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Element contents in shoots of sunflower (*Helianthus annuus*): Prediction versus measuring

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ABSTRACT

Sunflower (*Helianthus annuus*) is cultivated as food and feed crop as well as for bioenergy production. It is also investigated towards its ability to remove contaminants from soil and accumulate them in the shoots (phytoextraction). So the reliable prediction of element contents in shoots based on soil contents would be advantageous to easily decide whether plants grown on a certain area could be either used as food and feed or for phytoremediation in combination with bioenergy production. However, it is desirable to predict element contents in plants based on only a few numbers of predictors. This would mean on the one hand a reduced effort in time and costs for analysis and on the other hand existing data on soil quality could be than used for estimations of the element uptake of plants on larger scales. Samples of sunflowers were used, that were grown in plots situated at two different sites in Germany and treated with different amendments (NPK-fertilizer, Streptomyces + Mycorrhiza, Rendzina). One site (heavy metal polluted) was the test field "Gessenwiese", which is situated on the area of the former uranium leaching heap "Gessenhalde". The other site (non-contaminated) was the lysimeter station Falkenberg. Shoot contents of Ca, Cd, Co, Cu, K, Ni, Pb, and Zn were correctly predicted by the mobile soil fraction extracted with 1 M NH_4NO_3 solution (simple regression), whereas for Mg, S, and U the specifically adsorbed soil fraction (extraction with 1 M NH_4OAc solution) needs to be added as predictor (PLS regression). Mn was the only element in the data set for which simple regression based on total soil contents (digestion with HF, $HClO_4$, and HNO_3) had to be used be used for correct prediction in the studied data set.

1. Introduction

Plants take up macro and micro elements from soil. Macro nutrients like Ca, K, Mg, and S are required in high amounts by plants and no negative effects are known even when they are applied in excess (Marschner, 2005). In contrast micro nutrients such as Co, Cu, Mn, Ni, and Zn can become harmful when a certain level in plants is exceeded (Prasad, 1999). Furthermore other micro elements exist that are not essential, but can enter the plant and are toxic even in small amounts, e.g., Cd, Pb, and U. Uptake and storage of toxic elements means harm for humans and animals which take them up via the food chain. Thus, cultivation of food and feed crops on contaminated areas is not carried out, due to the risk of high uptake into the plants. However, the cultivation of bioenergy plants might be a possibility to use these areas. Additionally conceivable is the simultaneous remediation of contaminated land with these bioenergy plants. Sunflower (*Helianthus annuus*) is both a promising bioenergy and phytoremediation plant (Cutright et al., 2010; January et al., 2008; Alaru et al., 2011; Mursec et al., 2009; Willscher et al., 2013). It is investigated towards its ability to remove contaminants from soil and accumulate them in the shoots (phytoextraction) (Adesodun et al., 2010; Fässler et al., 2010; Marchiol et al., 2007). A reliable prediction of element contents in this plant part by soil contents is necessary to decide whether harvested plants grown on a certain area could be used as food and feed or for phytoremediation in combination with bioenergy production.

Plants take up elements with their roots from the soil solution. The direct sampling of soil solution is very difficult and can be very time consuming. Thus, extraction methods were developed to estimate the bioavailable share of elements in soils (Tessier et al.,

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1979; Zeien and Brümmer, 1989) of different origin under comparable conditions. Many extraction schemes have been developed and different solvents have been proven to extract the bioavailable fraction of soil (Kennedy et al., 1997), e.g. NH_4NO_3 (DIN-ISO-19730, 2009; Gryschko et al., 2005), NH_4OAc (Kennedy et al., 1997) or both in combination (Zeien and Brümmer, 1989).

Both, total contents of elements in soil and their bioavailablity are anthropogenically influenced by mining and heavy industry, by the application of fertilizers and sewage sludge (Amir et al., 2005; Carlsson and Büchel, 2005; Mortvedt, 1995). Also soil conditions like pH, carbonate content, organic carbon content, presence of microbes, and plants themselves (release of root exudates) influence the ratio of bioavailable and total element contents in soil (Brümmer et al., 1986). To rate if harmful soil changes and thus potential health risks are caused by a soil substrate, e.g., precaution values based on total contents of elements are defined in federal ordinances and laws (e.g., BBodSchG, 1998; BBodSchV, 1999). However, these values also include the share of the elements that is strongly bound to minerals and therefore not easily bioavailable. In nature it could take hundreds to thousands of years until this share would be released and become available for the plant (Ernst, 1996). So it seems problematic using total contents for risk assessments or prediction of element contents in plants and bioavailable soil fractions should be used instead.

