SJSS
SPANISH JOURNAL OF SOIL SCIENCE

# Remediation of a copper mine soil with organic amendments: Compost and biochar versus Technosol and biochar

*Recuperación de un suelo de mina de cobre con enmiendas orgánicas: compost y biochar versus tecnosol y biochar*
*Recuperação dum solo de mina de cobre com materiais orgânicos: composto e biochar versus tecnosolo e biochar*

**AUTHORS**

**Rodríguez-Vila A.**[@, 1]
fonso@uvigo.es

**Asensio V.**[1, 2]

**Forján R.**[1]

**Covelo E.F.**[1]

[@] Corresponding Author

[1] Departamento de Bioloxía Vexetal e Ciencia do Solo, Facultade de Bioloxia, Universidade de Vigo. Lagoas, Marcosende. 36310 Vigo, Pontevedra, Spain.

[2] Department of Plant Nutrition, CENA, Universidade de São Paulo (CENA-USP). Av. Centenário 303. 13400-970, Piracicaba, SP, Brasil.

**Received:** 19.09.2014 | **Revised:** 05.03.2015 | **Accepted:** 23.03.2015

## ABSTRACT

The contamination produced by metal mining activities is a major environmental problem; for this reason, sustainable management strategies are required for remediating mine soils. The present study focused on the effect of applying organic amendments combined with vegetation in a settling pond soil of a depleted copper mine of Touro (Spain). Two different amendments were applied in different soil/substrate ratios: (1) a mixture made of Technosol and biochar and (2) a mixture of compost and biochar. A 3-month greenhouse experiment was carried out to evaluate the effect of both amendments and mustard plants on the chemical characteristics of the mine soil and the metal uptake by plants. The results showed that the addition of both amendments and planting mustards significantly increased soil pH as well as C and N soil concentrations. The treatments also reduced the $CaCl_2$-extractable metal concentrations in the soil. However, the amendments increased the pseudototal concentration of Zn in the mixtures, since Zn was present in the Technosol and the compost used. Mustard plants extracted Ni from the soil amended with compost and biochar, and Zn from the soil amended with Technosol and biochar. These results suggest *Brassica juncea* L. is a potential candidate to revegetate mine soil for their tolerance to Ni and Zn.

## RESUMEN

*La contaminación producida por las actividades derivadas de la minera metálica representa un grave problema medioambiental y, por tanto, se requieren estrategias sostenibles para la recuperación de los suelos de mina. En este estudio se comparó el efecto de la aplicación de enmiendas orgánicas en un suelo de balsa de flotación de cobre de la mina de Touro (Galicia, España). Para ello, se aplicó al suelo de balsa dos tipos de enmiendas en diferentes proporciones suelo/sustrato: (1) una mezcla de tecnosol y biochar y (2) una mezcla de compost y biochar. Se llevó a cabo un experimento en invernadero durante 3 meses para evaluar el efecto de ambas mezclas de enmiendas y de las plantas de mostaza en las características químicas del suelo de mina y en la extracción de metales por las plantas. Los resultados mostraron que la adición de ambas enmiendas aumentó significativamente el pH y las concentraciones de C y N en el suelo, y redujo las concentraciones de metales extraíbles con $CaCl_2$. Sin embargo, las enmiendas aumentaron la concentración pseudototal de Zn en el suelo, el cual fue proporcionado por el tecnosol y el compost utilizados. Los resultados también mostraron que las mostazas extrajeron Ni en el suelo enmendado con compost y biochar; y Zn del suelo con tecnosol y biochar. Estos resultados sugieren que Brassica juncea L. es un candidato potencial para ser empleado en restauración, por su tolerancia a Ni y Zn en suelos de mina.*

DOI: 10.3232/SJSS.2015.V5.N2.03

**RESUMO**

*A poluição das atividades mineiras representa um grave problema ambiental e, consequentemente, são necessárias estratégias sustentáveis para a recuperação dos solos de mina. Neste estudo foram comparados os efeitos da aplicaçao de materiais orgânicos combinados com vegetação numa lagoa de sedimentação de um solo empobrecido de uma mina de cobre em Touro (Galiza, Espanha). Com esse objetivo, aplicaram-se dois tipos de materiais orgânicos ao solo em duas razões solo/material orgânico: (1) uma mistura de tecnosolo e biochar e (2) uma mistura de composto e biochar. Foi realizado um ensaio numa estufa de vegetação durante três meses para avaliar o efeito dos dois materiais orgânicos e de plantas de mostarda nas propriedades químicas do solo de mina e na extração de metais pelas plantas. Os resultados mostraram que a adição de qualquer um dos materiais orgânicos aumentou significativamente o pH e as concentrações de C e N no solo e reduziu as concentrações de metais extraíveis com $CaCl_2$. No entanto, os materiais orgânicos aumentaram a concentração pseudototal de Zn nas misturas, o qual estava presente no tecnosolo e composto utilizados. Os resultados também mostraram que as plantas de mostarda extraíram Ni do solo corrigido com composto e biochar; e Zn do solo corrigido com tecnosolo e biochar. Estes resultados sugerem que a Brassica juncea L. é um potencial candidato para revegetar os solos de minas dada a sua tolerância ao Ni e Zn.*

**KEY WORDS**
**Contamination, metals, *Brassica juncea***

**PALABRAS CLAVE**
Contaminación, metales, *Brassica juncea*

**PALAVRAS-CHAVE**
Contaminação, metais, *Brassica juncea*

## 1. Introduction

Mining activities have negative impacts on the environment due to the deposition of a huge amount of waste material. Soils on mine tailings usually present unfavourable conditions for the survival of living organisms (Vega et al. 2005; Asensio et al. 2013a). If the metals are in a vegetation-available form, they may accumulate in plants and be transferred to humans and grazing animals (Misra et al. 2009), posing a threat to environment and public health (Cui et al. 2005).

Copper was extracted from 1973 to 1988 in the mine at Touro (Spain). This activity produced a tailings area and a settling pond consisting of fine materials from the accumulation of wastes following the copper flotation process. Currently, another company extracts material for road construction. In 1988, work began on remediating this mine by planting *Pinus pinaster* Aiton and *Eucalyptus globulus* Labill, and later by amending with waste such as sewage sludge and paper mill residues, among others (Asensio et al. 2013a).

The application of organic amendments to mine sites improve the quality of their soils and allows the establishment of vegetation (Asensio et al. 2013b). Organic amendments such as Technosol and compost may be an important and feasible method for reusing waste products, recycling essential nutrients, stabilising the organic matter present in such materials and restoring degraded areas (Camps Arbestain et al. 2008; Karami et al. 2011; Yang et al. 2013). Biochar has been described as a possible material to improve soil fertility, carbon sequestration and other ecosystem services (Sohi et al. 2010). Biochar is expensive and, therefore unsuitable for use in huge areas such as mine sites, and its concentrations of nutrients are probably not enough for most plant species. Consequently, it is better to combine biochar with an organic amendment.

