezza Lake, Tarn Flat Lake and Edmonson Point. Fresh-snow samples were taken from two Stations: Melbourne Mount, along a transept at

three altitudes, (360 –710 – 1490 mt.) and Rennick Glacier, also at three altitudes (1350 –1650 –2100 mt.). Lake water was collected 30–50 cm deep, in two-litre polyetylene screw-capped bottles, immediately frozen at –20°C and kept at this temperature until the analysis procedure.

Snow was melted before freezing and storage at –20°C in the bottles. Before use, polyetylene containers were washed three times with a 5% Extran solution (Merck, Darmstadt, Germany), then rinsed with warm tap-water, deionized and ultra pure water, in order to avoid pre-sampling organic contaminations, finally heater-dried at 70°C.

Each sample was melted and filtered once by 47 mm.-0.45 μm HAWP filters (Millipore, Bedford, MA, USA) just before the extraction procedure. Filtration residues were collected, air dried and then weighed before analysis procedure. Figure 1 shows the sampling points near the Italian Antarctic Base.

Chemicals

Nitrophenols were purchased from Fluka (Buchs, Switzerland.), Sigma – Aldrich (Milwaukee, WI, USA) and Riedel de Hæn (Seelze, Germany.). Nitrobenzenes were from Sigma-Aldrich, except nitrobenzene (Alltech Associtates, Inc.) and 3,4-dichloronitrobenzene (Dr. Ehrenstorfer, Augsburg, Germany). All the standards were of analytical grade. Ultrapure water (Maxima apparatus Elga Ltd, England) was used to prepare the solutions. Methanol, acetonitrile, dichloromethane (Riedel de Hæn) were of chromasolv grade; glacial acetic acid (Merck, Darmstad, Germany) was of extra pure grade, sulphuric acid and sodium-hydroxide were analytical grade reagent from BDH (Pole, England). Tetrabutylammonium phosphate (PIC-A) and propylen-glycole were extra-pure reagents obtained from Carlo Erba.

Instruments

For a preventive screening of nitroarenes, a 5300HRGC Megaseries, (Carlo Erba Instruments, Milan, Italy) equipped with a FID detector was used; confirmation analysis were then performed by a GC-MS AutoHRGC -MS-QMD 1000 (CE Instruments).

Nitrophenols were first analyzed by a Merck (Darmstadt, Germany) L6200A HPLC pump system equipped with an Hitachi L 4250 UV-Vis Detector and, for confirmation, by an Hewlett-Packard Series 1100 (Palo Alto, CA, USA) LC-DAD-MSD System.

