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Acute phytotoxicity of seven metals alone and in mixture: Are Italian soil threshold concentrations suitable for plant protection?

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ABSTRACT

Metals can pollute soils in both urban and rural areas with severe impacts on the health of humans, plants and animals living there. Information on metal toxicity is therefore important for ecotoxicology. This study investigated the phytotoxicity of different metals frequently found as pollutants in soils: arsenic, cadmium, chromium, lead, mercury, nickel and zinc. Cucumber (*Cucumis sativus*), sorghum (*Sorghum saccharatum*) and cress (*Lepidium sativum*) seeds were used as models for other plants used in human nutrition such as cereals, rice, fruits and vegetables. The 72-h germination rate and root elongations were selected as short-term ecotoxicological endpoints in seeds exposed to single metals and mixtures. Metals were spiked onto OECD standard soils in concentrations comparable to current Italian contamination threshold concentrations for residential and commercial soils. Arsenic, chromium, mercury and nickel were the most toxic metals in our experimental conditions, particularly to cress seeds (5.172, 152 and 255.4 mg/kg as 72 h IC50 for arsenic, mercury and nickel respectively). Italian limits were acceptable for plant protection only for exposure to each metal alone but not for the mixtures containing all the metals concentrations expected by their respective legislative threshold. The effects of the mixture were class-specific: trends were comparable in dicots but different in monocots. The response induced by the mixture at high concentrations differed from that theoretically obtainable by summing the effects of the individual metals. This might be due to partial antagonism of the metals in soil or to the formation of complexes between the metals, which reduce the bioavailability of the pollutants for plants.

1. Introduction

Soil protection is one of today's main challenges and priorities. Soil, in fact, is a non-renewable resource essential to the well-being of the ecosystem as, first of all, it provides support and conveys water and nutrients to plants, many of which are not only necessary for the sustenance of mankind, but also regulate the distribution of substances in the environment through a delicate balance of storing, filtering and buffering processes. Different thematic strategies have been developed on national, European and international levels for the protection of soil resources (Commission of the European Communities, 2002, 2006; European Commission, 2005, 2013).

One of the most important soil degrading factors is the widespread presence of organic and inorganic xenobiotics and pollutants, which arouse global concern because these agents could affect human and environmental health (Oliver, 1997; Senesil et al., 1999; Abrahams, 2002; European Commission, 2013). Contaminated cereals and vegetables enter the diet of humans and animals, with adverse effects on their health. Chemical contamination derives not only from the application of pesticides, sludges and soil amendments to the soil but also from anthropogenic (industrial activities, traffic, drains and waste) and natural sources.

Chemical analytical techniques, chemical-based risk assessment and biological evaluation are the most widely applied approaches to define soil quality and establish soil protection guidelines and acceptable threshold concentrations of pollutants. Focusing on the biological approach, the organisms most frequently used for the assessment of soil quality are microorganisms, plants, nematodes, mites, springtails and some cell models (Klaine et al., 2003; Mendoza et al., 2006; Picer et al., 2006; Leitgib et al., 2007; Lah et al., 2008; Mattsson et al., 2009; Taffetani and Rismondo, 2009; Vidic et al., 2009; Rocco et al., 2011; Sforzini et al., 2012; Khan et al., 2012; Moore et al., 2012; Oleszczuk et al., 2012; Baderna et al., 2013, 2014; Roubalová et al., 2014.).

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Seeds and higher plants are used as model organisms in ecotoxicology in the short or long term (Sawidis et al., 2011; Malizia et al., 2012). The plants constitute an essential trophic level for terrestrial ecosystems and are closely dependent on their environment: their growth is regulated very precisely by a series of ecological factors (light, nutrients, pollutants, and temperature) that can modify the presence, growth and spread of plant species in a territory (Feoli, 1976; Pignatti et al., 2001; Klaine et al., 2003). The 72 h phytotoxicity test (phytotest) is probably the most widely used acute test assessing chemical-induced adverse effects by measuring seed germination rates and root elongation (USEPA, 1996; ASTM, 2002; Nappi and Jacomini, 2002; Klaine et al., 2003; Baderna et al., 2014).

In the present study we investigated the acute phytotoxicity of seven metals frequently found as pollutants in soil (arsenic, cadmium, chromium, lead, mercury, nickel and zinc) which were investigated by the application of 72 h phytotest with cucumber, cress and sorghum seed exposed to OECD soil contaminated with different concentrations of each metals and evaluating their effects on seed germination and root elongation. Subsequently, the same approach was applied to study the plant toxicity of a mixture in which each metal was present in concentrations equal to its respective legislative limit provided by the Italian Consolidated Environment Act (D.Lgs, 152/2006, Ministry for Environment and Territory and Sea, 2006) to evaluate if the Italian soil threshold concentrations are suitable for plant protection. This second part of the study is the novelty of the research: several publications were published in the recent years about phytotoxicity of single metals or binary mixtures but studies about multi-metals mixtures are very few.