Phytoremediation is defined as the use of plants to remove pollutants from the environment or to reduce their hazardous effect (Raskin et al. 1994). Phytoextraction is a specific type of phytoremediation that is based in the use of plants to remove contaminants from soils (Salt et al. 1998). Some plants can extract, accumulate and tolerate high levels of metals that are toxic to other organisms. These plants are called hyperaccumulators (Evangelou et al. 2004). Successful phytoremediation requires plants with high metal uptake capacity and high biomass production (Hsiao et al. 2007). Plants such as Indian mustard (*Brassica juncea* L.) have the capacity to generate a large amount of biomass and can be used to extract metals from the soil in a reasonable period of time (Quartacci et al. 2006).

The aim of this study was to assess the potential of amendments produced from organic waste (Technosol, compost and biochar) and vegetation (*Brassica juncea* L.) on the recovery (pH, C and N, concentration of metals) of a copper mine soil.

# 2. Materials and Methods

## 2.1. Soil sampling

The sampling area was located at the settling pond from a depleted copper mine located in Touro (Galicia, north-west Spain) (Lat/Lon (Datum ETRS89): 8° 20′ 12.06″ W 42° 52′ 46.18″ N). Copper was extracted in the mine between 1973 and 1988. This activity produced a settling pond of 71 ha resulting from the accumulation of waste after the process of concentrating the metal in flotation banks, and it has been completely dry for several years. Some parts of the settling pond were colonized by native vegetation: gorse (*Ulex* sp.), heather (*Erica* sp.), *Agrostis* sp. and bryophytes, among others (Asensio et al. 2013c).

The mine soil (S) was collected from an untreated area of the settling pond, covering 1.20 ha and without vegetation. The soil had an AC horizon 20 cm deep and was classified as Spolic Technosol (Asensio et al. 2013d). The materials used as amendments for the greenhouse experiment were: a Technosol made of waste, supplied by Tratamientos Ecológicos del Noroeste S.L. (Santiago de Compostela, Spain), a biochar provided by PROININSO S.A. (Málaga, Spain) and a compost supplied by Ecocelta Galicia S.L. (Ponteareas, Pontevedra, Spain). The Technosol (T) was made of sewage sludge (60%), sludge from an aluminium company (10%), waste from canning and rabbit farms (10%), sands from water treatment plants (5%) and ash from a paper mill (5%). The percentages are only approximate, as the total does not account for 100% of the weight of the Technosol. The biochar (B) was made from holm oak wood submitted to a pyrolysis temperature of 400 °C for 8 hours. The compost (CP) was a commercial material marketed for soil amendment, made of rabbit and horse manure, fruit wastes, pruning residues and seaweeds.

## 2.2. Greenhouse experiment

The greenhouse experiment was carried out in 0.5 L pots filled with settling pond soil treated with 20%, 40%, 80% and 100% (in a weight basis) of the amendment (Technosol or compost and biochar) as indicated in **Table 1**. The amendments were mixed with the mine soil before filling the pots with 200 g of the corresponding soil mixture. Pots filled only with mine soil (A0, with 0% amendment) were used as controls. Control pots were supplemented with drinking straws cut into 1 cm length in order to minimize the difference in the substrate volume between the control soil and the amended soils (Puig et al. 2013). Six pots were filled with each type of mixture: three were used for planting mustard (labelled with the letter P) in order to evaluate the effect of the vegetation on soils and the other three were left unplanted.

Three seedlings of *B. juncea*, previously germinated from seeds until they grew two fully expanded leaves, were transferred to each pot. After 45 days, the plants were thinned to one per pot following uniform criteria. The plants, with three replicates per treatment, were harvested 90 days after sowing.

**Table 1.** Labels for the mixtures of soil and amendments, with or without plants

| Soil mixture | Pots without plant | Pots with plant |
|---|---|---|
| **Control: settling pond soil** | A0 | A0P |
| **80% soil + 20% amendment[1]** | T20 | T20P |
| **60% soil + 40% amendment[1]** | T40 | T40P |
| **20% soil + 80% amendment[1]** | T80 | T80P |
| **100% amendment[1]** | T100 | T100P |
| **80% soil + 20% amendment[2]** | C20 | C20P |
| **60% soil + 40% amendment[2]** | C40 | C40P |
| **20% soil + 80% amendment[2]** | C80 | C80P |
| **100% amendment[2]** | C100 | C100P |

[1] Amendment is made of 95% Technosol + 5% biochar. [2] Amendment is made of 95% compost + 5% biochar.

**Table 2.** General characteristics of the mine soil and the 3 amendments

| | | S | B | T | CP |
|---|---|---|---|---|---|
| | pH $_{H_2O}$ | 3.0±0.1d | 9.9±0.2a | 5.6±0.1c | 9.0±0.1b |
| | pH $_{KCl}$ | 2.7±0.0d | 9.6±0.1a | 5.5±0.1c | 8.8±0.1b |
| | C (g kg$^{-1}$) | u.l | 667.0±57.2a | 218.9±15.9b | 214.3±24.1b |
| | N (g kg$^{-1}$) | u.l | 5.8±0.9c | 22.9±3.2a | 18.4±1.4b |
| | C/N | - | 115a | 9b | 11b |
| Pseudototal (mg kg$^{-1}$) | Cu | 452.3±52.6b | 28.7±2.8d | 656.0±47.6a | 142.5±7.8c |
| | Ni | 18.9±3.4c | 27.9±8.7b | 82.4±17.8a | 19.9±2.4c |
| | Pb | 20.9±3.2b | u.l | 94.0±3.0a | 26.6±8.6b |
| | Zn | 65.4±20.8c | 85.8±11.4c | 1445.9±91.5a | 552.9±32.3b |
| $CaCl_2$-extractable (mg kg$^{-1}$) | Cu | 188.3±10.9a | u.l | 4.0±0.1b | 1.3±0.4b |
| | Ni | 8.3±0.1a | 0.3±0.0c | 4.3±0.2b | 0.2±0.1d |
| | Pb | u.l | u.l | 0.4±0.0 | u.l |
| | Zn | 2.6±0.1c | 1.3±0.3c | 735.4±3.8a | 5.32±1.1b |

Mean ± CI values (n = 3). Values followed by different letters differ significantly with P < 0.05. u.l.: undetectable level; S: mine soil; B: biochar; T: Technosol; CP: compost; C: soil total carbon; N: soil total nitrogen.

## 2.3. Soil analysis

The soil samples collected from the field (mine soil) and from the pots were air dried, passed through a 2 mm sieve and homogenized in a vibratory homogeniser for solid samples (Fritsch Laborette 27 rotary sampler divider) prior to analysis.

General soil characteristics were determined (Table 3). Soil reaction was determined with a pH electrode in 1:2.5 water or KCl soil extracts (Porta 1986). Soil total carbon (C) and total nitrogen (N) were determined in a LECO CN-2000 module.