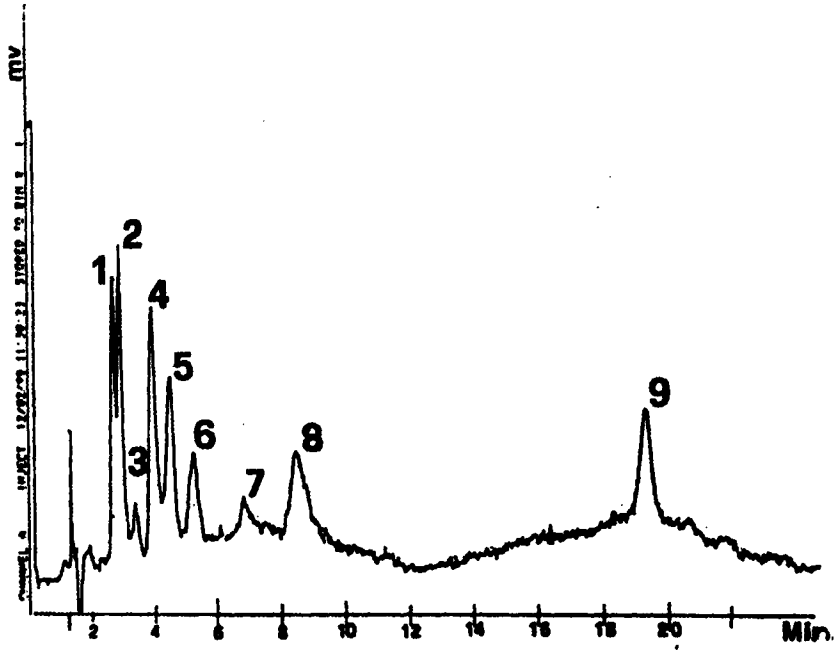

FIGURE 2 Standard solution of 30 ng/L of nine NP extracted according to Table III conditions. Peak Assignement, for 405nm. detection: 1) 2,4-Dinitrophenol 2) 3,4-dinitrophenol 3) 2,5-dinitrophenol 4) 4-Nitrophenol 5) 2-Methyl-4,6-Dinitrophenol (DNOC) 6) 2,4,6-Trinitrophenol (Picric Acid) 7) 2-nitrophenol 8) 3-Methyl-4-Nitrophenol 9) 2-secButyl-4,6-Dinitrophenol (DINOSEB)

pH measurements were made with an E-500 Metrohm Digital pH-Meter (Herisau, Switzerland). Extractions were performed by an Heidolph disperser (Schwabach, Germany) Diax 600 or by a magnetic stirrer (for lake water suspension). Pre-concentration steps were performed by a Kuderna-Danish-like concentration system and by a Rotavapor (Büchi, Flawil, Switzerland).

Extraction and enrichment processes

One of the crucial points in LL-extraction procedures is the choice of solvent. Previous works [11] have been reporting n-hexane as the best extractant for nitroarenes, but we found comparable extraction values with dichloromethane. On the other hand, acetone or methanol, utilized mainly for nitrophenols SPE [15], are not so suitable for nitroarenes; furthermore, they form azeothropes with aqueous solutions, a limiting factor for a free-loss evaporation step. Given the necessity of analyzing either NA or NP from the same aqueous matrix, we finally

chose dichloromethane, already widely utilized for NP[16], in order to obtain good recoveries for both kinds of substances without changing solvent between subsequent extraction processes.

In order to separate nitroarenes from nitrophenols, Aqueous matrix (1 L) was basified at pH 12 with NaOH 1N, before liquid-liquid discontinue (3x5 min.) extraction by Diax 600 disperser (8000 rpm) directly in a separatory funnel with 40–30–30ml of dichloromethane. The organic fractions were then collected and dehydrated with Na_2SO_4 before pre-concentration.

The solvent was then evaporated in a Kuderna-Danish-like apparatus, in a cooled bath at 50°C, until the residue was 1 ml (Enrichment Factor E.F.: 1000). 1μL of this extract was subsequently injected in FID-GC for analytical elution of NA (Figure 3).

