Assessment of heavy metal bioaccumulation in sorghum from neutral saline soils in the Po River Delta Plain (Northern Italy)

Dario Di Giuseppe[1], Massimiliano Melchiorre[2], Gianluca Bianchini[1], Alessandra Giurdanella[1], Massimo Coltorti[1], Barbara Faccini[1], Giacomo Ferretti[1]

[1] Department of Physics and Earth Sciences, University of Ferrara, Via Saragat 1, 44122 Ferrara, Italy

[2] Group of Dynamics of the Lithosphere, Institute of Earth Sciences Jaume Almera ICTJA – CSIC, Lluís Solé i Sabarís s/n, 08028 Barcelona, Spain

Abstract

Po River Delta is situated in the Padania Plain at the confluence with the Adriatic Sea. Since the second half of the XIX century, human activities have been focused on the reclamation of wetlands, in order to use them for agricultural purposes. Nowadays these reclaimed soils are mainly used to cultivate cereals and horticultural crops. The mail problem related to this kind of soils is their capability to retain heavy metals. These can be transferred to the cultivations and to the humans, being in some cases very dangerous. It is therefore fundamental to investigate the amount of heavy metals present in reclaimed soils and that which could be transferred to the plants. With this aim, a reclaimed soil that has been used for sorghum cultivation was chosen as test field. Heavy metal concentrations of the soil, the rhizosphere and the seeds, as well as the roots and some aerial parts of the sampled sorghum were measured, respectively. High Cr and Ni concentrations were detected in the soil and rhizosphere samples, related to their natural availability due to the presence of chlorite and serpentine in these soils. On the contrary in the seeds, micronutrients such as Zn and Cu have higher concentrations with respect to Cr and Ni, but all the metals (except Cd) are below the national admitted limits. Metals classified as non-essential for humans (As, Cd, Cr, Ni) are scarcely absorbed by the plant and their average concentrations decreased according to this pathway: soil > rhizosphere > root > aerial parts > seed. Cd, Zn and As are richer in the rhizosphere with respect to the bulk soil.

Keywords

Heavy metals; reclaimed soils; food safety; phyto-availability.

Introduction

The Po River Delta constitutes a vulnerable and fragile environment characterized by a very high human pressure. One of the most important effects of these human activities in the last decades has been the reclamation of wetlands. Reclaimed soils from this area are critical environments where water salinization and accumulation of heavy metals can easily occur (Molinari et al. 2013; Di Giuseppe et al. 2014a; Mastrocicco et al. 2016). Therefore, it is quite important to estimate the bioavailability of metals in these reclaimed soils and their transferability to vegetables, in order to evaluate the exposition of living receptors to potentially toxic elements.

This study is focused on the easternmost part of the Padania Plain (Fig. 1A) where reclaimed soils within the Po River Delta (Fig. 1B) are mainly used for cereals cultivations (such as maize, sorghum and wheat) and horticultural crops. Soils of this area were widely investigated (Amorosi et al. 2002; Amorosi and Sammartino 2007; Dinelli et al. 2007; Amorosi 2012; Di Giuseppe et al. 2014a,b) emphasizing the presence of high natural geochemical anomalies such as Ni and Cr related to the presence of chlorite and serpentine minerals (Bianchini et al. 2012; 2013).

Amorosi and Sammartino (2007) and Di Giuseppe et al. (2014c) have shown that these metals are not bioavailable and thus potentially non-hazardous. However, these authors focused only on heavy metals contents on soils using specific extraction tests, without paying attention to accumulation in cultivated plants. Toxic elements uptake by crops from the soil may in fact stored in the roots or transfer to the atmosphere (Li et al. 2009; Bini et al. 2013; Kelepertzis 2014). The consumption of food contaminated with heavy metals can cause serious health problems (Thomas et al. 2009) and eventually a decrease of the amount of available food (Kabata-Pendias 2011).

The understanding of soil-plant interaction is important also because in the last years many remediation techniques are taking into account the phytoremediation as an environment-friendly and ecologically responsible approach. These remediation techniques are cheaper, preserves soil’s properties and microflora, and have not any secondary pollution consequence (for a review see Ali et al. 2013).

This work explored the relationships between soils and plants analysing *Sorghum vulgare* grown on a reclaimed neutral saline sulphate soils that constitutes a large part of the eastern part of Po river Delta lowlands. The analyses were performed on different underground (roots) and sub-aerial (stem, leaves and seeds) plant organs as well as on soil portions strictly associated with the plant roots, hereafter called rhizosphere. The approach was repeated in two distinct vegetative phases of plant development, allowing to define a more precise soil-plant interaction.

