POLYCYCLIC AROMATIC HYDROCARBONS (PAHs) IN VULCANO ISLAND (AEOLIAN ARCHIPELAGO) MUD UTILIZED FOR THERAPEUTIC PURPOSE

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Abstract

This paper examines the possible presence, distribution, nature and sources of 18 Polycyclic Aromatic Hydrocarbons (PAHs) as constituent of the muds pool collected in Vulcano Aeolian Island. PAHs are important from environmental and toxicological point of view. Analysis was performed by gas chromatography/mass spectrometry (GC/MS) in selected ion monitoring (SIM). The total concentration of Polycyclic Aromatic Hydrocarbons ranged from 112 to 154 µg/Kg of dry matrix. The volcanic muds, used for therapeutic purposes, are moderately contaminated.

1. Introduction

Vulcano is the most southern island and one of two active volcanic centres of the Aeolian Archipelago, located in southern Tyrrhenian Sea, north of Sicily, Italy (Fig.1).

Figure 1. Location map of the

The last eruption in the island occurred in 1888–1890 from the Gran Cratere. Recent volcanic activity is limited almost entirely thanks to high temperature fumarolic exhalations around and inside the crater called La Fossa, and to an extensive hydrothermal activity near the isthmus of the Baia di Levante. At the beach, the outlet temperatures of fumaroles are quite constant and close to 100°C. Boiling mud-flows and smoke-holes are used often for therapeutic purpose, in particular to treat rheumatism and respiratory disorders. The black and shiny mud, rich in salts is applied to a part or to the whole body to treat skin diseases and rheumatoid arthritis. The beneficial effectiveness of mud bath treatment, especially when combined with sulphur baths, has been extensively documented in the medical literature [1-3]. Moreover, muds are used often for esthetical purposes only. In spite of therapeutical effects of these treatments, organic contaminants can be present in the mud as reported in the literature. Since Polycyclic Aromatic Hydrocarbons can be also formed by condensation processes [4], we paid our attention in these class of compounds by considering the favourable conditions for their formation and the literature data on their toxicological effects. PAHs are ubiquitous environmental contaminants, being formed from both natural, as volcanic eruptions, and anthropogenic sources, largely by the incomplete combustion of organic materials. In the aquatic environment, PAHs can also be formed by microbial synthesis from certain precursors [5]. The main scientific interest has been addressed to understanding the environmental sources, distribution and fate of PAHs because they are known or suspected carcinogens. PAHs, like other semi-volatile compounds, are widely distributed and enter the aquatic environment by wet and dry deposition, direct and indirect discharges, and surface runoff. Owing to their hydrophobic and lipophilic properties, PAHs are relatively insoluble in water and tend to accumulate on surfaces or in non-polar matrices [6]. The carcinogenicity of PAHs has been suspected from many years [7-8]. More recently, other risks, such as alteration of immune functions and neurological development has also been examined [9].

Toxicological and epidemiological studies show that dermal penetration of these molecules may be an important way of exposure; however, until now, this has been mostly determined indirectly. For example, the exposure level of workers wearing protective gloves and clothing is lower than that measured for workers without any protection [10]. On the other hand, in the urine of patients with psoriasis disease, treated with a coal-tar product, a biomarker of PAHs, the 1-hydroxypyrene, has been found. The maximal excretion occurred approximately 15 hours after treatment [11]. Quantitatively, dermal absorption could represent up to 90% of the total dose penetrating into the body in specific conditions [12-13]. Kinetics studies reported that PAHs penetration through the skin involved both diffusional and metabolic processes. An evaluation of the US Superfund program indicates that predicted dermal absorption for adults and children pose the greatest risk attributable to contaminated soils at 15.7% of the sites [14]. In about 50% of these cases, this way of exposure contributes largely to carcinogenic risk. Because this conclusion is based only on predictions from mathematical modelling rather than on measurements carried out in the population, its strength is questionable [14]. In addition, the amount of soil adhering to the total body burden skin is generally extrapolated from hand measurements; it seems to be overestimated and depends on the type of activity: rugby playing, farming and children playing in the mud of the shore offer conditions for the greatest soil contact while soccer and professional grounds maintenance were shown to be the lowest [**15-16**]. There are no studies that take in consideration dermal penetration of dangerous compounds during mud bath treatment.