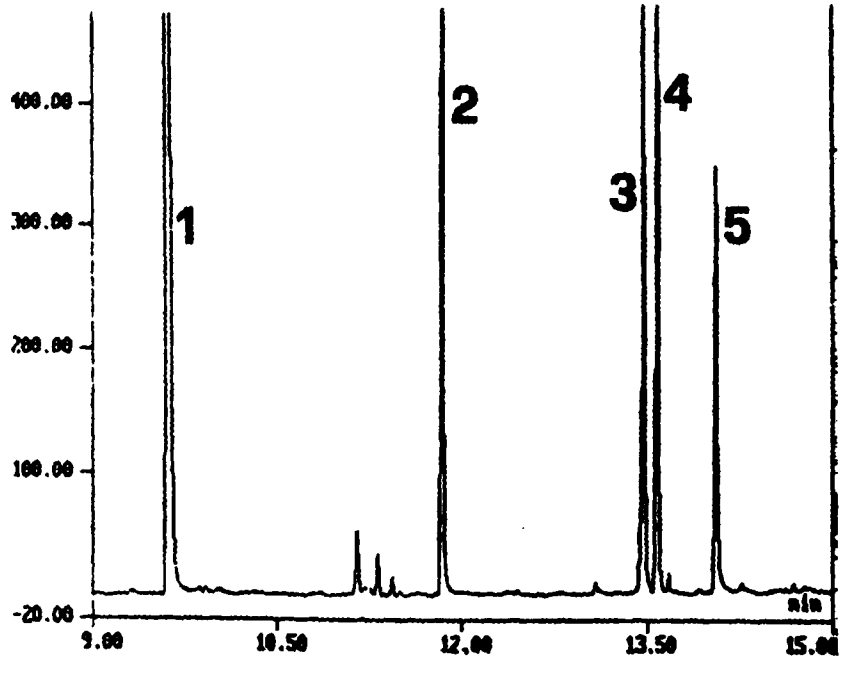

FIGURE 3 Standard solution of 1μg/L of five NA extracted according to Table III conditions. Showed Chromatogram Time – range: 9–15min. Peak assignement for GC-FID-detection: 1)Nitrobenzene 2)1-Chloro-2-Nitrobenzene 3)3,4-Dichloronitrobenzene 4)2,3-Dichloronitrobenzene 5) 1,3-Dinitrobenzene

Before GC-MS confirmation analysis, extracts were collected and further halved by evaporation, until reaching an Enrichment Factor of 2000. Moreover,

to obtain an increase of E.F., a SPME was attempted from extracts. After unsatisfactory attempts with a Polydimethylsyloxane[17] fiber, a Supelco 85μm-layer polyacrylate-coated fiber was used for this purpose. Briefly, an aliquot of the extract (500μl) was transferred in a 4 ml. screw-tread vial and evaporated in a weak N_2 flow, until dichloromethane was blown off. The residue was then resuspended in 2ml. of NaCl-saturated Ultrapure water (to obtain Salting Out effect) and submitted to a 20 min. microfiber-absorption by stirring (300 r.p.m.) and heating (60°C).

Desorption time was of 5 min. directly in the GC injection chamber. Unfortunately, extracts of micro-fiber absorption, in our conditions, were far from quantitative recovery of nitroarenes. The limited sensibility-gain arising from this further step was opposed by a remarkable increasing of matrix-effects in FID-chromatograms, emphasizing interference-peaks of substances with a major affinity for micro-fiber. Therefore, preventive analyses on nitroarenes were performed without this additional enrichment.

Aqueous matrix obtained from the first (NA) basic extraction was subsequently acidified at pH < 2 with sulphuric acid and then extracted by Heidolph Diax 600 disperser for recovery of nitrophenols. Discontinue extractions (5x10 min., 8000 rpm) were performed in the separatory funnel with 5x 40 mL of dichloromethane. Organic fractions were dehydrated by Na_2SO_4 and collected in a pear-shaped flask, as for NA.

TABLE II Extraction recovery values for a standard mixture of three NP at two different rates of backpressure. Analytical conditions of Table III

Analyte (50ng/L)	*25°C; – 40KPa*		*25°C; – 60KPa*	
	Recovery%	*(RSD%)*	*Recovery%*	*(RSD%)*
2,4-dinitrophenol	69.8%	(13.2%)	74.4%	(12.8%)
4-nitrophenol	70.9%	(4.6%)	43.5%	(10.3%)
2-nitrophenol	11.5%	(8.3%)	89.6%	(16.3%)

Average values obtained from five replicates.

To prevent loss of nitrophenols during the evaporation step, 70 μL of propylene-glycole were added, before connecting the flask to a Rotary vapor. Solvent was removed by a constant, moderate depression up to 0.5 ml. and the residual dichloromethane was finally blown off by a weak N_2 flow. Solvent evaporation is a discriminant for extraction processes, since it can determine a marked loss of analytes if the sum of temperature and backpressure leads to their distillation: in particular, 2-nitrophenol forms an intramolecular hydrogen bond and presents

opposite distillation recoveries to those of p-nitrophenols, producing instead extra-molecular hydrogen bonds[3]. Therefore, since recovery values of nitrophenols with diverging characteristics of polarity were differently conditioned much more by pre-concentration backpressure rates (as described in Table II) rather than by concentration levels of these analytes (Table I), in order to optimize pre-concentration conditions, which should yield acceptable recovery values for the whole of examined analytes, we opted for a compromise (25°C-60KPa; Table II).