**Table 3.** Selected chemical properties in soil samples after 3 months experiment duration

| Soil sample | pH $H_2O$ | pH KCl | C (g $kg^{-1}$) | N (g $kg^{-1}$) | C/N |
|---|---|---|---|---|---|
| A0 | 2.83±0.08m | 2.72±0.04m | u.l | u.l | - |
| A0P | 2.70±0.17n | 2.60±0.18n | u.l | u.l | - |
| T20 | 4.56±0.11l | 4.49±0.11l | 26.2±1.0hi | 2.6±0.4gh | 10d |
| T40 | 5.16±0.13k | 5.12±0.15k | 56.5±2.5g | 5.5±1.8e | 10d |
| T80 | 5.97±0.28i | 5.94±0.26i | 130.7±4.9e | 11.4±3.7c | 11c |
| T100 | 6.02±0.10hi | 5.98±0.10i | 238.2±2.5a | 20.2±2.8a | 11c |
| T20P | 4.58±0.05l | 4.54±0.07l | 21.0±2.5i | 2.0±0.4h | 10d |
| T40P | 5.56±0.26j | 5.52±0.21j | 59.1±3.3g | 5.0±0.7e | 11c |
| T80P | 6.18±0.24g | 6.17±0.23g | 110.1±2.1f | 9.5±1.2d | 11c |
| T100P | 6.07±0.10h | 6.06±0.07h | 235.7±1.2a | 20.5±0.9a | 11c |
| C20 | 7.04±0.22f | 7.02±0.27f | 20.2±1.4i | 1.8±0.2h | 11c |
| C40 | 7.79±0.13d | 7.74±0.10d | 33.8±1.2h | 3.0±0.3g | 11c |
| C80 | 8.66±0.10b | 8.41±0.03b | 116.7±5.1f | 9.6±1.0d | 12b |
| C100 | 9.03±0.08a | 8.80±0.06a | 187.5±6.4c | 15.3±1.8b | 12b |
| C20P | 7.11±0.16e | 7.07±0.15f | 24.5±1.0hi | 2.1±0.1gh | 11c |
| C40P | 7.75±0.16d | 7.56±0.15e | 50.9±1.2f | 4.1±0.1f | 12b |
| C80P | 8.27±0.26c | 8.09±0.23c | 149.0±3.6d | 11.1±0.4c | 13a |
| C100P | 8.71±0.14b | 8.47±0.39b | 206.9±1.7b | 15.3±0.5b | 13a |

Mean ± CI values (n = 9). Values followed by different letters in each column of each treatment differ significantly with $P < 0.05$. u.l.: undetectable level; C: soil total carbon; N: soil total nitrogen.

## 2.4. Concentrations of copper, nickel, lead and zinc

Cu, Ni, Pb, Zn were extracted with acidified 0.01M $CaCl_2$ (Houba et al. 2000). This metal fraction is operationally defined as phytoavailable (Houba et al. 2000). Pseudototal concentrations of Cu, Ni, Pb and Zn were extracted with *aqua regia* by acid digestion (1:3 v/v) in a microwave oven (Milestone ETHOS 1). The certified reference material CRM026 was analysed in parallel to samples to check the effectiveness and precision of the extraction analysis. Metal concentrations were determined by ICP-AES (Optima 4300 DV; Perkin-Elmer).

## 2.5. Pore water analysis

One week before harvesting the mustards, a “Rhizon” soil pore water sampler (Eijkelkamp Agrisearch Equipment, The Netherlands) was carefully inserted into the soil of each pot at an angle of approximately 45º. Vacuum tubes (10 mL) were attached through a Luer lock system with hypodermic needles to extract pore water. Pore water samples were analysed for metal concentrations by ICP-AES, carried out in triplicate (data not shown). Soil pore water values were used to calculate the bioconcentration factor (BF = shoots concentration / soil pore water concentration).

## 2.6. Plant growth and determination of metals in plant tissues

After 90 days, both roots and shoots were sampled and carefully washed with deionized water. Fresh biomass was immediately weighed and dry mass was assessed after oven drying for 48 h at 80 °C and cooling down to room temperature.

The plant tissues were ground and the total concentrations of Cu, Ni, Pb and Zn extracted by acid digestion using a mixture of $H_2O_2$ and $HNO_3$ (1:6 v/v) in a microwave oven. The certified reference plant material ERM-CD281 was analysed in parallel with the samples.

The transfer coefficient (TC) for the plants was calculated using the following equation:

$$TC = C_s / C_{so}$$

where $C_s$ is the metal concentration in shoots (mg kg$^{-1}$) and $C_{so}$ is the metal content of soil (mg kg$^{-1}$) (Peijnenburg and Jager 2003). A TC indicates a high shoot concentration of the element compared to the pseudototal concentration in soil (Karami et al. 2011). A plant is considered as an accumulator or hyperaccumulator biosystem when TC > 1 (Busuioc et al. 2011).

The translocation factor (TF) for the plants is expressed by the following equation:

$$TF = C_s / C_r$$

where $C_s$ and $C_r$ are metal concentrations (mg kg$^{-1}$) in shoots and roots, respectively. A TF indicates a relatively high shoot metal concentration compared to its root concentration. A plant species translocates metals effectively from the roots to shoots when TF > 1 (Baker and Brooks 1989).

The bioconcentration factor (BF) for plants is expressed by the following equation:

$$BF = C_s / C_{pw}$$

where $C_s$ is the shoot metal concentration (mg kg$^{-1}$) and $C_{pw}$ is the metal concentration in the soil pore water (mg L$^{-1}$) (data not shown) (Peijnenburg and Jager 2003). The BF describes the ratio of metal in pore water concentration that is taken up into shoots. High BF values indicate high shoot element concentration compared to the element concentration in the soil pore water (Karami et al. 2011).

The biotransfer factor (BTF) measures the efficiency of mustard plants in taking up metals from the soil, assessed by considering the phytoavailable forms instead of the soil pseudototal concentrations as a reference, as expressed by the following equation:

$$BTF = C_s / C_{ph}$$

where BTF represents the biotransfer factor of the plants, $C_s$ is the metal concentration in the shoots (mg kg$^{-1}$) and $C_{ph}$ is the $CaCl_2$-extractable (phytoavailable) metal concentration in the soil (mg kg$^{-1}$).

For calculating the TC, BF and BTF, we used the shoot metal concentrations of the mustard plants, as under field conditions this is the plant part that would be removed by a combine harvester. Moreover, the analysed roots had low harvestable amount of metal.

### 2.7. Statistical analysis

All analytical determinations were performed in triplicate. The data obtained were statistically treated using SPSS version 19.0 for Windows. An analysis of variance and homogeneity of variance test were carried out. In case of homogeneity, a post-hoc least significant difference test was carried out. If there was no homogeneity, Dunnett's T3 test was performed. We also carried out a correlated bivariate analysis.

## 3. Results and Discussion

### 3.1. Characteristics of the mine soil and amendments used

The untreated settling pond soil used in this study had an extremely acid pH and undetectable concentrations of soil total carbon and nitrogen (Table 2). The soil acidity was corrected by adding Technosol and especially compost and biochar to the settling pond soil (Table 2). The amendments used also have high C and N contents. The Technosol used is polluted by Cu, Ni, Pb and Zn (Table 2) and the compost used has high pseudototal Cu and Zn concentrations (Table 2).