On this light we planned our investigation on the determination and characterization of PHAs in the mud of Vulcano Island, in order to evaluate their nature and source, i.e. the toxicity level, by considering their distribution ratios, according to a recognized scheme reported in the literature [17-24]. Analysis of PHAs, extracted by means of a well tested methodology already used in previous investigations [24,25], has been carried out by GC-MS in Selected Ion Monitoring (SIM) mode.

2.   Materials and methods

Chemicals - All chemicals used were of analytical grade with high purity. In particular: n-pentane and dichloromethane, from Fluka, were of 99.8% purity. Acetone (Envisolv for analysis of dioxins, furans and PCB) from Fluka was of ≥ 99.8% purity, HCl suprapur for trace analysis from Fluka, Standard PAHs mixture (EPA 610 PAHs mix, lot LA-96245) and perdeuterated internal standards (fortification solution B Lot N° LA-92479) were from Supelco (Milano, Italy).

Sampling -. Four mud samples were collected in the spring of 2004. Figure 2 shows the location of the sampling sites. A total of 200 g of mud were collected of each site and were placed in plastic bags. The samples were immediately refrigerated (4° C) on site, stored avoiding the exposure to light, and then rapidly carried to the laboratory where they were frozen before analysis.
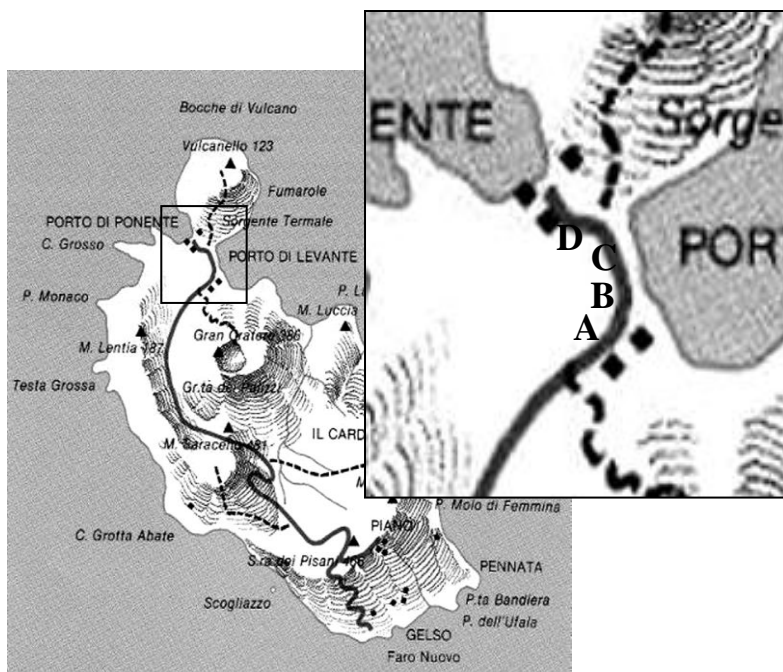
Figure 2. Location map of sampling sites

Sample treatment - About 5 g of mud, after centrifugation for 10 minutes, were treated with precleaned (Soxhlet extracted with dichloromethane for 24 h) anhydrous Na_2SO_4 (Fluka). Activated copper (200 mg) was added to the extraction vessel to remove elemental sulphur. The copper (40 mesh, 99.5% purity by Aldrich) was activated with HCl 1N and then washed with water, acetone and CH_2Cl_2. A solution of a perdeuterated internal standard (benzo(a)anthracene-d_{12}) was added.