TABLE III Chromatographic conditions and detection for Screening Analysis

Nitroarenes: Gas-Chromatograph	*Nitrophenols: HPLC Merck-Hitachi 6200L*
HRGC 5300 Megaseries Carlo Erba	Detector Hitachi L4250Uv-Vis
Column DB5 30m. x 0.32 mm. I.D.	Inj. Rheodyne, (Cotati, CA, USA)
Carrier H2 2ml/min	DP700 CE Instruments Integrator.
Make-Up N_2 65KPa	Column Lichrospher® 100 RP-18 (5μm) 250mm x3mm I.D
Inj. Temp. 275°C	Eluent solution B: CH_3OH 38% – H_2O 62%-
FID-Detector Temp. 300°C	PIC-A 5 mM 3% (pH 7.5); C: CH_3OH
Oven Temperature Program:	Vol. Injection 100 μl Flow 1ml./min.
50°C (5min)	Gradient:
15°C/min. to 250°C (5 min.)	0→8min. 100%B (isocratic).
15°C/min. to 275°C	8→15min. to 85%B
Inj. Split-Splitless (1:200). 1μl.,direct.	15→24min. 85%B
	Detection: 405vm wavelenght (280nm UV)

Chromatographic analysis

The extract was resuspended in 1,5 mL (Enrichment Factor 650) of eluent B, filtered by a 0.22μm Millex LLL (Millipore, Bedford, MA, USA) disposable filter and an aliquot (100μL) was injected on to HPLC (Figure 2). Since Athmospheric Pressure Chemical Ionization (APCI-MS) Mass Spectrometry required some different conditions (Table IV), for this analysis the extracts were resuspended in 1mL (E.F. 1000) of elution mixture (CH_3CN/ H_2O 50:50 v/v).

Particulate suspensions >0.45μm, collected from filtration of 2 lt of water samples, were air-dried (50–140 mg/L moist substance) for 1/2 hour and then extracted with 5 ml. of dichloromethane in a closed vial first by sonication (10 min.) and then by stirring (1 hour), previously adding a drop of diluted sulphuric

acid. The extract was subsequently dehydrated, filtered (0.22μm), added of propylene-glycole (50μL), and the solvent was finally evaporated by a weak N_2flow, before resuspension and analysis. For both nitroarenes or nitrophenols, we first developed such analytical conditions (summarized in Table III) to obtain a ng/L-level sensibility and a good selectivity for a "screening" quality-quantitative detection of analytes. Subsequently, we submitted "suspect positive" (e.g. Figure 4a, 4b) samples to a confirmation by mass-spectrometry, according to the conditions explained in Table IV.

TABLE IV Chromatographic and Analytical conditions for GC- and LC-MS Confirmation

Nitroarenes: AutoHRGC C E Instruments	*Nitrophenols: LC-DAD-MSD Series1100*
Column: DB5 30m × 0.25mm. ID film 0.25μm.	HewlettPackard
Carrier: Helium (60KPa)	Eluent: CH_3COOH 0.05M pH3 / CH_3CN
Injection: 1μl Splitless (40 sec.)	Gradient: 30% CH_3CN (2')→ 60% CH_3CN (10')
Detector: MSquadrupole MD 1000	Column: Lichrospher® 100 RP-18 (5μm) 250mm × 3mm I.D.
C E Intruments	
Ionization Source: Electron Impact (70eV)	Flow: 1ml / min. Injection: (Rheodyne) 20μl
Polarity: Positive Mode: Selected Ion Monitoring	Detector: MSDquadrupole Hewlett-Packard
	Ionization Source: APCI Polarity: Negative
	Mode: Selected Ion Monitoring

RESULTS AND DISCUSSION

In Antarctic Environment organic pollutants are usually present in trace or ultra-trace levels [12,13]. Before analysing samples, it is therefore necessary to ensure an appropriate pre-concentration of analytes, then reaching the required sensitivity of detection. Today, many extractive techniques such as liquid-liquid extraction (LLE), off-line and on-line solid phase extraction (SPE), Solid Phase Micro-Extraction (SPME) are available to perform good enrichments.

