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Enhanced Bioavailable Contaminant Stripping: a Case Study of Hg Contaminated Soil

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Despite the numerous articles appearing in scientific journals, very few field applications of phytoextraction have been successfully realized. To overcome the imbalance between the technology's potential and its drawbacks, there is growing interest in the use of plants to reduce only the fraction that is the most hazardous to the environment and human health, that is to target the bioavailable fractions of metals in soil. Enhanced Bioavailable Contaminant Stripping (EBCS) is a new remediation approach aimed to remove all the bioavailable metal fractions, also considering the soil ability to replenish the bioavailable pool in the long term. EBCS have been used in a mercury contaminated soil.

1. Introduction

Phytoextraction is a technique that uses plants to remove metals and other contaminants from the soil..The technology is a typical "green remediation" strategy able to reduce the "footprint" of cleanup actions. It is characterized by a very low environmental impact and cost effectiveness and for these features it has obtained a wide public acceptance. However, despite intensive research over the past decade, only few large-scale applications have been successfully carried out. The discrepancy between the theoretical and the actual application of technology has led to the belief that phytoextraction is not practical (Van Nevel et al., 2007).
Certainly, if a metal is not bioavailable it can not be removed by plants, since metal uptake is function of the root exposure to bioavailable metal in soil (Robinson et al., 2006). Moreover, after several crops, the amount of metal bioavailable for plant uptake will decrease, even if this reduction could be offset by the release of bioavailable metal from soil surfaces (Fischerova et al., 2006).
To overcome the imbalance between the technology's potential and its drawbacks there is a growing interest in the use of phytoextraction to reduce only the most hazardous fraction to the environment and the human health, that is to target the mobile fractions of metal in soil. In this way plants can be used to decrease the bioavailable metal fractions while clean up time can be substantially shortened.
Following this strategy, the Enhanced Bioavailable Contaminant Stripping (EBCS) was introduced (Petruzzelli et al., 2011), this new approach, derived from a work of Hamon and McLaughlin (1999), considered also the possible long term release of metals to solution from soil surfaces.
EBCS approach falls in the field of the Green Remediation as defined by U.S.EPA, with the aim to reduce contaminant bioavailability that represent the highest risk for human health and environment. Fundamental to green remediation is the need to minimize soil disturbance and bioavailablle

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contaminants (U. S. EPA, 2008). Therefore, according to this approach, the target of the plant based remediation is the bioavailable pool of contaminants.
The aim of this work was to determine the EBCS efficiency to remove bioavailable fractions from a mercury contaminated soil. Mercury is of greatest concern in many environmental fields from atmospheric emissions (Karata et al., 1996), to water (Di Natale et al., 2011) and soil pollution (Pedron et al., 2011). Studies on mercury speciation are essential to evaluate its behaviour in the environment (Di Natale et al., 2006; Bozske et al., 2008).To reduce environmental hazards from mercury, adsorption processes have been evaluated (Karatza et al., 1996; Lancia et al., 1996),while phytoremediation has been proposed as soft technology for soil decontamination (Moreno et al., 2005; Pedron et al., 2011). In this study, two plant species have been selected *Brassica juncea* and *Helianthus annuus*. The study was carried out at greenhouse scale using the soil sampled in the contaminated site.

2. Materials and methods

2.1 Soil

The used soil derived from an industrial site located in northern Italy. Soil samples were air dried and ground to pass through a 2 mm sieve before laboratory analysis. Soil analysis were carried out according to the methods of soil analysis (Sparks, 1998). Soil was characterized by: pH 7.1, organic matter 1.1 %, cation exchange capacity 17.3 cmol(+) kg^{-1}. The texture was: sand 58.4 %, clay 22.5 %, silt 19.1 %. Mercury was the only contaminant. The total Hg concentration was 26.7 mg kg^{-1}.

2.2 Enhanced Bioavailable Contaminant Stripping (EBCS)

EBCS is a green remediation procedure aimed to eliminate the bioavailable fractions of contaminants by the use of plants and chemical additives, such as agricultural fertilizers, with no adverse effect on soil quality. In this work the scheme of the EBCS approach consisted in the following a, b, c, d steps:
a) Evaluation of the original Hg bioavailable soil fraction by the use of the sequential extraction with H_2O and NH_4Cl (Bozske et al., 2008).
b) Evaluation of the long-term potential Hg release from soil surfaces with time. This step was performed by extraction with a specific solubilising agent: ammonium thiosulphate $(NH_4)_2S_2O_3$. The action of this extractant is much stronger than any natural process, and the amount released in this step can be safely considered the maximum possible amount of metal available to plants.
c) Evaluation of plant removable mercury. Mesocosm experiments were carried out selecting the most efficient species on the basis of their ability to uptake both the original bioavailable and the newly created fraction brought into solution by the addition of the mobilizing agent.
d) Assessment of the existence of a residual bioavailable fraction in soil by both chemical extractions and further plant growing cycles. When the metal concentration in plants is negligible and no available fraction can be further extracted from soil by $(NH_4)_2S_2O_3$, the residual concentration of the metal in soil can be considered not bioavailable and can be safely left in soil.