Extraction of PAH - Among different techniques and various solvents, tested to find the better extraction method, we used the Soxhlet extraction because it allowed us to obtain the highest recovery percentage of PAHs [**24**]. All samples were extracted in a Soxhlet extractor for 24 hours by using the solvent mixture dichloromethane-pentane 1:1 (Carlo Erba, Milano, Italy). The extracts were filtered through pre-cleaned Pasteur's pipette filled with solvent-rinsed glass wool and pre-cleaned anhydrous Na_2SO_4, rinsed and concentrated in a rotary evaporator with the thermostatic bath at T = 35 ($\pm$ 0.5) °C. The final volume was around 2 ml. Finally, the solution containing PAHs was taken to dryness under a weak nitrogen flow. The dry residue was dissolved in 1 ml solution containing the following perdeuterated internal standards in cyclohexane (0.2 mg/L each): Acenaphthene d_{10}; Phenanthrene d_{10}, Chrysene d_{12} and Perylene d_{12} .

PAHs analysis - The qualitative and quantitative determinations were carried out by means of a gas chromatograph (Shimadzu mod. GC-17A) coupled to a mass spectrometer (Shimadzu, quadrupole detector mod. GCMS-QP5000), operating with acquisition data (Shimadzu, CLASS 5000 system). GC separations were achieved on an Equity-5 (30 m x 0,25 id, 0,5 μm) fused-silica capillary column from Supelco (Milano, Italy). The injector mode was splittless (0,61 min) and a total flow 20.6 ml/min was used. The injection of both extracts from samples and standard solutions (1 μl) was performed by hand. The injector temperature was maintained at 280°C. The GC temperature program was: from 40°C (2 min) to 100°C at 40°C/min, to 200°C at 10°C/min, to

325°C (8 min.) at 30°C/min. The carrier gas was helium (flow rate 2.6 ml/min). The use of Equity-5 column allowed us to obtain the complete separation in a shorter time (about 25 min) compared to previous investigations [**24, 25**]. The interface temperature was 325°C. The analysis was operated in Selected Ion Monitoring (SIM) mode. The identification of the components of the standard mixture was carried out by comparing retention times of each component of the mixture with those of pure components, analysed under the same experimental conditions. Identification was confirmed by comparing the spectra of the single components with those stored in the library of the acquisition system. The identification of PAHs in the solutions extracted from sediment was carried out on the basis of previously determined retention times, and it was confirmed using mass spectra. The PAHs content in the sample was quantified relatively to perdeuterated PAH added to the dry residue. The response factors of different compounds were measured by injecting a mixture containing 18 standard compounds and having the same concentration of perdeuterated PAHs as that used for spiking the sample.

Water content analysis – About 2 g of mud, previously centrifuged, was dried at 105°C for a night. The water content was determined by weight loss and was utilized to refer all the results to dry weight.

Organic Matter – Total organic matter in the mud was determined by ignition at 450°C for 4 h. Since this procedure is known to over-estimate the organic matter content owing to the simultaneous elimination of carbonates, we carried out the method after carbonates were taken off as CO_2 by means of addition to sample of HCl 1:1.

3.   Results and discussion

Investigated muds consisting of augite, plagioclase, magnetite, ilmenite, K-feldspar, and glass; pyrite, alunite, sulfur, and opal are precipitating from the thermal fuids. The deeply black colour of the sediments is due to the presence of poorly crystallised iron monosulphide.

In Table 1 we report the concentration of each individual PAH detected in muds of Vulcano. In all the sampling sites the same distribution of 18 polycyclic aromatic compounds (expressed as weight percentage) is observed.

Compounds	Sampling sites			
	A	B	C	D
Naphthalene	5,1	10,1	6,0	10.0
Acenaphthene	0,5	0,4	0,1	0,1
Acenaphthylene	0,7	0,5	0,5	0,3
Fluorene	1,5	1,7	1,1	1,9
Phenanthrene	15,1	10,6	9,1	17,9
Anthracene	1,4	0,9	0,7	1,5
Fluoranthene	16,8	9,1	9,3	16,0
Pyrene	13,4	9,0	9,1	13,6
1 methyil pyrene	32,2	41,3	34,4	35,1
Benzo (a) anthracene	11,5	9,6	9,8	11,0
Crysene	9,6	6,2	6,6	11,1
Benzo (b) fluoranthene	4,3	2,1	6,1	6,4
Benzo (k) fluoranthene	2,0	1,1	2,2	4,0
Benzo (a) pyrene	3,7	3,2	8,8	3,5
Perilene	1,0	1,3	1,4	1,1
Indeno(1,2,3-cd) pyrene	4,9	1,6	4,8	5,0
Dibenzo (a,h,) anthracene	4,5	1,4	2,9	6,1
Benzo (g,h,i) perilene	6,3	1,7	2,8	9,1