SPE-techniques are actually among the most employed extraction procedures because of their simplicity and solvents saving [9,18,19]. In a preliminary study, we utilised IEC-SPE, followed by an on-line pre-chromatographic focalization onto a C-18 Guard Column (Supelco, Bellefonte, USA), to obtain a selective extraction of some nitrophenols (2-NP; 2,4-DNP and 4-NP) and nitroarenes (1,3-DNB; NB). Whereas with the first we had good recoveries, the latter were

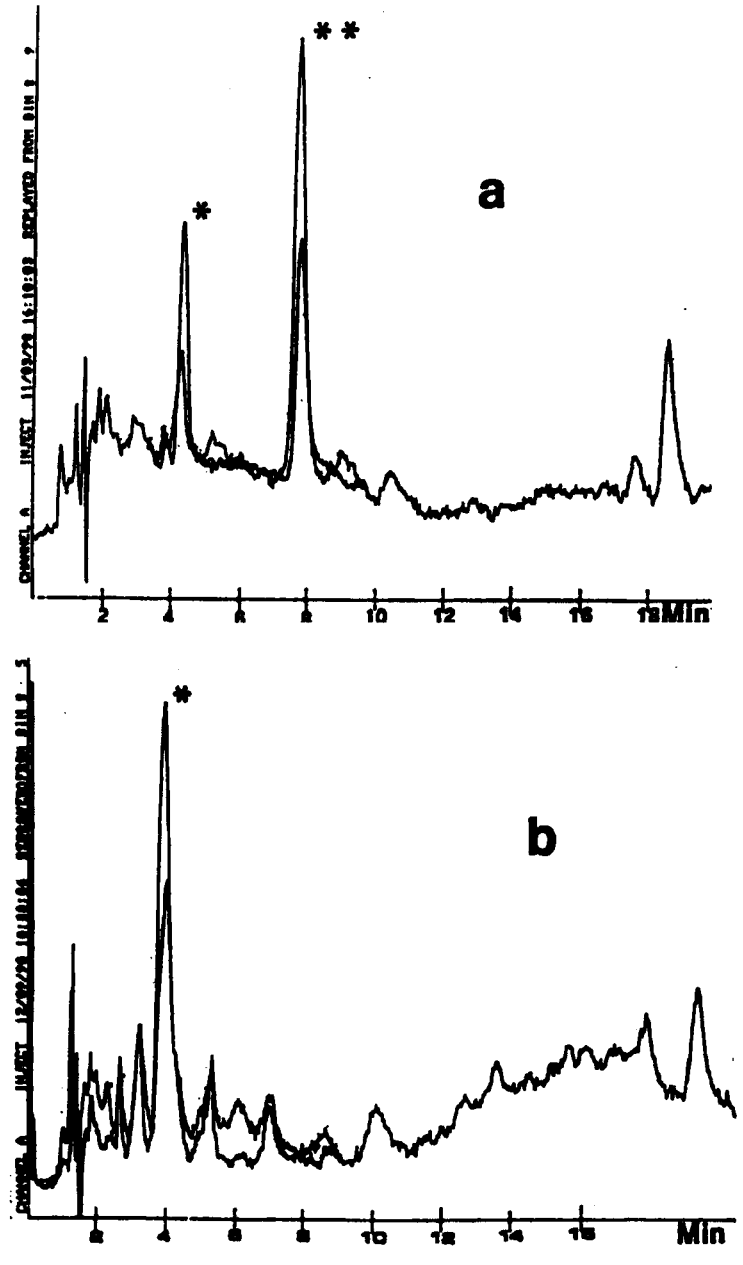

FIGURE 4 (a): Carezza Lake water 95/96: 4-nitrophenol and 2-nitrophenol, real and spiked with, respectively, 20ng/L * and 150ng/L * * of homologous Standard. (b): Edmonson Point water 96/97: 4-nitrophenol, real and spiked with 30 ng/L * (Analytical conditions of Tab. III)

almost totally lost in the extraction phase. Direct SPME on untreated matrices, tested with different microfibers (polydimethylsyloxane; polyacrylate) and performed by HPLC- and HRGC-desorption, did not lead to a satisfactory result, confirming the difficulty to obtain simultaneous selective extractions of NA (nearly apolar) and NP (polar and ionizable) from the same matrix for distinct analyses, without cross-interference or lost of analytes. In our study, instead, this was a necessary condition, as a consequence of limited availability of antarctic water samples. With sequential discontinue liquid-liquid (LL) extractions [10,16] we found a better correspondence for all these requirements.