Results were processed using the Translocation Factor (TF) that allows to evaluate the Sorghum ability to accumulate or exclude heavy metals in their tissues. The TF is defined as the ratio between the element concentration in the aerial parts of the plant and the concentration in the corresponding tissue or soil (Singh et al. 2010).

The main goals of this research were: i) to determine soil and plant contamination, if any, ii) evaluate bioaccumulation processes and iii) estimate the hazard for the human health.

Study area

With its 38,110 km^2, Po River Plain is the largest Italian plain, bounded to the north by the Alps and to the south by the Apennine Mountains. It is a sedimentary basin containing a succession of Pliocene-Quaternary marine and continental sediments up to 9 km in thickness (Toscani et al. 2009). The uppermost Quaternary succession can be mainly ascribed to the Po River deposition and secondarily to the sediments carried out by the Apennine Rivers (e.g. the Reno River; Fig. 1A). The evolved soils of this area have poorly weathered profiles and are characterized by young depositional ages and extensive agricultural activities (Amorosi et al. 2014). Calcaric Fluvic Cambisols have developed on top of the channel and proximal levee deposits, Haplic Calcisols at distal levees and Calcaric Stagnic Cambisols at back swamp deposits.

The study site is an agricultural field located near Codigoro (eastern province of Ferrara, Italy; Figs. 1A and B), only about 13 km from the Adriatic Sea coast and it is characterized by a microclimate influenced by the sea. Rainfalls locally reach the regional pluviometric minimum, with an average annual value varying between 500 and 700 mm. Temperatures are affected by the proximity of the sea. This is evident in particular during the cold seasons, when marine thermoregulation contains the minima over zero, reducing the number of night frosts (Mollema et al. 2012). In 1860 the surroundings of the actual city of Codigoro were almost entirely occupied by lagoons (Fig. 1B) that have been gradually dried up in the subsequent decades (Bondesan 1990); today the entire Codigoro Municipality lays on dry land (Fig. 1B).

Materials and Methods

Pedological, geological, mineralogical and physico-chemical features of the soils investigated in this work are reported in Di Giuseppe et al. (2014b).

Plants of *Sorghum vulgare* have been sampled in the experimental field (in Codigoro town) implemented during the ZeoLIFE project (Faccini et al. 2015), in two distinct vegetative phases of the plant development (13/06/2013 and 16/07/2013). The first sampling was carried out one month after planting and the plants were 30 cm high. The second sampling was realized two months before sowing when the plants reached one meter of height.

For each sampling, three samples of plants were taken, every sample being the combination of about 10 plants. In addition, before seeding four bulk soil samples (0–30 cm below the ground level) were taken in order to determine the background level of heavy metal composition.

Plants were uprooted and then sectioned dividing roots and aerial parts (stem and leaves); rhizosphere was also collected shaking the roots and recovering the soil particles that were intimately associated. Rhizosphere samples were taken only on 16/07/2013. The sorghum seeds were sampled at the harvest the 23rd of September 2013.

Roots were washed with distilled water to remove any trace of soil, before the analysis. Samples were then dried at 30°C for 2 days, sieved with a 2-mm sieve and finally ground in an agate mill.

Analyses were carried out using an X Series Thermo-Scientific inductively coupled plasma mass spectrometry (ICP-MS) at the Department of Physics and Earth Sciences of the University of Ferrara. In order to analyse the Cr, Ni, Zn, As, Cd and Cu contents 0.15 g of powder of each sample was totally digested with suprapure grade HF and HNO_3 (Merck, Darmstadt, Germany) acids on a hot plate. Plant samples were dissolved starting from 1 g of sample with H_2O_2 and HNO_3 (proportion 2:1). Rh, In and Re were added to the analysed solutions as internal standard, in order to correct the instrumental drift. Accuracy and precision, based on replicated analyses of samples and standards, are better than 10% for all elements, well above the detection limit. As reference standards, the NIST SRM1567a (wheat flour powder), NIST SRM1547 (peach leaves powder), NIST SRM1573a (tomato leaves powder), E.P.A. Reference Standard SS-1 (a type B naturally contaminated soil) and the E.P.A. Reference Standard SS-2 (a type C naturally contaminated soil) were also analysed to cross-check and validate the results.