Soil–plant relations of elements were already investigated using multiple linear regression (MLR) (Li et al., 1998; Qian et al., 1996; Efroymson et al., 2001). This regression method has the disadvantage, that redundancies due to correlations in the predictor variables cannot be recognized or have to be excluded before MLR is carried out. Partial-least-squares (PLS) regression, used in this study, is a more sophisticated method, which is able to handle noisy and correlated data (Wold et al., 2001).

The objective of the present study was to find out if contents of Ca, Cd, Co, Cu, K, Mg, Mn, Ni, Pb, S, U, and Zn in shoots of sunflowers grown at different sites (both contaminated and non-contaminated and treated with various amendments) can be predicted just based on the mobile soil contents extracted with NH_4NO_3 or if other predictors, such as specifically adsorbed and total soil contents have to be taken into account. The sole use of the mobile soil fraction for predicting element contents in sunflower shoots could be used for monitoring/screening with minimum effort in time and costs for analysis. This could be of high interest due to its utilization as food and feed crop, as well as bioenergy plant and for phytoremediation.

2. Materials and methods

2.1. Site and plot description

The sunflowers investigated in this study were cultivated at two sites in Germany. One site (heavy metal polluted) is the test field "Gessenwiese", which is situated on the area of the former uranium leaching heap "Gessenhalde" (Jakubick et al., 1997). This heap was part of a former Uranium mining site (1949–1989) in the eastern part of the German federal state Thuringia near the city of Ronneburg (N 50° 51′ 15.872″, E 12° 8′ 49.625″). The heap "Gessenhalde" was used for Uranium ore leaching from the late 1970s until 1989 by the Soviet/German limited company (SDAG) WISMUT. The underlying heap barrier consisted of compacted loam, however was permeable. Thus leachates enriched with U and other metals could penetrate into the underlying soil (Runge and Wolf, 2006; Grawunder et al., 2009). In the early 1990s the leaching material and soil to a depth of up to 10 m were removed. Afterwards the area was recontoured and covered with allochtonic soil substrate (Runge and Wolf, 2006). Carlsson and Büchel (2005) investigated the post-remediation situation and found a slight rest-contamination of metals and radionuclides on the area of the former heap. The test field Gessenwiese was installed in 2004, with the specific objective to test several phytoremediation approaches to manage and remove the remaining contamination (Mirgorodsky et al., 2010). Four different kinds of plots were installed, each having a surface area of 4 m^2 and a depth of 1 m. First: homogenized untreated soil substrate (GW) from the Gessenwiese, second: homogenized fertilized soil substrate (TF) from the Gessenwiese, third: homogenized, fertilized soil from the Gessenwiese amended with mycorrhiza *Glomus intraradices* (Amykor GmbH) and bacteria *Streptomyces acidiscabis* E13 and *Streptomyces tendae* F4 (SM), fourth: homogenized, fertilized soil from the Gessenwiese mixed with 20 kg m^{-2} calcareous Rendzina (MIX) in the top soil layer (0–30 cm). Plots 2–4 were treated with 100 kg per hectar NPK-fertilizer, and exist in triplicates. Further details are given in (Mirgorodsky et al., 2010). The other site (non-contaminated) was the lysimeter station Falkenberg (N 52° 51′ 36.457″, E11° 48′ 44.417″), where from 1981 to 1983 small lysimeters with a surface area of 1 m^2 and depth of 1.25 m were installed (Godlinksi, 2006). They were first used for investigations regarding crop yield maximization and since 1991, they have been used to investigate the influence of land use on soil water and solute balance (Meißner et al., 1998; Godlinksi, 2006). For this study lysimeter 30 (Lys 30, not fertilized) and lysimeter 117 (Lys 117, NPK-fertilized, annually varying type and amount of fertilizer) were used.