As shown in Figures 2 and 3 and reported in Table I (R_t), after preventive extraction and pre-concentration steps, we obtained an effective chromatographic separation for eleven NP and five NA in our analyses. Recovery values yielded by the described procedures, for both nitrophenols and nitroarenes, are summarized in Table I. Extractive efficiency was generally better and more reproducible for nitroarenes. As previously mentioned in the Experimental section, this is in agreement with the non-polar nature of dichloromethane, the solvent utilized for extractions, which is more suitable for NA than for NP. Nevertheless, nitrophenols recovery and their Relative Standard Deviations are satisfactory, if we consider the very low detection limits (LOD) obtained for most of them.

The semi-quantitative recoveries of some compounds, such as 4-nitrophenol, pointed out by other works[20], are also probably due to the enrichment-evaporation step conditions (Tables I-II) described in the Experimental section.

In Table V the analytical results on antarctic lake water and snow collected during 94 / 95 – 98/ 99 Italian P.N.R.A. expeditions are reported. They assess the effective presence of 4-nitrophenol (4-NP) and 2-nitrophenol (2-NP) in lakes and of 4-nitrophenol in snow. MS-confirmation was performed monitoring 138 and 139 m/z peaks by athmospheric pressure chemical ionization (A P C I) in the negative polarity mode (Table IV), according to Jauregui et Al.[21]. For higher concentration values (e.g. 94/95 Carezza Lake water, 2-nitrophenol: purity grade 996.7) DAD iso-absorbance maps indicated appreciable purity values. For lower concentrations, because of background noise, the system was not able to evaluate enough data for this parameter. Figures 5a -5b show DAD spectra of detected analytes in some water samples. Figures 6a-6b report the Total Ionic Current -MSD profiles of the same. 4-Nitrophenol was ubiquitous in lakes, with higher values in the Carezza Lake water (70–30 ng/L) and lower in the Tarn Flat lake water (10–20 ng/L). On the other hand, minimal but significative (5–15 ng/L) amounts of 4-NP were verified in snow samples, except for Mount Melbourne's snow which was collected at higher altitudes. 2-Nitrophenol sporadically appeared in lake waters above detection limits: a particular situation was found