Table. 1. Individual PAHs concentration (µg/Kg dry weight) [a)]

The total concentration of PAHs in the Vulcano muds is quite homogeneous, ranging from 112 µg/Kg (dry weight) at site **A** to 154 µg/Kg (d.w.) at site **D**, as shown in figure 3.
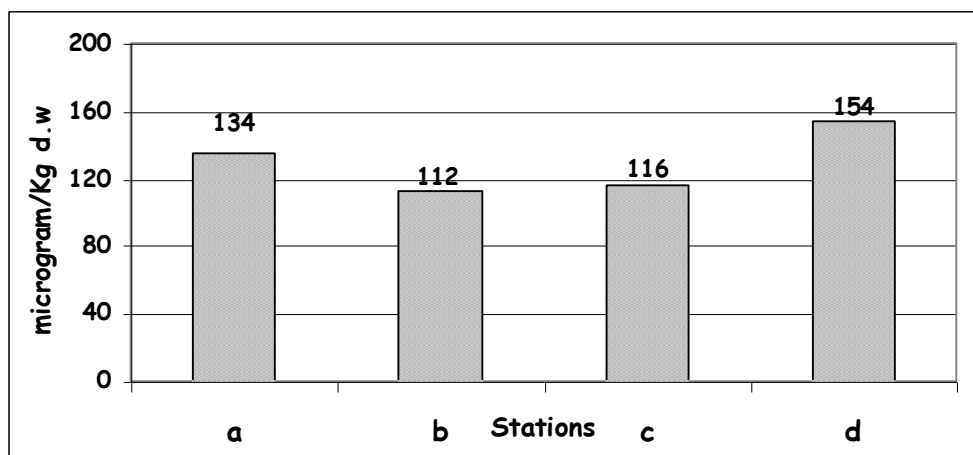

Figure 3. Total concentration of PAHs in the different sampling points

The organic matter content is in the range from 5.1 to 8.0 %. The results show that the concentration of polycyclic aromatic compounds in muds generally is higher in correspondence with the increasing of organic matter content. This trend has been already pointed out by different authors for different matrices [24,26,27]. In order to find a relationship between the content of organic matter and the concentration of PAHs determined in samples investigated, we carried out a linear regression analysis whose results are reported in Figure 4.
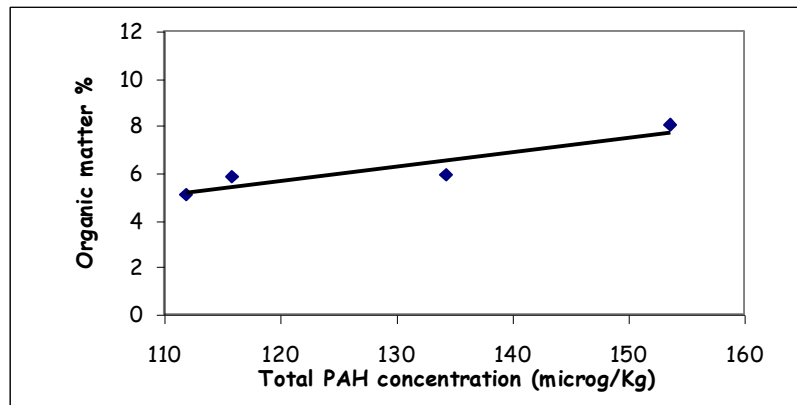

Figure 4. Linear regression curve of total PAHs concentration (18 compounds) *vs*. total organic carbon content of muds.

The results show an improved linear correlation coefficient ($r = 0.93$). From these results we can suppose that PAH identified in samples collected from all the stations would originate from the same origin.