2.3 Soil sequential extraction

The mercury available fractions were determined by a two steps sequential extraction procedure (Millán et al., 2006) with H_2O and NH_4Cl. In the first step, 0.5 g of soil were treated with 25 mL of deionised water at pH 7.0 for 1 h at room temperature, the soil residue from water extraction was treated with 25 mL of ammonium chloride 1 M at pH 7.0 for 1h at room temperature. Mercury concentration was determined in the surnatants from both extractions. The total available fraction was calculated as the sum of the water soluble fraction and the exchangeable fraction. The long term potential release from the soil solid phase was determined by 0.27 M ammonium thiosulphate extraction at pH 5.0 with a ratio soil/extractant 1:20 (Moreno et al., 2005; Pedron et al., 2011).

2.4 Mesocosm experiments

The trials were carried out at mesocosm scale in a greenhouse where the temperature was kept between 18 and 26 °C. Mesocosms were polypropylene containers (height 30 cm, volume 8.15 L) that are arranged to collect leachates by a hole in the bottom connected to a plastic tank with a PVC tube. Two plant species were selected: *B. juncea* var. scala, and *H. annuus* var. paola. Coarser materials (>

2 cm) were eliminated from soil before filling the mesocosms. The amount of soil per pot was 5 kg. Plants were sowed using 0.5 g of seeds for *B. juncea* and 9 seeds for *H. annuus*. During the growing period, plants were watered daily with deionized water.
Treatment with mobilizing Hg additive started 45 days after sowing, with the same solution used for Hg extraction, 0.27 M ammonium thiosulphate. The solution was added to mesocosms by splitting the total dose, 250 mL in five consecutive days to avoid or at least to minimize possible toxic effects on plant species. Three replicates of treated mesocosms (TS) were prepared, with controls (CT) (untreated soil) run simultaneously. Experiments lasted 60 days. Plants were harvested 15 days after additive treatment. Aerial parts were separated from the roots and all samples were washed with deionised water. The roots were subjected also to a washing in an ultrasound bath (Branson Sonifier 250 ultrasonic processor) for 10 min to eliminate soil particles that could have remained on root surfaces. Vegetal samples were left in a ventilated oven at a temperature of 40 °C until a constant weight was obtained. The dry mass of shoots and roots was gravimetrically determined. Materials were grinded and homogenised by the use of Knife Mill Grindomix GM 300 Retsch for analysis.

2.5 Mercury analysis

Hg concentrations in soil, plant samples, and surnatants from extraction procedures were determined by atomic absorption spectrophotometry with Automatic Mercury Analyzer (AMA 254, FKV, Bergamo). The analysis was performed directly on the sample both liquid and solid, using SW-846 Method 7473 (USEPA, 1998). The Hg concentration was determined in duplicate, by two optical paths of different sensitivity, at wavelength of 253.65 nm.

2.6 Quality assurance and quality control

Quality assurance and quality control were performed by testing standard solution every 10 samples. Certified reference material was used both for soil (CRM 024-050) and plants (CRM 281) to control the quality of analytical procedure. The detection limit for Hg was 2 $\mu g\ L^{-1}$. The recovery of spiked samples ranged from 95 to 101 % with a RSD of 1.93 of the mean.

2.7 Statistical analysis

All statistical analysis was performed using Statistica version 6.0 (Statsoft, Inc.). Treatment effects were analyzed using one way analysis of variance. Differences among means were compared and a post-hoc analysis of variance was performed using the Tukey's honestly significance difference test ($p < 0.05$).

3. Results and discussion

3.1 Results from EBCS

Step a

Among the several reagents used for evaluating the bioavailable Hg fractions the sequential extraction with H_2O and NH_4Cl was selected (Millán et al., 2006) since properly individuates the amounts in the Hg soil solution (H_2O) or easily solubilizable (NH_4Cl) available for plant uptake.
The analysis on extracts from the sequential extraction procedure showed that Hg soluble and exchangeable fractions represented a very low portion of the total concentration, 2.6 and 9.6 $\mu g\ kg^{-1}$ respectively. The sum of these two fractions was considered the "total available mercury", that can be uptaken by plants.