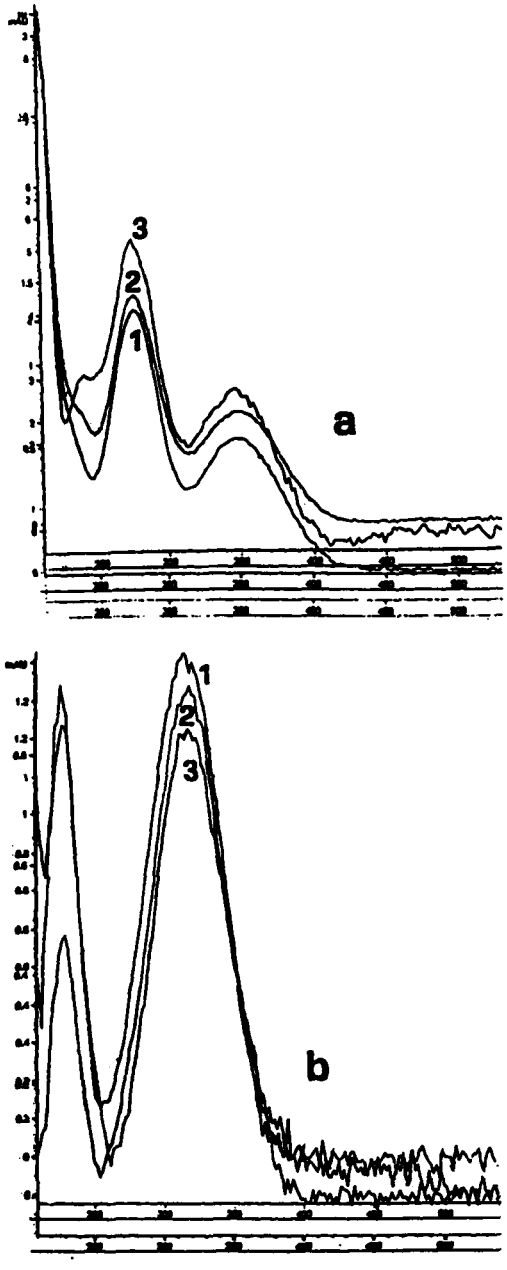

FIGURE 5 (a): 2-Nitrophenol DAD spectra relative to Carezza Lake 94/95(1) and 95/96(2) water, compared with 500ng/L 2-Np Standard(3). (b): 4-Nitrophenol DAD spectra of Carezza Lake water 94/95 (1) and 95/96 (2). 4-Np Standard (3) spectrum is referred to 80ng/L

in the Carezza Lake 94–96 waters, with noteworthy mean concentration values (200–450 ng/L) of this last compound which, however, had a noticeable negative trend in its concentration values during 94–99 period. No 2-NP instead was found in snow samples above LOD.

TABLE V Analytical results for lake waters, suspensions and snows sampled during the 94–99 Antarctic Italian Expeditions. Average values and Standard Deviations arise from three distinct replicates

Lakes (Water)		*4-Nitrophenol (ng/L)*	*2-Nitrophenol (ng/L)*
CAREZZA LAKE	94/95	70 ± 7	428 ± 78
" "	95/96	40 ± 19	225 ± 37
" "	96/97	63 ± 8	<LOD
" "	98/99	28 ± 7	63 ± 7
EDMONSON POINT	96/97	34 ± 11	<LOD
" "	97/98	11 ± 2	<LOD
" "	98/99	28 ± 7	28 ± 4
TARN FLAT	97/98	12 ± 4	38 ± 7
" "	98/99	17 ± 4	<LOD
Lakes (Suspension >0.45μm)		*4-Nitrophenol ng/Kg*	*2-Nitrophenol ng/Kg*
CAREZZA LAKE	98/99	42735 ± 561	<LOD
EDMONSON POINT	98/99	<LOD	<LOD
TARN FLAT	98/99	<LOD	<LOD
Highgrounds 96/97 (Snow)[a]		*4-Nitrophenol (ng/L)*	*2-Nitrophenol (ng/L)*
Mount MELBOURNE	360m.	12 ± 8	<LOD
" "	710m.	<LOD	<LOD
" "	1490m.	<LOD	<LOD
RENNICK GLACIER	1350m.	8 ± 2	<LOD
" "	1650m.	13 ± 4	<LOD
" "	2100m.	13 ± 6	<LOD

a. Data refers to only transepts carried out on Mount Melbourne and Rennick Glacier at three altitudes, in the 96/97 antartic sampling expedition.

In a general view of data reported in Table V, a time-persistent, even if fluctuating, presence of 4-NP at ng/L levels in all the matrices analyzed can be shown, whereas 2-NP was only checked episodically in lake waters. Quantitative values

