Electronic Supplementary Material (ESI) for Environmental Science: Processes & Impacts

Materials and Methods. Supplementary information.

Vegetation categories in sampling sites

In the Songe area we sampled in the "crops" category that includes *Zea mais* fields and the "pasture" category including grazing areas for the local cattle and goats (1500-2500 m a.s.l). In the Meru area we could found the "shrubs" category which includes the savannah bushes at low altitude (1500 m a.s.l) and the vegetation of the ericaceous zone with *Philippia* and *Erica* as the dominant genus at 3200-3600 m a.s.l. It is obvious that savannah and ericaceous zone has different ecological features, but the vegetation density is similar. Then we crossed the "forest" category (1700-3200 m a.s.l.) that includes a wide arrange of forest areas following the increment of altitude (dry mountain forest, dominated by *Juniperus procera* trees; moist mountain forest with trees of the *Croton* genus; upper mountain forest with *Hagenia abyssinica* as the main tree). Again we put together ecological areas that are different, but characterized by multi layered forest cover. Above 3600 m a.s.l. there is the afroalpine zone, characterized by freezing and thawing cycles and by very little precipitation that support only desert-like grasses of the genus *Pentaschistis,* which survive in pockets of soil inside rock fractures and volcanic ashes.

Soil taxonomy in sampling sites

In the lower dry plains, Cambisols are widespread, with Salic and Sodic features, showing strong evapotranspiration and salt accumulation in the upper soil horizons. These soils show alkaline or strong alkaline reaction. In the alluvial plains also Fluvisols are present, often with Sodic features. Going up the slopes, Cambisols acquire Andic characteristics, due to the presence of pyroclastic materials (due to the past eruptive activity of Mt. Meru) in the substrate. In a central altitude belt, that for the eastern side of Mt. Meru is between about 2000 and 3100 m, the abundant rain and the thick pyroclastic cover allow the formation of true Andosols, rich in organic matter and with Melanic features (their pH is acid-subacid).
Higher, in a belt till about 3600 m, Andic Cambisols appear again. At higher elevation they leave space to Regosols with Tephric characteristics, rich in pyroclastic material with little weathering, and to Lithic Leptosols, very shallow over hard rock.

Chemicals and instruments

All solvents used were pesticide grade. Florisil adsorbent for chromatography (100–200 mesh) was obtained from Fluka (Steinheim, Germany). Silica gel for column chromatography (70–230 mesh) was supplied by Sigma–Aldrich (Steinheim, Germany). The p,p’-DDE D8 (deuterated p,p’-DDE) used as an internal recovery standard was purchased from Dr. Ehrenstorfer (Augsburg, Germany).
A GC chromatograph (TRACE GC, Thermo-Electron, Austin, Texas, USA) equipped with a Programmed Temperature Vaporizer injector (PTV) and an AS 2000 autosampler (Thermo Electron) was used coupled with a PolarisQ Ion Trap mass spectrometer. A Rtx-5MS (Restek, Bellefonte, PA, USA) capillary column (30 m length, 0.25 mm I.D., 0.25 µm film thickness) was used for the chromatographic separation. Helium for gas-chromatographic analyses was purchased from Sapio, Monza, Italy.

Extraction and cleanup procedure

Samples (~20 g) were lyophilised and extracted for 12 h using 100 mL acetone/n-hexane (1:1 v/v) in a soxhlet apparatus (FALC Instruments, Lurano, Italy). Samples were then concentrated to the final volume of 3 mL initially by a rotary evaporator (RV 06-LR, IKA, Staufen, Germany) and then by a gentle nitrogen flow. Organic matter was digested adding 6 mL of sulfuric acid 95% and

digesting overnight. The supernatant solution of acetone and hexane was then concentrated with gentle nitrogen flow to the volume of 1 mL.
Cleanup was performed using a multilayer column (40 cm x 1.5 cm I.D.) composed of 10 g of silica gel (activated overnight at 130 °C, then deactivated with water, 5% w/w), followed by 10 g of Florisil (activated for 16 h at 650 °C). The phase-filled columns were washed with n-hexane/acetone/ dichloromethane (8:1:1 v/v). Elution was carried out first by collecting 50 mL of n-hexane and then 50 mL of 1:1 n-hexane/dichloromethane (v/v). 1 mL of isooctane was added to the fractions that were then concentrated by rotary evaporator to 10 mL and then to 1 mL under gentle nitrogen flow.

Quantification conditions

Samples were analysed using GC/MS/MS methodology under the following instrumental conditions: PTV in solvent split mode with split flow of 20 mL min^{-1} and splitless time at 2 min; carrier gas helium at 1 mL min^{-1}; EI mode with standard electron energy of 70 eV; transfer line at 270 °C; damping gas at 1 mL min^{-1} and ion source at 250 °C. Chromatographic separation of the PCB congeners was obtained by the following conditions: initial oven temperature starting at 100 °C and maintained for 1 min, then ramped to 180 °C (no hold time) at 20 °C min^{-1}, to 200 °C (no hold time) at 1.5 °C min^{-1}, to 250 °C (no hold time) at 3 °C min^{-1} and finally to 300 °C (held 5 min) at 30 °C min^{-1}. Chromatographic separation of DDX, HCHs and HCB was obtained by the following conditions**:** initial oven temperature starting at 70°C and maintained for 1 min, then ramped to 220 °C at 8 °C min^{-1} and finally to 300 °C (held 7 min) at 20 °C min^{-1}. DDX, PCB, HCH and HCB quantification was performed by external standard calibration curves, ranging from 1 to 100 pg μL^{-1} for each compound.

Quality assurance (QA) and quality control (QC)

Samples and blanks were spiked with 10 μL of the deuterated recovery standard p,p'-DDE D_8 (and so with 5 ng of DDE-D_8) prior to solvent extraction to monitor methodological analyte losses, as in Sarkar *et al*. (2008). Recoveries over 80% were accepted. The suitability and the stability of deuterated standard response were evaluated as in Sarkar *et al*. (2008). A procedural blank was run in parallel with every batch of three samples and then extracted in a manner identical to that of the samples. No significant concentrations of analysed compounds were found in blanks. LODs (limits of detection) were estimated by the signal-to noise ratio (3:1) and ranged between 0.15 and 0.35 pg (injected amount) depending on the compound. Considering 20 g of extracted soil and the ratio 1:1000 or 1:500 of the injected vs. sample volume, LOQs (limits of quantification) are not higher than 0.001 ng g^{-1} d.w. for PCB and DDX, while it's non higher than 0.005 ng g^{-1} d.w. for HCH and HCB.

Statistical analysis

All statistical elaboration of our data (Box plots, GLM analysis, PCA) were performed with the SPSS 19 software pack and the STATISTICA software pack

Bibliography

S. K. Sarkar, A. Binelli, C. Riva, M. Parolini, M. Chatterjee, A. K. Bhattacharya, B. D. Bhattacharya and K. K. Satpathy (2008): Organochlorine Pesticide Residues in Sediment Cores of Sunderban Wetland, Northeastern Part of Bay of Bengal, India, and Their Ecotoxicological Significance. Archives of Environmental Contamination and Toxicology, 55, 358-371.

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