2.2. Experimental setup and sampling

The sunflowers (*Helianthus annuus*, variation "Peredovick") investigated in this study were grown in 2009 and 2011. In 2009 sunflowers were sown in April and harvested 96 days after sowing. For the present study the data of sunflowers (single samples) grown on plots TF I to III, SM I to III, and MIX I to II at the test field Gessenwiese were used. In 2011 sunflowers were sown in May on the plots GW (only 1 m^2 used), Lys 30, and Lys 117. For the present study data of sunflower samples (duplicates) taken 84 days after sowing (Lys 30 I, Lys 30 II, Lys 117 I, Lys 117 II) and 96 days after sowing (GW I, GW II) were used. The harvested shoots were washed with deionized water and dried to constant weight in the oven (40 °C). The samples were stored in tubes (CELLSTAR®, Greiner Bio-One) at room temperature until further processing. In 2009 soil samples were taken 96 days after sowing of sunflowers. In 2011 soil samples were taken in October. Soil samples were dried to constant weight in the oven (40 °C) and afterwards stored at room temperature in PVC bottles (Kautex) until further processing.

2.3. Sample preparation and analysis

2.3.1. Plant samples

Sunflower samples from 2009 were ground with a rotor mill (ZM 100, Retsch, Titanium ring sieve). Sunflower samples taken in 2011 were ground in a mixer mill (MM 400, Retsch) for 2 min at 25 Hz (grinding jars made of zirconium oxide) or with mortar and pistle (both made of agate). Up to 200 mg of plant material was weighted and digested in a microwave pressure digestion system (2009: CEM, Mars 5; 2011: CEM, Mars 5 XPRESS) after addition of 5 mL HNO_3 (subboiled).

2.3.2. Soil samples

Before analysis the aggregates in the soil samples were softly chopped with a pistle (agate) and then the soil was sieved to <2 mm. Bioavailability of elements in soil from 2009 and 2011 was determined with a sequential extraction method according to (Zeien and Brümmer, 1989). 2 g of dried, sieved soil (<2 mm) were weighted in tubes (CELLSTAR®, Greiner Bio-One) and 50 mL of 1 M NH_4NO_3 (p.A., Merck) solution were added to extract the "mobile soil fraction". The suspension was shaken (25 rpm) for 24 h overhead (ELU safety lock, Edmund Bühler). The supernatant was removed and 50 mL 1 M NH_4OAc (p.A., Merck) solution were added to extract the "specifically adsorbed fraction". Again, the suspension was shaken for 24 h overhead. The supernatants were stabilized with 0.5 mL HNO_3 (subboiled) and stored at 4 °C in tubes (CELLSTAR®, Greiner Bio-one) until further processing. The detailed procedure is described in Grawunder et al. (2009). For total content analysis the soil (<2 mm) was ground with a mixer mill (MM 400, Retsch) for 2 min at 25 Hz using grinding jars made of zirconium oxide. Total digestion was carried out with about 100 mg ground soil in a pressure digestion system (DAS 30, PicoTrace) using HF (suprapur, 40%, Merck), $HClO_4$ (suprapur, 70%, Merck), and HNO_3 (subboiled).

2.3.3. Chemical analysis

In soil and plant samples Ca, K, Mg, and S (except S total content in soil) were analyzed by ICP-OES (725 ES, Varian), and Cd, Co, Cu, Mn, Ni, Pb, U and Zn were analyzed by ICP-MS (X-Series II, Thermo Scientific). Each sample was measured three times. The precision and accuracy of the ICP-MS measurements were proven by analyzing standard reference material SPS–SW2 (Spectrapure Standards AS) and NIST 1643e (NIST) each in dilution 1:5 (v:v) and comparison to the certified values. Typical precision for triplicate measurement of ICP-MS was ≤2%. The precision and accuracy of the ICP-OES measurements were proven by measuring multi element standard solution (500 mg L^{-1} Ca, K, Mg Bernd Kraft) in dilution 1:5 (v:v) and comparison to the certified values. Typical precision for triplicate measurement of ICP-OES was ≤5%.

2.4. Data pre-treatment

All contents determined to be below the limit of detection (LOD) were substituted against a random value between 0 and the limit of detection. To exclude a significant influence of this substitution on the result of the prediction this was done ten times. An influence was excluded if no change for the result of the *F*-test and *t*-test (see Section 2.5), which were carried out to compare the predicted and measured values of the validation data set, could be found. The variables were logarithmically transformed to normalize their distribution and auto-scaled to guarantee, that

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