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Mobility and distribution of arsenic in contaminated mine soils and its effects on the microbial pool

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ABSTRACT

Three soils, coming from a former mining site and characterized by a different degree of pollution, were analysed in terms of Arsenic (As) content, using three different analytical approaches, and its distribution in various soil fractions. The effect of As on soil microbial biomass (size, respiration and microbial quotients) was also analysed. Total arsenic concentration between soil fractions was significantly different and ranged from 189 to 4357 mg kg^{-1}, indicating a high level of pollution. Soil sequential fractioning showed that more than 60 percent of total As was bound to Fe–Al oxides, suggesting a minor availability and environmental risk regardless the total concentration of As in the sample. On the contrary, water soluble As fraction showed a significant difference among the three samples. The largest water soluble As concentration was found in the sample with intermediate total As amount. As far as microbial biomass is concerned, it was found that bioavailable As negatively impacted microbial metabolism in terms of basal and cumulative respiration, and microbial quotients, suggesting a strong selection within microbial pool.

1. Introduction

In the last decades Arsenic (As) became a major environmental problem due to its high toxicity, mobility and bioaccumulation; its presence in the environment is mainly geogenic, i.e. naturally occurring (Nriagu et al., 2007), but contamination threats are associated with anthropic activities too (Krysiak and Karczewska, 2007). As is present in various forms in soils due to the interactions with soil components (Ghosh et al., 2004b).

As toxicity and its effects, on both environment and human health, are due to its labile forms. One of the most debated issues related to As toxicity is the adsorption-desorption process which depends on soil physico-chemical characteristics. As shows a high affinity to oxidic surfaces and to other soil particles depending on several factors such as redox potential, pH, soil texture, organic matter and competing ions nature. In a pH range from 2 to 7 the As (V) form H_2AsO_4 is usually present while, in a pH range from 4 to 8, the As(III) form H_3AsO_3 is present depending on the soil's redox potential (Aguilar et al., 2006).

In soils affected by mining activities, As was found to be primarily associated with amorphous Fe, Al, and Mn oxides and/or hydroxides, (Ahumada et al., 2004; Filippi et al., 2004; Ghosh et al., 2004a; Morin and Calas, 2006), and with organic matter (Kabata-Pendias, 2001; Reimann et al., 2003).

The choice of the proper analytical methodology for the determination of heavy metals in soils must be done considering the advantages and disadvantages of various techniques related to: (i) sensitivity (limit of detection) of the technique, (ii) the degree of pollution of the soil, (iii) type of matrix. For instance very sensitive methods may not be suitable for the measurement of soil containing high concentrations of analyte as the measures may be affected by dilution errors.

Studying available forms of chemical elements in soils is of great importance. The different chemical elements forms in soil, soil components they are bonded to, and bond strength, can inform on the element reserves accessible to plants and microorganisms, as well as the scale and possible ecological effect of soil pollution (Siromlya, 2009). Recently, it was demonstrated that the total concentration of As in soils does not necessarily represent its biological availability or potential toxicity measures (Newman and Jagoe, 1994; Anawar et al., 2006; Villalobos-Castañeda et al., 2010; Larios et al., 2012), which are much more important for assessing possible environmental impacts. Most risk from As is, in fact, associated with As forms biologically available for absorption, or "bioavailable" to plants, soil microbes and finally to humans. Bioavailability is a function of the abundance, chemical form (i.e. oxidation state), the nature of its binding to soil particles, and biological factors (Violante et al., 2010; Márquez-García, et al., 2012).

Chemical fractionation methods, based on sequential extraction procedures, were used to determine the amount of contaminants

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in specific chemical pools (Lagomarsino et al., 2010; Vodyanitskii, 2006), allowing thus a more correct evaluation of the potential health, environmental risk and proper waste disposal strategies. These methods are based on the rational use of a series of selective reagents chosen to solubilize successively the different mineralogical fractions thought to be responsible for retaining the larger part of the trace elements. Although a considerable body of literature has been produced on this subject, there is still no standard practice for extracting available forms of chemical elements and even no standard terminology. Furthermore there still exists ambiguity on a universally accepted definition for "bioavailability" (Vodyanitskii, 2006). Most chemical fractionation methods were used to measure heavy metals in ion-exchangeable, superficially adsorbed, precipitated, organic chelated, and occluded chemical pools in baseline soils (Lagomarsino et al., 2010; Tessier et al., 1979). Sequential Extraction Procedures (SEPs) are also usually employed to fractionate arsenic in solid materials and evaluate the potential of arsenic leaching and diffusion into the environment. In this study, the sequential extraction procedure reported by Wenzel et al. (2001), and developed for *P*, was preferred considering the similarities of *P* and As chemical behaviour with respect to specific soil particles (Violante et al., 2010).

As may directly influence soil microbial populations. It is toxic to almost all bacteria, by inhibiting basic cellular functions, which are linked to energy metabolism (Walker et al., 2000; Sheik et al., 2012). The inorganic forms are arsenate (As(V)) and arsenite (As (III)); As(V) is analogous to phosphate what makes it able to interfere with the essential cellular processes. These forms are interconvertible depending on the redox status of the environment (Tripathi et al., 2007).

Biochemical and microbial assays such as the basal respiration rate, microbial biomass content, *C* mineralization processes, appear to be very useful in monitoring the effects of soil pollution. Microorganisms and microbial activities can provide an integrated measure of soil quality, an aspect that cannot always be obtained with physical and chemical measures and/or analyses of higher organisms. Microbial communities adapt sensitively to changing environmental conditions by varying individual activity, by increasing reproduction of species with favourable abilities, and by spreading new capabilities via horizontal gene transfer.