### 3.2. Characteristics of the greenhouse soil samples

#### *3.2.1. Chemical characteristics*

The chemical characteristics of the mine soil changed significantly after application of the amendments or amendments plus mustard plant vegetation (Table 3). After 3 months of experiment, the pH of the soils without amendment (A0 and A0P) was still extremely acid. The amended soils showed significantly higher pH values than the untreated soils. The highest pH was observed in the samples of compost + biochar not vegetated with mustard plants (C100). Both C and N concentrations were undetectable in the two control soils. Both C and N increased significantly with the application of both amendments, and it was observed that the higher the percentage of amendment, the higher the concentration of C and N.

#### *3.2.2. Metal concentrations in soils*

All of the samples had concentrations of Cu above the GRL for Galician soils (Macías and Calvo de Anta 2009) (Table 4), as the untreated mine soil and the Technosol and compost used have a high total Cu concentration (Table 2).

**Table 4.** Pseudototal and $CaCl_2$-extractable (phytoavailable) Cu, Ni, Pb and Zn concentrations in soil samples after 3 months of experiment; concentrations of the reference soil CRM026

| | Pseudototal (mg kg$^{-1}$) | | | | $CaCl_2$-extractable (mg kg$^{-1}$) | | | |
|---|---|---|---|---|---|---|---|---|
| Soil sample | Cu | Ni | Pb | Zn | Cu | Ni | Pb | Zn |
| A0 | **341.0±53.7b** | 11.9±3.8ghi | 29.9±11.2g | 46.5±9.7j | **151.8±34.9a** | 7.2±2.7a | u.l | 2.8±0.1j |
| A0P | **344.0±88.1b** | 9.3±2.9i | 27.9±9.2gh | 49.3±12.9j | **149.2±38.5a** | 6.9±2.2a | u.l | 3.1±0.4j |
| T20 | **418.8±40.7a** | 14.8±2.8fgh | 42.8±14.4f | 127.2±17.4ghi | **98.2±13.6c** | 5.1±1.4c | 4.8±0.8b | 69.4±18.1h |
| T40 | **408.2±98.2a** | 22.1±6.0cd | 52.4±10.3e | **245.9±59.4f** | **54.0±17.0d** | 6.1±1.8b | 3.1±0.8c | 167.7±48.8e |
| T80 | **351.9±84.5b** | 32.0±8.6b | **91.6±24.7c** | **577.2±200.3c** | 3.4±0.7f | 3.2±0.2e | 0.6±0.0f | **298.7±8.0a** |
| T100 | **349.0±27.1b** | 46.4±8.6a | **125.4±20.5a** | **875.6±186.8a** | 2.0±0.5f | 2.1±0.1f | 0.7±0.2e | **291.3±12.0b** |
| T20P | **408.1±134.1a** | 19.4±1.2de | 37.6±9.4f | 175.3±52.7g | **110.6±20.1b** | 6.0±1.9b | 5.3±0.7a | 86.6±31.2g |
| T40P | **350.2±60.9b** | 23.2±5.6cd | 52.0±16.9e | **244.2±84.3f** | 30.5±6.0e | 4.3±0.6d | 2.8±0.9d | 155.7±29.6f |
| T80P | **300.5±63.6cd** | 26.1±5.8c | 60.8±19.5d | **408.5±127.6d** | 3.3±0.9f | 3.1±0.3e | 0.6±0.1e | **280.8±8.6c** |
| T100P | **314.5±41.9c** | 42.1±4.8a | **105.5±18.9b** | **773.4±72.7b** | 1.7±0.2f | 2.0±0.2fg | 0.6±0.2f | **271.5±26.9d** |
| C20 | **287.6±36.5d** | 12.0±4.1ghi | 23.8±7.1gh | 73.6±21.5ij | 30.5±10.4e | 2.8±0.7e | 0.3±0.1h | 52.2±9.9i |
| C40 | **263.4±27.8e** | 13.9±5.1fgh | 21.9±4.7h | 117.6±24.4hi | 5.1±1.6f | 1.8±0.4fg | u.l | 90.8±11.4g |
| C80 | **190.9±60.6f** | 16.9±4.6ef | u.l | **252.2±67.2f** | 1.2±0.2f | 0.2±0.1h | u.l | 4.1±1.0j |
| C100 | **82.8±22.0g** | 14.1±2.3fgh | u.l | **333.5±91.9e** | 0.9±0.1f | 0.2±0.1h | u.l | 2.8±0.6j |
| C20P | **296.0±63.7cd** | 15.0±4.5efg | 18.0±5.0hi | 92.0±16.3ij | 31.0±3.9e | 3.0±0.6e | 0.4±0.1g | 50.5±4.1i |
| C40P | **291.6±46.5cd** | 11.9±4.2ghi | 14.3±4.3i | 161.1±23.4gh | 3.3±0.7f | 1.4±0.3g | u.l | 87.0±4.2g |
| C80P | **197.8±28.2f** | 13.3±4.6fghi | u.l | **329.8±62.3e** | 1.1±0.1f | 0.2±0.0h | u.l | 4.1±0.3j |
| C100P | **99.7±11.8g** | 10.4±3.3hi | u.l | **404.3±43.8d** | 0.9±0.1f | 0.2±0.0h | u.l | 3.1±0.9j |
| GRL | 50 | 75 | 80 | 200 | - | - | - | - |
| CRM026[a] | 23.1±3.3 | 19.5±2.0 | 45.3±6.3 | 174.5±17.7 | - | - | - | - |
| CRM026[b] | 18.8±0.9 | 14.4±1.5 | 25.6±1.9 | 140.0±7.1 | - | - | - | - |
| SD | 3.0 | 3.6 | 13.9 | 24.4 | - | - | - | - |

Mean ± CI values (n = 9). Values followed by different letters in each column of each treatment differ significantly with P < 0.05. u.l.: undetectable level; GRL: generic reference level established for Galician soils (Macías and Calvo de Anta 2009). Values in bold are over the GRL.

[a] Values obtained from this study.

[b] Certified values.

The highest pseudototal Cu concentration was detected in T20 and T40. Nevertheless, there is a decreasing concentration of Cu in the studied soils when amendments are added (**Table 4**), which may be due to the dilution effect of mixing amendments with soil. The highest $CaCl_2$-extractable Cu was observed in the unamended samples, attributable to their low soil C concentration and the extremely acid pH (**Table 3**). It was observed that the higher the proportion of amendment in the soil mixture, the lower the Cu $CaCl_2$-extractable concentration, decreasing the risk of this metal being transported to other soils, surface water or groundwater. The pseudototal and $CaCl_2$-extractable Ni concentrations were lower than the GRL in all soil samples (**Table 4**), which may be due to the low concentration of this element in both the mine soil and in the biochar and compost used (**Table 2**). However, it can be observed that the higher the amendment rate, the higher the Ni pseudototal concentration in the soils amended with Technosol and biochar. Adding Technosol to the mine soil increased the concentrations of pseudototal Pb, which exceeded the GRL in the samples with the highest amendment rate (**Table 4**). This may be due to the Pb in the Technosol used (**Table 2**). The addition of amendments to the settling pond increased the concentration of pseudototal Zn in the soil, being above the GRL in the samples the highest amendment rate (**Table 4**). It can be seen that the higher the percentage of amendment, the higher the pseudototal Zn concentration in the soil. This is because of the Zn provided by the compost and Technosol used (**Table 2**), probably due to the use of this metal as a nutritional supplement in feed for intensive animal production (Bolan et al. 2004). Caution should be exercised when adding organic wastes, as they can lead to increase pseudototal concentrations of Zn in the treatments with the highest proportion of amendments.