Step b

To assess the potential ability of the soil to replenish the available metal pool over time, extraction with a highly specific Hg mobilizing agent 0.27 M ammonium thiosulphate was performed. Ammonium thiosulphate extracted, as a mean, 0.12 mg kg^{-1} Hg before plant growth. A further extraction on the residual soil showed negligible amount of Hg below the detection limit. This extraction gives an estimate of the likely long term bioavailable Hg. Since EBCS aims to remove the bioavailable fraction, which is considered extremely dangerous for humans and the environment, the amount of mercury released by thiosulphate extraction can reasonably be considered the quantity that must be removed from soil.

Step c

EBCS efficiency is evaluated by determining the ability of plants to reduce original and also this artificially created bioavailable amount of Hg. Due to the very low original available fraction, to enhance the efficiency of the technology it was necessary the addition of mobilizing agents that can increase the amount of the metal in the soil solution in potentially available forms. The extractant 0.27 M ammonium thiosulphate used to evaluate the release of mercury in the long term has been used also as the additive to increase plant uptake. In this way a new bioavailable pool is created from which plants can uptake larger amounts of the contaminant. The efficiency of plant removal was determined by the ratio between total accumulation and total available mercury in soil. For both species efficiency was higher than 95 %.

As a matter of fact there was a slight increase in biomass production probably due to the fertilizing effect of ammonium thiosulphate. In the growth period of 60 days in the untreated soils the mean values of the aerial biomass were 10.4 and 30.2 g DW for *B. juncea* and *H. annuus*, respectively. After ammonium thiosulphate treatment the obtained results were 16.1 and 34.5 g in the case of *B. juncea* and *H. annuus*, respectively. Root biomass was not affected by treatment with mean values of 1.5 g DW for *B. juncea* and 2.8 g DW for *H. annuus* both in CT and TS soil. The results obtained for original (CT) and thiosulphate treated (TS) soils are reported in Figure 1, where bars indicate standard deviation of n = 3 replicates and means with different letters for the same plant tissue are significantly different from each other ($p < 0.05$) according to the Tukey's test.

The addition of the mobilizing agent promoted Hg uptake by plants of both species. The data (Figure 1) clearly show that the uptake of the plants was dependent on Hg concentration in the soil solution that determines the metal bioavailability.

The mean Hg concentrations in plants grown in the original soil were 2.20 and 32.9 mg kg^{-1} for aerial and root portions of *B. juncea* and 0.80 and 14.0 mg kg^{-1} in the case of *H. annuus*.

The addition of TS solubilised greater quantities of Hg, this in turn increased the Hg uptake by plants. For *B. juncea* the mean Hg concentrations increased to 29.1 and 74.6 mg kg^{-1} in the aerial and root portions, respectively, while for *H. annuus* results of Hg concentration were 10.5 and 73.4 mg kg^{-1}.

The increase in Hg concentrations in plants after TS addition has been reported also in different soils and plants (Moreno et al., 2004 and 2005; Pedron et al., 2011). The lower concentrations of Hg in shoots reflects the defence mechanisms of plants that store the toxic metal in the root portion (Moreno et al., 2004 and 2005). At the end of the experiments the control analysis of leachates showed negligible Hg concentration, below the detection limit (2 μg L^{-1}), in any mesocosms.

The time required to achieve reduction of bioavailable concentration required in soil can be calculated by:

$$t = \frac{W_s \cdot \Delta C}{C_v \cdot B} \quad (1)$$

where t is time (years), W_s weight of soil (kg), and ΔC the decrease of metal concentration necessary to achieve the remediation target (mg kg^{-1}) i.e. elimination of the bioavailable fractions. C_v is the concentration of metal in plants (mg kg^{-1}), and B is the annual biomass production per mesocosm (kg y^{-1}). Both C_v and B depend on the soil characteristics and bioavailable forms of metals. These are the two essential parameters that determine the applicability of plant based remediation. Considering one growing cycle per year, and inserting the specific values of this study in equation 1, the resultant time required is one year.

Step d

To confirm the removal of all the bioavailable Hg, a second growing cycle (without any further treatment) using the same plants was carried out. Results showed a negligible uptake of the metal. Mesocosms were left to rest for one year and then they were reseeded. The new trials were prepared with the following scheme: some pots were sown with the same type of plants used in the past, in others plant species were reversed, sowing *B. juncea* in pots where *H. annuus* grew in previous experiments and vice versa. At the end of the experiment, plants were collected and analysed. The results showed that also in this case Hg concentrations in plant sample were below the detection limit.