Three processes can generate Polycyclic Aromatic compounds: i) combustion at high temperature, ii) release of petroliferous products, and iii) diagenetic processes (degradation of the organic matter). The molecular patterns generated by each sources are like fingerprints and it is possible to hypothesize which processes generate PAHs by studying their distribution in samples, even if generally the source are various and some time occasional.

If we group the Polycyclic Aromatic Hydrocarbons in different classes depending on the number of aromatic rings present in their structure, it can be observed that PAHs having 4, 5 and 6-rings, found in samples of sites under investigation, contribute over 80 % of the total. This evidence suggests that PAH presence in mud pool might originate mainly from high temperature process: in fact, pyrogenic Polycyclic Aromatic Hydrocarbons are generally characterized by the dominance of high molecular mass (4-6 rings) over the 2-3 rings low molecular mass PAHs [**22**], generally present in petrogenic sources.
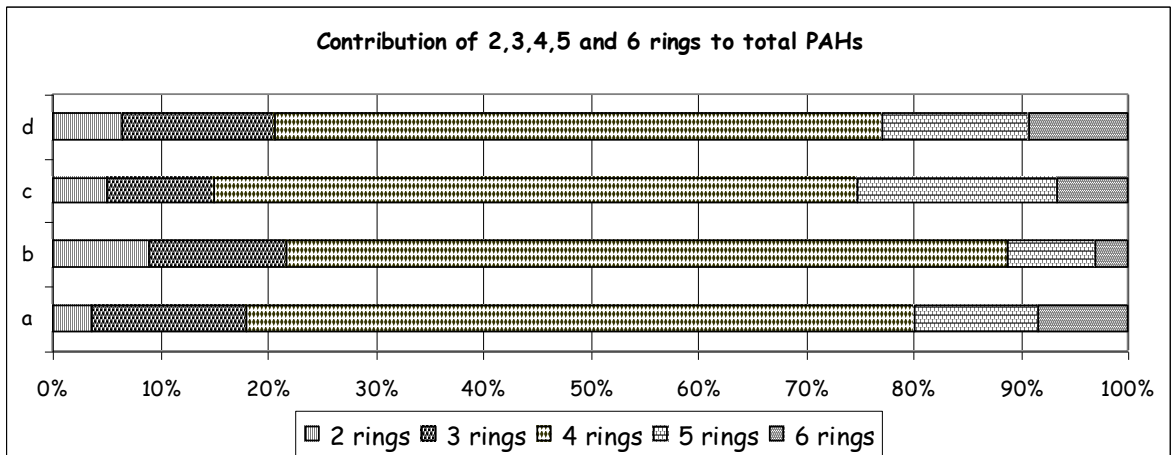
Figure 5. Contribution of 2,3,4,5 and 6 rings to total PAHs

Phenanthrene/Anthracene (P/A) and Fluoranthene/Pyrene (Fl/Py) are the most common ratios used as clues to source and delivery routs of PAH. Samples (sediments, muds, etc.) from sites in urban areas are prone to contamination from urban runoff that contains both raw fuel and combustion products, whereas samples of remote sites are primarily contaminated by atmospheric deposition of combustion materials. For example, sediments from urban areas tend to have lower Fl/Py ratios than those from remote sites [20]. Values of Fl/Py ratio of 1.3–1.7 have been recorded for sediments at remote sites and ca.1.0 for sites near urban centers [16, 21]. Values of P/A in the range of 3–11 are indicative of urbanized areas, especially those most impacted by urban runoff, while ratios greater than 11 are indicative of remote sites that are primarily influenced by atmospheric deposition. This may be due to the selective photooxidation of anthracene during long-range transport [22]. In this study, the P/A and Fl/Pyr ratios for all sites ranged from 11 to 13, and 1.0 to 1.3 respectively (Fig 5).