### *3.2.3. Plant biomass and metal uptake*

The mustard plants were not capable of growing in the untreated mine soil (A0P), probably due the high soil acidity, with all of them dying one week after being planted in this soil. Amendment incorporation into the S soil significantly increased the biomass produced in the soils amended with T + B compared to the soils amended with CP + B (**Table 5**). The highest biomass produced was observed in T80P (**Table 5**). There were no significant differences within the biomass produced in the soils amended with CP + B (**Table 5**).

**Table 5.** Biomass produced and concentration of Cu, Ni, Pb y Zn in *B. juncea* plants grown in the different soil treatments at 3 months of experiment

| Soil sample | Biomass (g) | Cu (mg $kg^{-1}$) | Ni (mg $kg^{-1}$) | Pb (mg $kg^{-1}$) | Zn (mg $kg^{-1}$) |
|---|---|---|---|---|---|
| **T20P** | 0.74±0.12d | 223.0±8.7a | 55.2±8.1bc | 6.7±0.9bc | 1113.1±33.4a |
| **T40P** | 2.40±0.23c | 67.1±8.9b | 25.0±0.9ef | u.l | 715.8±9.4c |
| **T80P** | 2.95±0.25a | 47.1±2.7c | 4.3±0.0g | 11.8±2.6ab | 728.4±23.8c |
| **T100P** | 2.55±0.67b | 34.5±3.6d | 19.1±4.3f | 11.7±2.5ab | 921.6±44.8b |
| **C20P** | 0.16 ±0.04e | 31.7±5.1d | 35.5±7.6de | 4.2±1.5c | 71.4±13.2d |
| **C40P** | 0.14±0.02e | 13.6±1.3e | 70.8±13.0a | 14.8±3.3a | 75.5±12.0d |
| **C80P** | 0.13±0.02e | 12.9±3.2e | 61.0±8.6ab | 12.5±1.3ab | 68.2±12.1d |
| **C100P** | 0.16±0.01e | 7.2±1.4f | 43.3±5.8cd | 6.9±0.5bc | 53.3±11.9d |

Mean ± CI values (n = 3 for biomass and n = 9 for metals). Values followed by different letters in each row of each treatment differ significantly with $P < 0.05$. u.l.: undetectable level.

It was observed that for copper, nickel, lead as well as zinc in the soils amended with T + B, the higher the amendment rate, the lower the TC value (Figure 1). TF showed an opposite pattern to TC for Cu and Zn, but the same for Ni. TC and TF were lower than 1 for Cu, Ni and Pb in the samples amended with T + B (Figure 1). TC for Zn was equal or higher than 1 in all of samples, indicating that mustards uptake this metal from soils (Busuioc et al. 2011) (Figure 1d). TF for Zn (Figure 1d) increases by adding organic amendment to the soil, probably because it promotes plant biomass, and was higher than 1 for all of the samples (Figure 1d), indicating Zn transfer from mustard roots to shoots (Baker and Brooks 1989). It was not possible to calculate TC or TF for Ni in T80P as Ni concentration was not detectable in shoots, neither was it possible

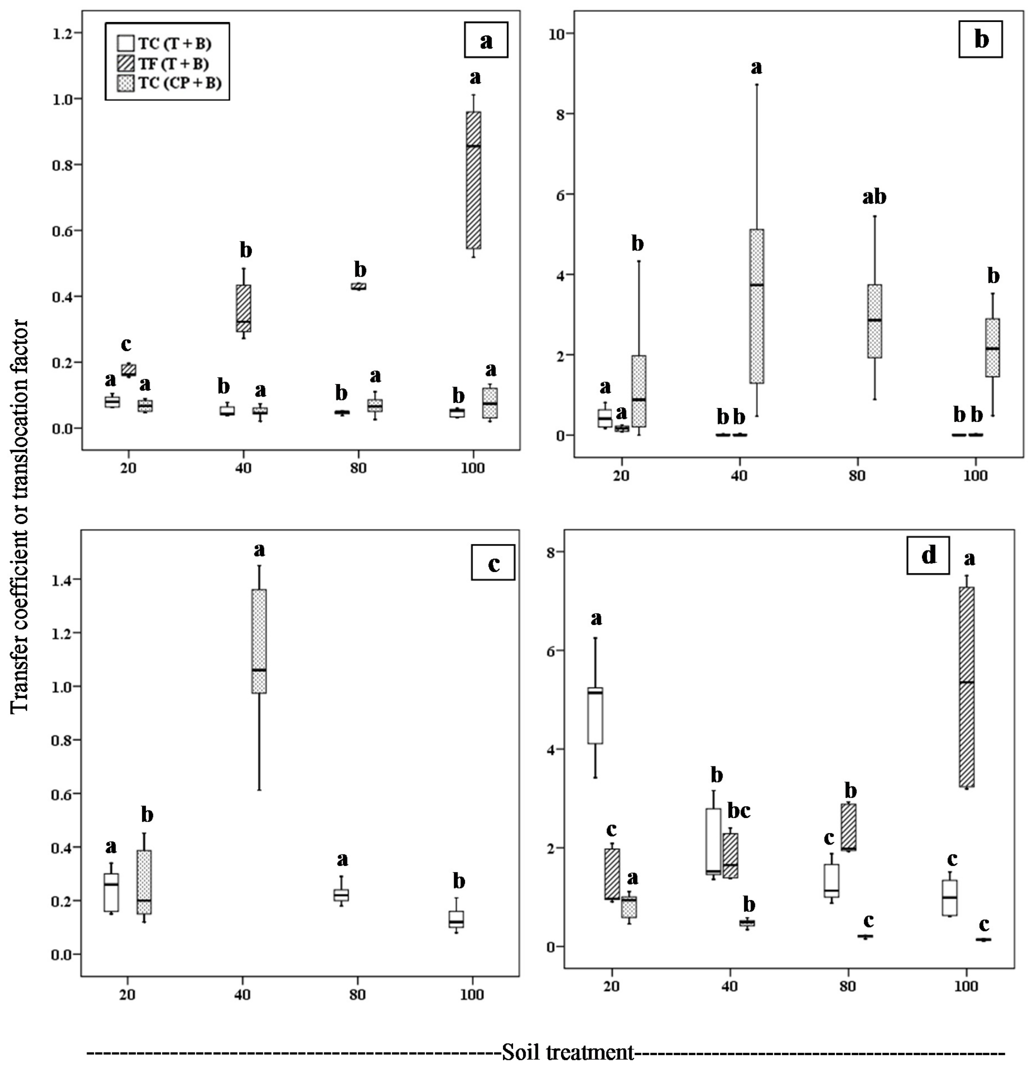


**Figure 1.** Transfer of Cu (**a**), Ni (**b**), Pb (**c**) and Zn (**d**) to and within mustard plants according to the transfer coefficient (TC) (shoots concentration / pseudototal soil concentration) and translocation factor (TF) (shoots concentration / roots concentration). 20 = T20P or C20P; 40 = T40P or C40P; 80 = T80P or C80P; and 100 = T100P or C100P. The solid line is the median, the box represents the upper and lower quartile and whiskers are the 10th and 90th percentiles (n = 3). Different letters indicate significant differences in TC or TF between treatments ($P < 0.05$). The TF for Pb in soils amended with T + B could not be calculated because Pb concentration in mustard roots was no detectable. The TF of mustard plants grown in soils amended with CP + B could not be calculated because metal concentration in mustard roots was not detectable.