Soil microbial biomass, its respiration activity and ecophysiological indexes (qCO_2 and *q*mic) were used as indicators of pesticide and heavy metal toxicity (Brookes, 1995), as index of microbiological activity, in soil contamination surveys under a range of different conditions (Tate, 1995).

Aim of the study, performed on soils deriving from a former mining area in northern Italy, was to:

(i) determine soil total As content using three different analytical methods, (ii) determine the distribution and potential toxicity of As in soils and fractions of ecological interest, (iii) determine soil microbial biomass, respiration activity, microbial indexes and interrelate the presence of As in the different fractions to microbial performances.

2. Material and methods

2.1. Site description, sample collection

The soil samples come from an abandoned ore mine in Piemonte region, northern Italy, very close to the Swiss border. Pestarena mine is located in Monte Rosa Mountain, on the left side of Anzasca river bench. The potential pollution factor is represented by the As concentration both in the tailings and soils surrounding the mining areas due to the oxidation of sulfide minerals. Furthermore mine tailings were accumulated beside the Anza river where the leaching of As can have dangerous outcomes. Soil samples were collected during Spring 2010 from three different plots (20 cm depth) characterized by different As content level, in the Anzasca valley, in Piemonte region (Northen Italy). The three soil plots were chosen with the aim to cover three levels of As contamination: high (H), medium (M) and low (L).

Low contaminated soils (L) were taken from an area outside the mining site under herbaceous vegetation (45°57′44″N, 8°1′28″E), soil cores were taken about 1 km far from the polluted area on the same mountainside as the other samples, medium contaminated soils (M) were taken few meters downwind a mine tailing deposit under herbaceous vegetation (′45°57′28″N, 8°0′51″E), high contaminated soils (H) were taken within the mining perimeter from an area covered with perennial vegetation (*Abies alba* and other conifers) (45°57′29″N, 8°0′56″E). In each plot, five soil cores (approx. 500 g) were taken within a 10×10 m homogeneous area at a maximum distance of 3–5 m from each other. All 15 samples were investigated separately

2.2. Reagents and standards

Analytical grade reagents were used throughout the investigation. High quality water (18.2 MΩ cm^{-1} resistivity) obtained from a Milli-Q system (Millipore, USA) was used. Stock solutions (As = 1000 mg l^{-1}) were Fluka Analytical. $NaBH_4$, K_2SO_4, $K_2Cr_2O_7$, HCl were Carlo Erba reagents. $CHCl_3$ and NaOH were Sigma Aldrich. HNO_3 was purchased from Merck (Darmstadt, Germany). All the standard solutions were prepared by successive dilutions of the stock solution to the required concentrations.

2.3. Samples preparation and chemical characterization

Soil samples were sieved (< 2 mm), oven dried at a temperature below 40 °C to minimize loss of volatile elements (7–15 days), and left at 4 °C. For biochemical analyses, soil moisture content was adjusted to 60 percent water holding capacity, then samples were left to equilibrate at room temperature in the dark for 1 day prior to analyses (microbial biomass and respiration). Active and exchangeable acidity were measured on sieved soil suspended in a solution of deionised water (active) or in 1 N KCl (exchangeable) in 1:2.5 ratio (w/v). The pH was measured in the supernatant with a pH meter (pH 211, Hanna Instruments). All analyses were performed on the original samples.

The total organic carbon (TOC) was determined by Shimadzu TOC VCSH analyzer, the principle of the method being based on the cathalitic oxydation of the organic compounds, at 680 °C. 1 g of soil was analyzed using the TC/IC method, where TOC is the result of the difference between total carbon and inorganic carbon. Cation exchange capacity (CEC) was determined after extraction with 10 percent $BaCl_2$ solution pH 8.1, according to Gillman (1979). Results were expressed as $cmol^+ kg^{-1}$ of soil.

All the results in the present study were presented on a dry mass basis. Dry mass data were obtained drying all samples to constant mass in an oven at (105 ± 5) °C. The difference in mass before and after the drying process is used to determine the dry matter and the water content.

2.4. Determination of total As in soils

Total As in soil samples was determined after microwave assisted acid digestion. 0.5 g of samples were placed in 100 ml PFA HP-500 Plus digestion vessels and 10 ml of concentrated HNO_3 were added to the samples.

Samples and reagents were digested in a CEM5 MARS plus microwave oven, at 165 °C (2 min)–175 °C (10 min).

In all analytical determinations, blanks and triplicate samples were used to ensure the quality and reproducibility of the results.

For the determination of total As in soils three different analytical approaches using FAA (Flame Atomic Absorption) were compared in order to find the most reliable analytical procedure. This screening was necessary as soils presented extremely high levels of contamination and interferences could likely arise also due to the presence of other heavy metals. In addition the high sensitivity of some methods (e.g. HGAAS, detection limit 1.0 μg l^{-1}) may provide errors due to dilution procedures.

Hydride Generation Atomic Absorption Spectroscopy (HGAAS): Total As determination was carried out HG-AAS technique (Perkin-Elmer 4000 atomic absorption spectrometer equipped with a MHS-15 hydride generation system, Perkin-Elmer Corp., Norwalk, CT, USA). The measurement was carried out according to EPA method 7062, the procedure allows the determination of As in concentrations ranging between 1 and 400 mg l^{-1} even in the presence of interferences, such as Co, Cu, Fe, Hg, and Ni, in concentrations up to 4000 mg l^{-1}.

Direct Flame: the measurement was carried out according to EPA Method (2000) metals. For this method the detection limit is usually < 1 mg l^{-1}, and thus it is less sensitive than HGAAS.

Method of Standard Additions: the measurement was carried out according to EPA Method (2000) metals.

The mean values obtained were compared with the certified values of CRM soil sample (Trace Metals - Loamy Sand 3 CRM034).