2. Materials and methods

2.1. Reagents and chemicals

Metals atomic spectroscopy and ICP standard solutions ($NaAsO_2$, $Pb(NO_3)_2$, $HgCl_2$, $NiSO_4 \cdot 7H_2O$, $CdCl_2$, $Cr(NO_3)_3$, K_2CrO_4 and $ZnSO_4 \cdot 7H_2O$) were purchased from Sigma (St. Louis, MO, USA). OECD standard soil, cress and sorghum seeds were purchased from Ecotox LDS srl (Italy) as the national distributor of MicroBioTests Inc. (Belgium) and the cucumber seeds were bought in a local seed store, selecting seeds with guaranteed germination and checking their characteristics of germination rates and root growth before use. Several bags from the same lot were purchased as stock to be used for the entire experiment.

Aqueous solutions of each metal were prepared from standard solutions to give the desired metal concentrations in soils.

2.2. Phytotoxicity test with higher plants

The acute phytotoxicity test was done according to the protocol of Martignon (2009) with slight modifications (Baderna et al., 2014) and using seeds of cucumber (*Cucumis sativus*), sorghum (*Sorghum saccharatum*) and cress (*Lepidium sativum*). The protocol is based on the UNICHIM 1651 guideline for phytotoxicity test (2003).

The concentration of metal solutions added to the OECD soil was prepared in order to obtain the desired levels in soil using a volume of solution equal to its water holding capacity (WHC), an agronomic feature of the soil that indicates the amount of water necessary to fully saturate the soil tested. Ten grams of OECD standard soil ((85% sand, 10% kaolin and 5% peat) was plated in a Petri dish (100 mm), wetted with 5 mL (WHC of the soil) of deionized water, metal solution or mixture at different concentrations to completely hydrate the soil and reach the desired metal concentration in soil.

Table 1
Concentrations tested and threshold concentrations according to the Italian Consolidated Environment Act.

Metals	Concentration tested in soil [mg/kg dry soil]	Italian contamination soil threshold [mg/kg dry soil]	
		Residential use	Commercial/industrial use
As	0.5–1–2–5–20–50	20	50
Cd	0.1–0.5–1–2–10	2	15
Cr^{3+}	10–50–100–150–300	150	800
Hg	0.5–1–5–25–125	1	5
Ni	10–50–100–150–300	120	500
Pb	10–50–100–150–300	100	1000
Zn	5–10–50–100–150–300	150	1500

The ranges tested for metal concentrations in soil are reported in Table 1 and include the Italian contamination threshold concentrations for each metal in residential soils. The preliminary analysis of the OECD soil used showed the presence of some metals in concentrations that may be considered negligible in this study (0.3 μg/kg for cadmium, 1 mg/kg for total chromium, and 0.1 mg/kg for arsenic) also because the same soil was used for the untreated/control plate so that, at any rate, these background concentrations could have affected seeds in the same way both in control and in treatments.

Wet soil was then spread over the whole surface of the test plate to obtain a flat homogeneous surface and covered with a wet paper filter (Whatman® qualitative filter paper, Grade 1, 90 mm). For each plate, ten seeds were placed on top of the filter paper and the plates were closed with the cover, sealed with plastic paraffin film and incubated for 3 days at 25 °C, without light. Five replicates were done for each plant species. OECD standard soil was used as control. At the end of the incubation, the number of germinated seeds on each plate was recorded and root elongation was measured.

For each plate, Germination Index (GI) was obtained as follows:

$$GI = \text{no germinated seeds} \times \text{roots length} \quad (1)$$

The mean GI was calculated for each soil sample and control and the percentage GI (GI%) was calculated as ratio between mean GI for the sample and the mean GI for the control soil as follows:

$$GI\% = 100 \times (\langle GI\ treatment \rangle / \langle GI\ OECD\ soil \rangle) \quad (2)$$

Finally the obtained GI% were compared with two scoring criteria: one proposed by Martignon (2009) and one adapted from Kapustuka et al. (2006) as follows: no toxicity = IG% > 90%, low toxicity IG% 75–90%, moderate toxicity IG% 51–75%, high toxicity IG% 26–50% and severe toxicity IG% < 25%.

The classification criteria slightly differ: scoring system 1 (Martignon, 2009) has three levels for toxicity and one level for biostimulation while scoring system 2 (modified from Kapustuka et al., 2006) has four classes of toxicity with tighter intervals but does not take account of the possible stimulating capacity of the treatment.

We calculated the theoretical GI% (Th.GI%) as below:

$$Th.GI\% = 100 - \sum \text{Inhibition induces by each metal}$$

The theoretical GI% was used to compare the effects of the experimental mixtures with those of the individual metals in order to assess whether the interaction of metals was synergistic, additive, or antagonistic.