to calculate TF for Pb because the root Pb concentration was not detectable in any of the samples. The highest TC for Pb was observed in T20P and T80P and the lowest in T100P. It was not possible to calculate TC in T40P because the Pb concentration in the plants was not detectable.

In the soils amended with CP + B, there were no significant differences in the TC values for Cu (Figure 1a), while for Ni were significantly higher in C40P and C80P, and were in all cases higher than 1 (Figure 1b). Previous studies have shown that plants of the gender *Brassica* are suitable for the phytomanagement of Ni in soils polluted by this metal (Purakayastha et al. 2008; Singh et al. 2013). For samples in which TC could be calculated, it was significantly higher in C40P than in C20P (Figure 1c). In C80P and C100P, TC for Pb could not be calculated because the pseudototal Pb concentration in soils was not detectable (Table 4). TC for Zn significantly decreased as the percentage of amendment increased, although there were no significant differences between the C80P and C100P treatments (Figure 1d).

Amendment incorporation into S soil only significantly increased BF for Cu in T20P compared to T80P (Figure 2a). For Ni the highest BF was observed in the treatments T20P and T100P (Figure 2b) while for Pb, the highest BF was found in T80P and T100P (Figure 2c). For Zn, the application of amendments significantly increased the BF in T20P compared to T40P and T80P (Figure 2d).

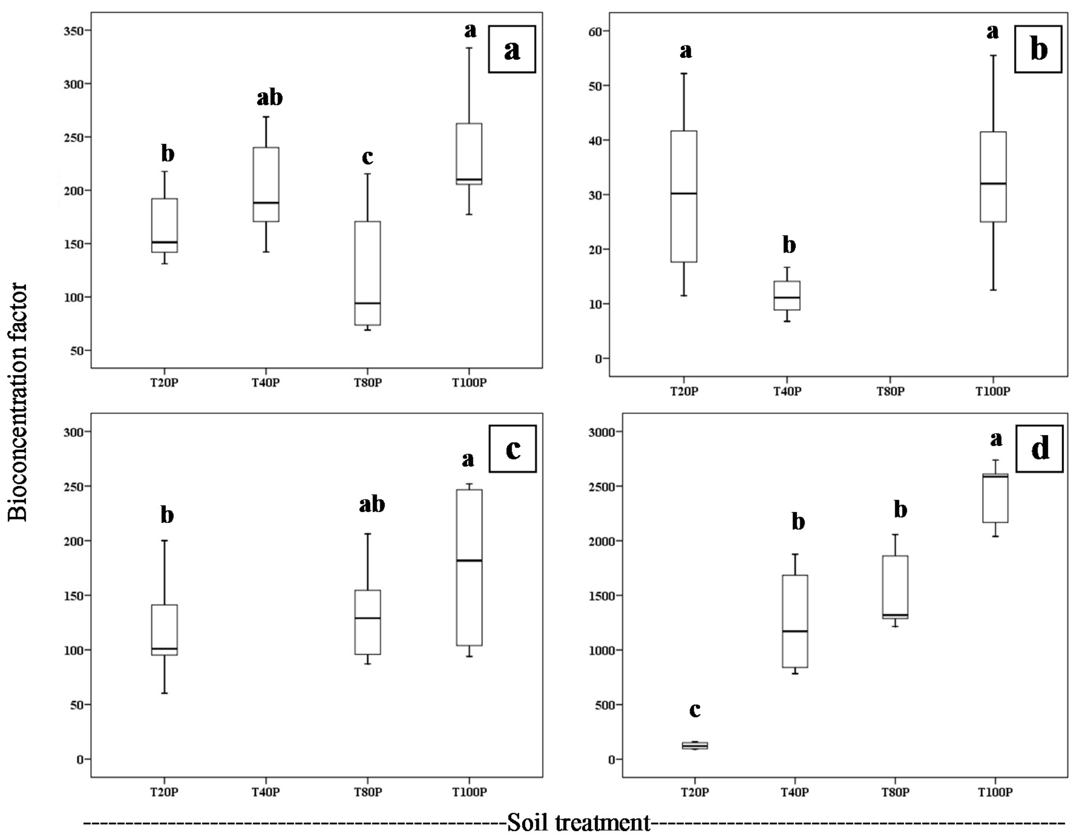


**Figure 2.** Transfer of Cu (**a**), Ni (**b**), Pb (**c**) and Zn (**d**) to mustard plants according to the bioconcentration factor (BF) (shoots concentration / soil pore water concentration). The solid line is the median, the box represents the upper and lower quartile and whiskers are the 10th and 90th percentiles (n = 9). Different letters indicate significant differences in BF between treatments ($P < 0.05$).

The highest BTF for Cu and Zn was observed in C80P and C100P (Figures 3a and c). This is probably because the treatments with the highest dose of amendment had low $CaCl_2$-extractable Cu and Zn concentrations (Table 4), as the organic matter added by the compost and biochar retains this metal. The highest BTF for Ni was observed in C80P (Figure 3b). In the case of Pb, BTF was not represented as it only could be calculated for C20P (11.1), since the $CaCl_2$-extractable Pb concentration in soils was not detectable in the other samples.

In order to take into account the different harvested plant weights in each treatment, the shoot metal removal (shoot dry weight yield x shoot metal concentration) of plant in each pot was calculated (Figure 4). In the samples amended with T + B, the higher the amendment rate, the higher the shoot Cu removal (Figure 4a). For Ni the highest shoot removal was observed in soils amended with 20% T + B (Figure 4b). For Pb the highest shoot removal was obtained for T80P plants, followed by T100P plants (Figure 3c). For Zn, a similar behaviour to Cu was observed, as the higher the amendment rate, the higher the shoot Zn removal (Figure 3d).

