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Synergic use of chemical and ecotoxicological tools for evaluating multi-contaminated soils amended with iron oxides-rich materials

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ABSTRACT

Abandoned waste piles from ancient mining activities are potential hot spots for the pollution of the surrounding areas. A pot experiment was carried out to check the potential toxicity of the dumping material present in one of these scenarios, and several amendments were tested to attenuate the spread of the contamination events. The waste material had an acid pH and a large total concentration of As and Cu. A dose-response experiment was performed with this material following OCDE 208 test. A proportion 90:10 uncontaminated soil: dumping material (% w/w) was selected for the following experiment, in order to surpass the amount of dumping material that caused 50% reduction in plant growth. Pots were filled with the 90:10 mixture, planted with seeds of *Brassica napus* and amended with the following materials: three iron oxides of Bayoxide® E33 series, iron (II) sulphate in combination with de-inking paper sludge (Fe + PS), iron oxide-rich rolling mill scale (ROL) and iron oxide-rich cement waste (CEM). Amendment effectiveness evaluation was based on chemical and biological assays: extractable trace element concentration, soil enzymatic activities, inhibition of light emission of *V. fischeri* and *Anabaena* sp., *B. napus* L. fresh weight and screening test for emergence of *B. napus* L. seedlings. Amendments E33HCF and Fe + PS were the most effective in reducing extractable As and Zn concentration. *B. napus* weight and dehydrogenase and β-glucosidase activities were positively increased with the two above mentioned treatments but they triggered more toxic effects for *V. fischeri* luminescence. E33P treatment was the only in which the EC_{50} was higher than in the control. *Anabaena* sp. was less sensitive than *V. fischeri* as its luminescence was not hampered by any treatment. Trace element concentration did not significantly affect the failure in seed emergence. E33HCF and Fe + PS could act as proper amendments as they decreased extractable As and Zn. Further, plant fresh weight, enzymatic activities and some of the bioassays identified the latter treatments as the best ones among those tested here to this type of multi-contaminated soil.

1. Introduction

Chemical analyses of contaminated soils do not provide evidence of the effects on the biota if they are not used in combination with ecotoxicological analyses, as chemical tests do not reflect the effect of pollutants on organisms and the interactions among contaminants. On the other hand, ecotoxicity tests provide an integrated measure of element bioavailability and the detrimental effects of contaminants in the ecosystem (Alvarenga et al., 2007; Fent, 2004). Enzyme activities are potential indicators of soil quality and degradation that aid to assess ecotoxicological risks resulting from the presence of trace elements (Hinojosa et al., 2008, 2004). Soil disturbances or changes in the soil management can trigger a rapid response of the activity of enzymes (Kandeler et al., 1996; Madejón et al., 2009). Bioassays using the marine luminescent bacterium *V. fischeri* are suitable to test toxicity in soils by adapting the protocols from the aquatic environments in order to evaluate the impact of soil composition on groundwater (Alvarenga et al., 2008a). *V. fischeri* are sensitive organisms to heavy metals and can detect acute and sublethal effects caused by a large number of chemicals (Ribo and Kaiser, 1983). The cyanobacteria *Anabaena* sp. PCC 7120 is largely used as a model in proteomic studies because its full genome sequence is known (Kaneko et al., 2001). In response to the presence of toxicants, *Anabaena* luminescence can undergo a negative impact, as it is directly proportional to the metabolic status of the cell (Muñoz-Martín et al., 2011). It has been used to test the toxicity of water samples potentially contaminated with antibiotics and metals or the degradation capacity of pesticides (Agrawal et al., 2015; González-Pleiter et al., 2013; Pandey et al., 2013). In this study, several

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amendments have been selected to elucidate whether their addition to soils contaminated with arsenopyrite residues will improve some soil biological activities and physico-chemical characteristics. The amendments were chosen according to their effectiveness in reducing arsenic and metal concentration in soils. Iron grit, iron oxide-rich rolling mill scale, iron-rich water treatment residues, furnace slag or iron sulphates are efficient in attenuating As in soils (de la Fuente et al., 2010; Hartley and Lepp, 2008a; Hartley et al., 2009; Koo et al., 2012; Nielsen et al., 2011). Recycled liming materials have been demonstrated to be suitable ameliorants to retain heavy metals in soils. Among them, paper sludge, a by-product of paper de-inking, contains a considerable proportion of calcium carbonate and its use has been reported to reduce the mobile fractions of metals in soils (He et al., 2010; Petruzzelli et al., 1998). The recycling of both iron-rich and liming materials contributes to the minimisation of their land disposal.

The aim of this work was to evaluate the improvement of soil quality after the addition of several amendments to As and heavy metal contaminated soils from the point of view of both soil physico-chemical properties and biological indicators.

2. Materials and methods

2.1. Description of the sampling area

Samples were collected from dumping tailings of a former and small arsenopyrite smelter established in the middle of the XXth century in North West Madrid (Spain). The area is composed by hydrothermal veins with arsenopyrite, scorodite, cassiterite, wolframite and quartz (Recio-Vazquez et al., 2011). During the Second World War, the smelting factory extracted large amounts of wolfram from the arsenopyrite rich-minerals (Helmhart et al., 2012). According to Recio-Vazquez et al. (2011), there is a potential risk for the catchment Area of the Madrid Detrital Aquifer (Spain) of being contaminated by arsenic. This area has been recently described by Gomez-Gonzalez et al. (2014), showing very low downward leaching of As in the waste pile, but some lateral spreading that makes advisable to undertake ecotoxicological and human risk assessment on one side, and also to look for strategies that may contribute to avoid As spreading with a low environmental impact.