The considered sites are far from most anthropical sources, so atmospheric deposition is likely to be the main contributor to the mud PAH contamination.
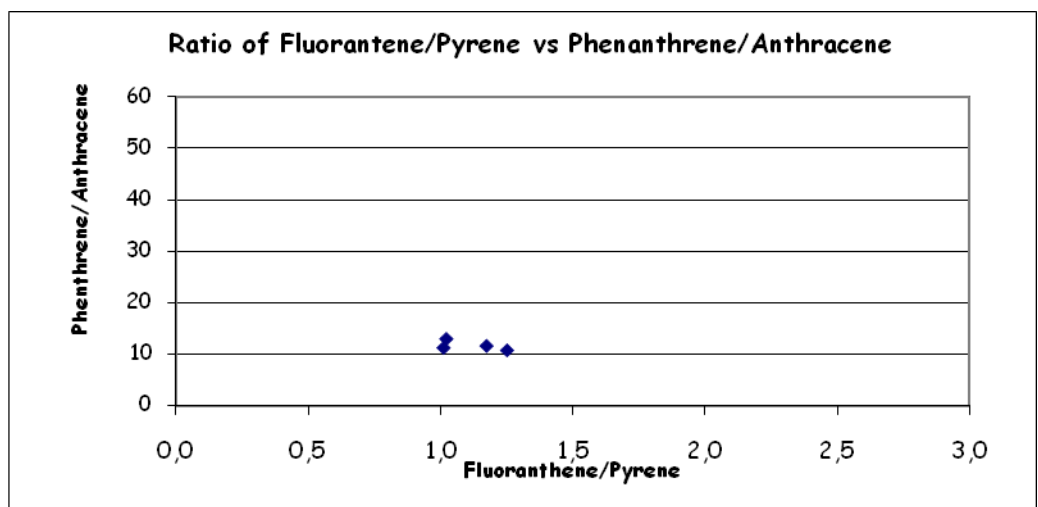

Figure 6. Plot of ratio of Phenanthrene/Anthracene versus ratio of Fluoranthene/Pyrene

The analytical data obtained by the investigation in the Vulcano muds indicate that polycyclic aromatic compounds found in all samples seem to be of pyrolytic input and that diffuse input, such as atmospheric

deposition, may be the major source of PAHs into the water bodies. In the atmosphere PAHs can be associated with particle phases. The aerosol particles are transported by the wind to distant locations and they are removed from the atmosphere by rain and dry fallout in seawater. In the course of sinking in the water column, PAHs are attacked by microbes, and some fraction should be decomposed. On the contrary, PAHs in the muds are more resistant to microbial degradation and therefore they accumulate [23].

To estimate the possible pollution sources, the concentration of perylene has to be considered, because the origin of this compound generally is attributed to terrestrial, marine or aqueous biogenic precursors [24], and the high concentrations of this compound have been measured in anoxic sediments where high biological productivity occurs [16, 25]. In the present study, perylene was found at low concentrations in all muds investigated (from 1.0 up to 1.4 μg/Kg, see Table 5). Therefore, we believe that the little amount of perylene found in samples may originate principally from diagenic process. This assumption is confirmed by the low redox potential (-75 mV) and by organic matter content [18].

The mean concentration of carcinogen PAHs in the four samples of Vulcano muds is 11 μg/Kg dw expressed as benzo(a)pirene.

4.   Conclusions

The main remarks of the present work can be summarized as follow:

•   The larger presence of PAHs with high molecular weight, found in all samples, and the values of Ph/An and Fl/Py ratios, as PAH distribution indexes, has demonstrated that all the samples owe their PAHs in Vulcano mud to a predominant single mode of origin, i.e. high temperature processes (volcanic activity), but negligible quantity of PAH can derive by biogenic sources because all sediments contain perylene [28].

•   In some cases, therapeutic properties of mud are due to the presence of bitumen, in Vulcano samples there is no evidence of coal-tar contamination; for coal-tar the ratio Fl/Py is ≥ 1.5 and, this ratio for mud collected is lower. The curative properties of the mud are probably due to its high salt content in the hyper saline interstitial brines, the absorption of sunlight and skin heating by the deep black material and by the interaction of the sulphides and elemental sulphur with the skin, and not due to the presence of organic matter.

•   From an toxicological point of view, the system investigated appears to be poorly contaminated. A complete determination of the risks linked to use of mud for therapeutic purposes remains far off, because exposure is complex and difficult to estimate.

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