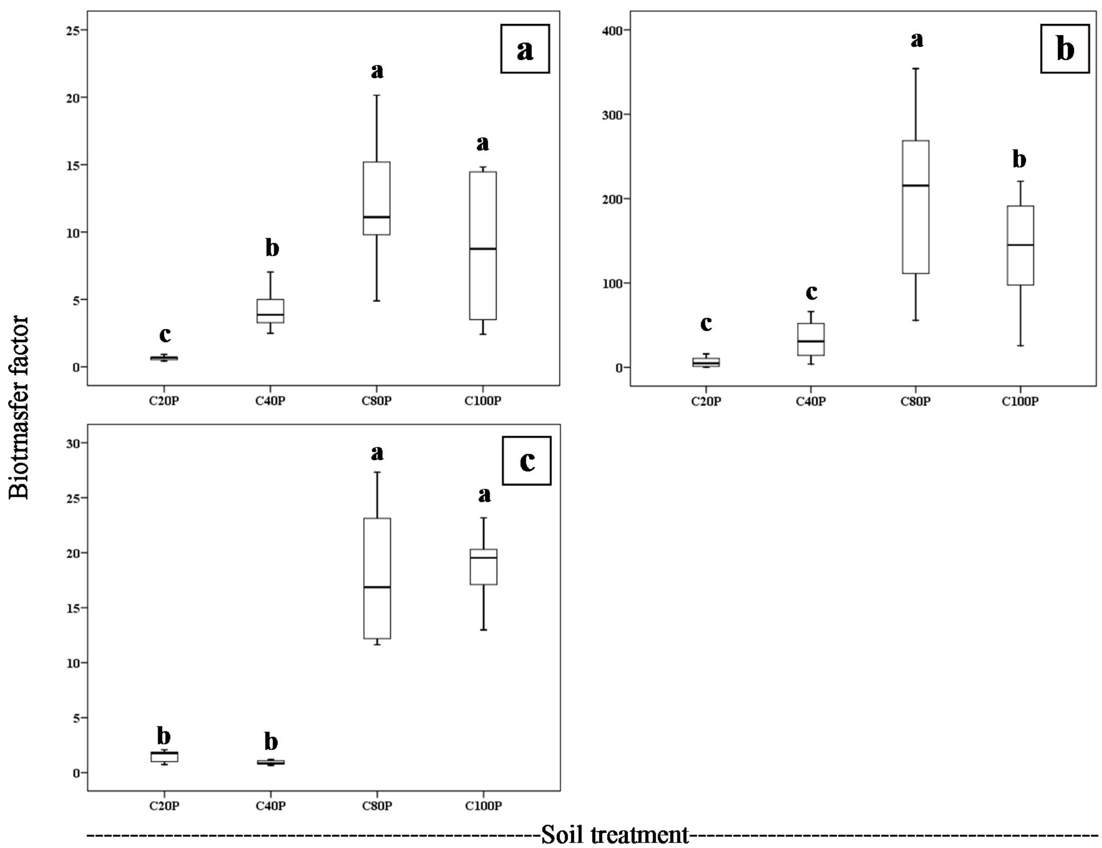


**Figure 3.** Transfer of Cu (**a**), Ni (**b**) and Zn (**c**) to mustard plants according to the biotransfer factor (BTF) (shoots concentration / soil phytoavailable concentration). The solid line is the median, the box represents the upper and lower quartile and whiskers are the 10th and 90th percentiles (n = 9). Different letters indicate significant differences in BTF between treatments (P < 0.05). The BTF for Pb was not represented because it only could be calculated for the soil C20P, since the $CaCl_2$-extractable Pb concentration in soils was not detectable in the others.

In the soils amended with CP + B, the highest total harvestable amount of Cu was obtained in C20P, and there were no significant differences between the other three treatments (Figure 4a). The highest harvestable amount of Ni (Figure 4b) and Pb (Figure 4c) was obtained in C40P. The same behaviour was observed in the harvestable amount for both Ni and Pb. The highest harvestable amount of Zn was obtained in C20P, and a similar pattern to that seen with Cu was observed (Figure 4d).

The combined use of Technosol and compost with biochar as soil amendments improved the quality of soils (e.g. pH, C and N concentrations) and reduced the $CaCl_2$-extractable metal concentrations, allowing the establishment of *Brassica juncea* L. plants. Mustard plants extracted Ni from the soil amended with compost and biochar, and Zn from the soil amended with Technosol and biochar, proving to be tolerant to these metals and to Cu.

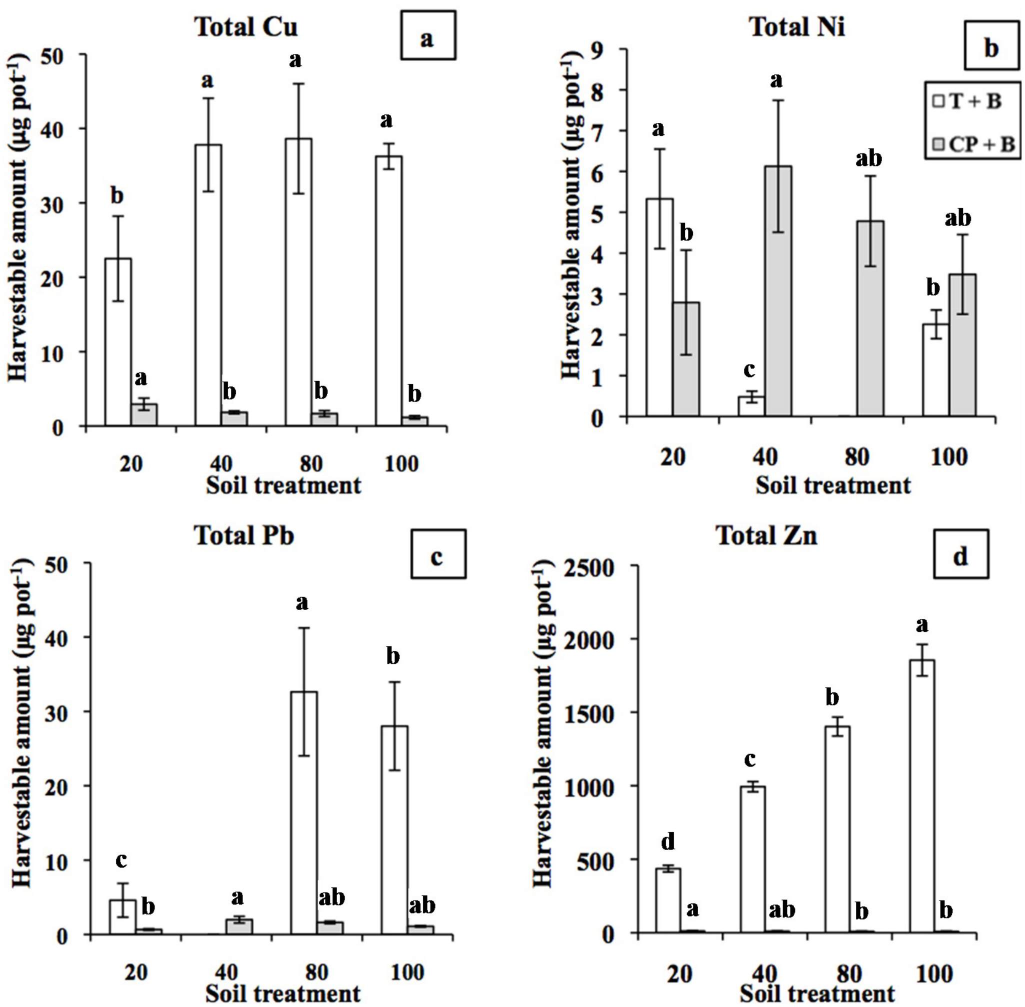


**Figure 4.** Harvestable amounts of total Cu, Ni, Pb and Zn in mustards shoots of the different soil treatments (20 = T20P or C20P; 40 = T40P or C40P; 80 = T80P or C80P; and 100 = T100P or C100P). Bars with the same letters are not significantly different at P < 0.05. Means of three replicates are reported with confidence interval (CI).