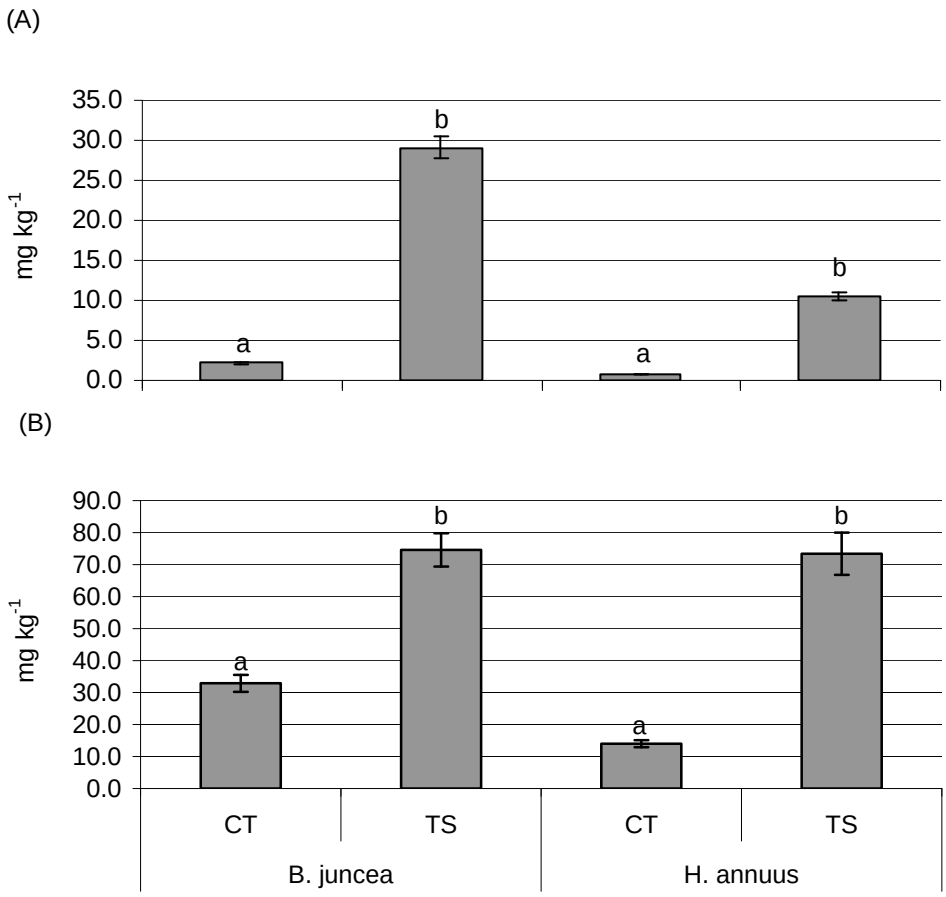

Figure 1: Hg concentrations (mg kg^{-1} DW) in the aerial part (A) and roots (B) of B. juncea and H. annuus grown in CT and TS soils.

The soil extraction with TS did not extract Hg amount over the detection limit. This confirms that all the Hg bioavailable portion has been removed and that new equilibria in the soil with subsequent release of bioavailable Hg were not created. Clearly these results are site-specific and it is possible that in different contaminated soils more than one growth growing cycle would be necessary to eliminate all the bioavailable metal fractions. Throughout the EBCS procedure the potential gaseous loss of Hg(0) has been controlled (Mercury tracker 3000IP Mercury Instruments GMBH) and the values were negligible. When used at field scale a monitoring program should be planned to control the absence of transport of mercury in the deeper soil layer**.**

Green remediation technologies are rapidly expanding in the world to reduce overall environmental impact of cleanup (Pedron and Petruzzelli, 2011). Green remediation is a completely new strategy of remediation that consider environmental impacts of remediation activities at every stage of the remedial process in order to maximize the net environmental benefit of a cleanup. Core elements of green remediation are the use of minimally invasive technologies with special attention to bioremediation and phytoremediation. These technologies appear promising also in the case of heavy metal contamination (Beolchini et al., 2011, Pedron et al 2011). EBCS appears promising, since it

removes the most dangerous metal forms while providing, if used as feasibility tests, a realistic estimation of the maximum amount of metal removable by phytoextraction. The residual metal in the soil following EBCS will remain non-available to plants over time, since it was not released in any mobile forms even with the use of strong mobilizing agents.

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