2.2. Dose response experiment

A composite sample of dumping material from the waste pile (upper 20 cm) was taken. Uncontaminated soil (upper 20 cm) was collected from the surroundings of the Universidad Autónoma de Madrid. All the materials were air-dried for 1 week, sieved to 4 mm and stored until their use. A dose-response experiment was carried out to test the toxicity of the dumping material for plants, following OECD 208 protocol (1984).

Dumping material was mixed in several proportions with uncontaminated soil (w/w): 0%, 12.5%, 25%, 50%, 75% and 100%, being 0% and 100% the proportions at which only uncontaminated soil or only dumping material were used respectively. Pots (0.6 L) were placed in a growth chamber under controlled conditions (night/day T 20/25 °C, photoperiod 13/11 h, relative humidity of 40/60%, and photosynthetic photon flux density of 520 µmol m^{-2} s^{-1}), filled with 500 g of the respective mixtures and left to equilibrate for one month at 70% water holding capacity (previously determined). Four replicates for each treatment were used. After one month, 0.2 g of *Lolium perenne* seeds were sown in each pot, which germinated five days after. Seedlings were sampled three weeks after emergence and straightaway 0.2 g of *Medicago sativa* L. seeds were planted in each pot, germinating after two days. *M. sativa* seedlings were sampled three weeks after emergence. During the experiments, pots were watered when necessary with tap water to reach 70% of their water holding capacity. Fresh weights of above ground tissues were recorded and plants were dried for five days at 60 °C. EC_{50} for both plants species were calculated using SigmaPlot 11.0 in order to determine the proportion of dumping material diluted with uncontaminated soil that caused a 50% reduction of shoot fresh weight.

2.3. Amendment application in soils

Uncontaminated soil and dumping material, dried and sieved to 4 mm, were mixed at a rate 90:10 (w/w) according to the outcome of the dose-response experiment described above. Several amendments were thoroughly mixed with the soil prior to being added to pots. 2–3 cm of gravel was placed at the bottom of 2 L-pots and filled immediately with 2 kg of the respective mixtures, with four replicates for each treatment. The different amendments were (treatment codes in brackets): Control (unamended); Bayoxide® E33P 1% w/w (E33P); Bayoxide® E33HC 1% w/w (E333HC); Bayoxide® E33HCF 1% w/w (E33HCF); $FeSO_4$ 1% w/w + recycled de-inking paper sludge 1% w/w (Fe + PS); iron oxide-rich rolling mill scale 3% w/w (ROL); iron oxide-rich cement waste 3% w/w (CEM).

Bayoxide® E33 series (LANXESS, Bayer) are synthetic granulated iron oxi-hydroxides (mainly goethite) with a very large surface area and high adsorption capacity. These iron oxides have been proposed by the manufacturer as an alternative for removing arsenic from drinking water and waste water serving as an in-line filter, adsorbing As when the water flows through a fixed bed of granules. The main differences among the three oxi-hydroxides are the particle size in the range 0.1–2.0 mm (E33P = E33HC > E33HCF), the specific surface area in the range 102–250 m^2 g^{-1} (E33P < E33HC = E33HCF) and the bulk density in the range 0.40–0.95 g cm^{-3} (E33P < E33HC = E33HCF). $FeSO_4$ and $CaCO_3$ were obtained from Sigma Aldrich and recycled de-inking paper sludge was supplied by Holmen Paper (Madrid, Spain). This latter was used to study a potential revaluation of this residue because of its pH and organic matter content, which may contribute to improve the physical, chemical and biological properties of soils. Iron oxide-rich rolling mill scale (36% magnetite, 35% maghemite and 29% plustite, de la Fuente et al., 2010) was originated from the hot rolling of steel and was supplied by CAESA (courtesy of PHYTOREC team, CEBAS-CSIC). The iron oxide-rich cement waste was provided by the local authorities, coming from a local cement manufacturer. This material was air dried and sieved to 2 mm before its application to the soils.

Once mixtures were transferred to pots, they were left to equilibrate for one month at 70% water holding capacity (previously determined). Prior to equilibration, soil was sampled for an initial characterisation using a driller to remove a significant amount of soil, dried and sieved to 2 mm. After equilibration, another portion of soil was sampled following the same procedure, soils were sown with 8 seeds of *B. napus* L. per pot and watered regularly. The emerged seedlings were grown for ten weeks. *Brassica* species has a high interest in phytoremediation techniques due to its relative high biomass yield and its moderate capacity to accumulate heavy metals (Ebbs et al., 1997). Further, this crop is interesting for biofuel production given the continuously rise of oil prices (Grispen et al., 2006). Ten weeks after emergence, above ground tissues were collected. Fresh weights were recorded and dried for five days at 60 °C before analysis. A portion of the soil was kept refrigerated (4 °C) at their field moisture to be used in the determination of soil enzymatic activities. The remaining soil was air-dried and sieved to 2 mm for analytical determinations and ecotoxicological evaluation. The experiment took place in a greenhouse under the following conditions: 10–28 °C and 60–80% relative humidity.

2.4. Analytical determinations

Pseudo-total concentration of As and metals in the materials were determined after autoclave digestion of 0.5 g material with H_2O:HNO_3:H_2O_2 (1.5:1:1 v/v/v) at 125 °C and 1.5 KPa for 30 min (Moreno-Jiménez et al., 2010). Extractable elements were determined