# 4. Conclusions

Without organic amendments it was not possible to grow mustard plants in the settling pond soil of a depleted copper mine, which is attributed to its extremely degraded conditions and high Cu concentration. The application of amendments made of Technosol, compost and biochar to a copper polluted soil improved the soil conditions for the establishment of mustard plants by reducing the extreme soil acidity, increasing the concentrations of C and N and reducing the $CaCl_2$-extractable concentration of metals. However, the Technosol and compost increased the Zn concentrations in the amended soils. The combined use of organic amendments and biochar were effective in reducing $CaCl_2$-extractable Cu, Ni and Pb concentrations. *Brassica juncea* L. plants proved to be tolerant to Cu, tolerant to Zn in the soils amended with Technosol and biochar and tolerant to Ni in the soils amended with compost and biochar. These results indicate that planting mustard in combination with organic amendments is an efficient method for the reclamation of mine soils polluted by metals.

# 5. Acknowledgments

This study was supported by the Spanish Ministry of Education and Science through project CGL2009-07843. We also thank the anonymous reviewers for their comments, which helped to improve the quality of this article.

## REFERENCES

• Asensio V, Vega FA, Singh BR, Covelo EF. 2013a. Effects of tree vegetation and waste amendments on the fractionation of Cr, Cu, Ni, Pb and Zn in polluted mine soils. Sci Total Environ. 443:446-53.

• Asensio V, Guala SD, Vega FA, Covelo EF. 2013b. A soil quality index for reclaimed mine soils. Environ Toxicol Chem. 32:2240-2248.

• Asensio V, Vega FA, Andrade ML, Covelo EF. 2013c. Technosols made of wastes to improve physico-chemical characteristics of a copper mine soil. Pedosphere 23:1-9.

• Asensio V, Vega FA, Andrade ML, Covelo EF. 2013d. Tree vegetation and waste amendments to improve the physical condition of copper mine soils. Chemosphere 90:603-610.

• Baker AJM, Brooks RR. 1989. Terrestrial higher plants which hyperaccumulate metallic elements - a review of their distribution, ecology and phytochemistry. Biorecovery 1:81-126.

• Bolan N, Adriano D, Mahimairaja S. 2004. Distribution and bioavailability of trace elements in livestock and poultry manure by-products. Crit Rev Environ Sci Technol. 34:291-338.

• Busuioc G, Elekes CC, Stihi C, Lordache S, Ciulei SC. 2011. The bioaccumulation and translocation of Fe, Zn, and Cu in species of mushrooms from *Russula* genus. Environ Sci Pollut Res Int. 18:890-896.

• Camps Arbestain M, Madinabeitia Z, Anza Hortalà M, Macías-García F, Virgel S, Macías F. 2008. Extractability and leachability of heavy metals in Technosols prepared from mixtures of unconsolidated wastes. Waste Manag. 28:2653-66.

• Cui Y, Zhu Y-G, Zhai R, Huang Y, Qiu Y, Liang J. 2005. Exposure to metal mixtures and human health impacts in a contaminated area in Nanning, China. Environ Int. 31:784-90.

• Evangelou MWH, Daghan H, Schaeffer A. 2004. The influence of humic acids on the phytoextraction of cadmium from soil. Chemosphere 57:207-213.

• Houba VJG, Temminghoff EJM, Gaikhorst GA, Van Vark W. 2000. Soil analysis procedures using 0.01 M calcium chloride as extraction reagent. Commun Soil Sci Plant Anal. 31:1299-1396.

• Hsiao K-H, Kao P-H, Hseu Z-Y. 2007. Effects of chelators on chromium and nickel uptake by *Brassica juncea* on serpentine-mine tailings for phytoextraction. J Hazard Mater. 148:366-376.

• Karami N, Clemente R, Moreno-Jiménez E, Lepp NW, Beesley L. 2011. Efficiency of green waste compost and biochar soil amendments for reducing lead and copper mobility and uptake to ryegrass. J Hazard Mater. 191:41-48.

• Macías F, Calvo de Anta R. 2009. Niveles genéricos de referencia de metales pesados y otros elementos traza en los suelos de Galicia. Santiago de Compostela: Xunta de Galicia.

• Misra V, Tiwari A, Shukla B, Seth CS. 2009. Effects of soil amendments on the bioavailability of heavy metals from zinc mine tailings. Environ Monit Assess. 155:467-75.

• Peijnenburg WJG, Jager T. 2003. Monitoring approaches to assess bioaccessibility and bioavailability of metals: Matrix issues. Ecotoxicol Environ Saf. 56:63-77.

• Porta J. 1986. Técnicas y experimentos en Edafología. Barcelona: Collegi Oficial D`Enginyers Agronoms de Catalunya.

• Puig CG, Álvarez-Iglesias L, Reigosa MJ, Pedrol N. 2013. *Eucalyptus globulus* leaves incorporated as green manure for weed control in maize. Weed Sci. 61:154-161.

• Purakayastha TJ, Viswanath T, Bhadraray S, Chhonkar PK, Adhikari PP, Suribabu K. 2008. Phytoextraction of zinc, copper, nickel and lead from a contaminated soil by different species of *Brassica*. Int J Phytoremediat. 10:61-72.

• Quartacci MF, Argilla A, Baker AJM, Navari-Izzo F. 2006. Phytoextraction of metals from a multiply contaminated soil by Indian mustard. Chemosphere 63:918-925.

• Raskin I, Kumar PN, Dushenkov S, Salt DE. 1994. Bioconcentration of heavy metals by plants. Curr Opin Biotechnol. 5:285-290.

• Salt DE, Smith RD, Raskin I. 1998. Phytoremediaton. Annu Rev Plant Physiol Plant Mol Biol. 49:643-668.

• Singh SK, Ramprakash, Kumari S, Duhan BS. 2013. Phytoextraction of Ni from contaminated soil by *Brassica juncea* as influenced by chelating agents. Ann Biol. 29:15-18.

• Sohi SP, Krull E, López-Capel E, Bol R. 2010. Chapter 2 - A Review of biochar and its use and function in soil. Adv Agron. 105:47-82.

• Vega FA, Covelo EF, Andrade ML. 2005. Limiting factors for reforestation of mine spoils from Galicia (Spain). L Degrad Dev. 16:27-36.

• Yang S, Cao J, Hu W, Zhang X, Duan C. 2013. An evaluation of the effectiveness of novel industrial by-products and organic wastes on heavy metal immobilization in Pb-Zn mine tailings. Environ Sci Process Impacts 15:2059-2067.