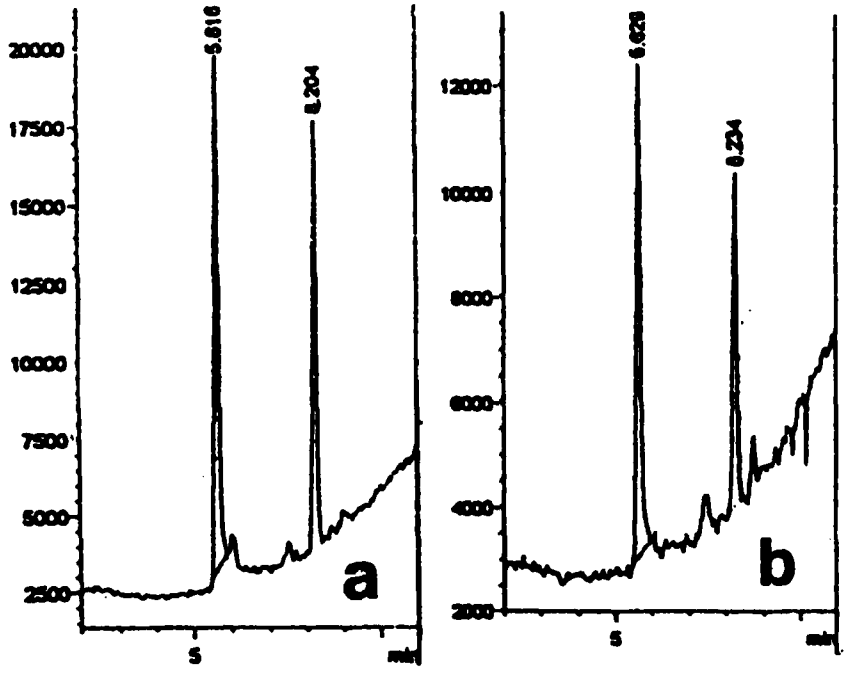

FIGURE 6 Total Ionic Current MSD profile of Carezza Lake water samples: 94/95 (a) and 95/96 (b). Analytical conditions as described in Table IV

found for both analytes were usually low (0–30 ng/L) except for those of Carezza Lake samples, higher for 4-NP and even greater for 2-NP. The very high amount (42μg / kg) of 4-nitrophenol detected in the particulate suspension of last expediton's (98 / 99) Carezza Lake water confirms this was the most polluted lake, since other lakes' particulate values did not exceed LOD.

Unpracticable sampling (completely frozen or dried lakes) is the reason of some lacking data (e.g. Carezza Lake water 97/98 or Tarn Flat Lake water 96/97). The hypothetical presence of a chloro-substitute of nitrobenzene (3,4-dichloronitrobenzene) in lake waters, at first suspected in the "screening" analyses, instead was not confirmed. In not one of the analyzed samples, did the characteristic GC-MS pattern (109, 191, 193 m/z values and their reciprocal quantitative ratios) of this compound occur, even if the limit of operative detection (LOD 100 ng/L) was abundantly below found values (340ng/L). Besides, the SCAN aquisition modality permitted us to deny the presence of other nitromonoaromatics, at least as concerns our working conditions.

CONCLUSIONS

This paper describes a sensible and selective method for simultaneous evaluation of nitrophenols and nitroarenes (nitrobenzenes) at ng/L levels in water samples. We utilized this method to investigate the presence of such pollutants in Antarctic environment and, in the affirmative, to assess a trend along a middle-term period, furthermore formulating hypotheses on the origin of eventually found analytes by sampling and analyzing other matrices. Our investigation did not find nitroarenes in antarctic fresh-water above our instrumental detection limits. This fact could be explained with the apolar and semi-volatile nature of such analytes, not much compatible with antarctic aqueous environment and perhaps more subject to absorption and accumulation by organic matrices (ashes, oils, sea particulates), since snow and lake water amounts, if present, could be very little and undetectable, in our conditions.

On the other hand, our study detected and confirmed the presence of trace levels of 4-nitrophenol and 2-nitrophenol in antartic fresh-waters (lakes and snow) without interruption for a period of at least five years. Some authors [22] suggested an autochthonous origin for these and other nitroaromatic compounds, by nitrosation and oxidation processes towards phenol or benzene, as recently proposed by others for antartic athmospheric nitro-PAH [14].

However, physico-chemical conditions to obtain a natural occurring in an Antarctic environment of such processes are very strict and hard to reach. On the contrary, presence of 4-nitrophenol in snow, at much lower values than those observed in lake waters, could be an indication of a possible external contribution by athmospheric agents, followed by a concentration process due to percolation and accumulation in to the lakes. With regards to this, noteworthy amounts of 4-NP in water particulate suspension of Carezza Lake, seems to be due to a bio-accumulation or a biochemical neo-formation rather than to a simple physico-chemical UV-mediated process [14,22], since we have not found relative (phenol, benzene) precursors above LOD. Analyses of other matrices (algas, sediments) should present clearer indications on the origin of these compounds.

Acknowledgements

The authors are very grateful to all members of the "Environmental Contamination Group" of the Italian Expeditions in Antarctica for their help in sampling operations. A particular acknowledgement for financial support is to be given to E.N.E.A. -Progetto Antartide and C.N.R. (Consiglio Nazionale delle Ricerche, Roma.)

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