In order to verify if within the data set there is a significant variation of heavy metal concentration (i.e. soils vs. roots), an analysis of variance (ANOVA test) was also performed. Differences between the groups were evaluated considering the p-values ($p \leq 0.05$).

Results

The total amount of the studied heavy metals (Cr, Ni, Zn, As, Cd and Cu) in the bulk soil samples (Table 1; Fig. 2A) fall within the level of local natural geochemical background (Di Giuseppe et al. 2014b), even though the concentration of Cr and Ni overcame the Italian threshold limits for green areas (ILD 2006). As previously mentioned, these two elements are likely associated with the peculiarly geochemical characteristics of the Po River drainage basin (Amorosi and Sammartino 2007; Bianchini et al. 2012; 2013). The average content of metals in soils (Table 1) were found in the following order: Cr (230) > Ni (143) > Zn (83) > Cu (41) >As (6.3) > Cd (1.3) mg*kg^{-1}. The same order of relative concentration was detected in the rhizosphere (Table 2, Fig. 2B): Cr (188) > Ni (120) > Zn (100) > Cu (51) >As (9.6) > Cd (3.0) mg*kg^{-1}. From their comparison significant differences can be highlighted for Cr ($p < 0.01$), Ni ($p < 0.01$), Cu ($p < 0.05$), As ($p < 0.01$) and Cd ($p < 0.01$) between the soils and rhizosphere. Cr and Ni have higher values in bulk soil, while Cu, As and Cd appear concentrated in the rhizosphere, likely due to the acidic environment (Coco et al. 2013).

The rhizosphere geochemical budget has been compared with literature guidelines (Kabata-Pendias 2011) and has no heavy metals concentration (except for Ni and Cr). The obtained rhizosphere concentration of metals are also below the Italian threshold limits.

As concerns the vegetal tissues, heavy metals mean level in seeds (Table 3; Fig. 2C) decreases in the following order: Zn (40) > Cu (7.4) > Ni (1.5) >As (0.9) > Cr (0.7) > Cd (0.2) mg*kg^{-1}. TF soil to seeds have this values: Zn (0.5) > Cd (0.2) > As (0.1) > Ni and Cu (0.01) > Cr (0.003).

The average metals content in roots were found in the following order (Table 4, Figs. 3A and C): for 13/06/2013 (Fig 3A), Zn (109) > Cr (33) > Ni (32) > Cu (26) > As (1.9) > Cd (0.6) mg*kg^{-1}; for 16/07/2013 (Fig 3C), Zn (35) > Ni (17) > Cr (15) > Cu (12) > As (3.5) > Cd (0.3) mg*kg^{-1}. TF soil to roots values shows the following trend, for 13/06/2013 are: Zn (1.3) > Cu (0.6) > Cd (0.5) > As (0.3) > Ni (0.2) > Cr (0.1); for 16/07/2013 are: As (0.5) > Zn (0.4) > Cu (0.3) > Cd (0.2) > Ni (0.1) >Cr (0.1).

It appears that metals are absorbed by plants in the early vegetative period, whereas they tend to be subsequently diluted in tissues mass in more mature stages of development.

Differently from the bulk soil and rhizosphere, in the roots the most abundant metal is Zn. Its concentration is so high that exceed that of the soil in the first phase of growth (TF=1.3). As mentioned, the average concentrations of metals in the roots seem to decrease from the first (13/06/2013) to the second (16/07/2013) sampling. Significant decreases can be observed for Zn ($p < 0.05$), Cu ($p = 0.05$) and Cd ($p < 0.05$).

Heavy metals in the aerial parts (Table 4, Figs. 3B and D) of the plants are : for 13/06/2013 (Fig. 3B), Zn (46) > Cu (13) > Ni (2.8) > Cr (2.0) > Cd (0.5) > As (0.3)

$mg*kg^{-1}$; for 16/07/2013 (Fig. 3D), Zn (23) > Cu (8.8) > Ni (1.2) > As (1.0) > Cr (0.9) > Cd (0.4) $mg*kg^{-1}$. TF root to aerial parts values shows the same trend with time (Table 4): for 13/06/2013, Cd (0.9) > Cu (0.5) > Zn (0.4) > As (0.2) > Ni and Cr (0.1); for 16/07/2013, Cd (1.7) > Cu (0.7) > Zn (0.7) > As (0.3) > Ni and Cr (0.1). The most abundant metals in the aerial parts are Zn, Cu and Ni. As already observed for the roots, the average metal concentrations in the aerial parts decrease with time. The elements preferentially transferred from the roots to the aerial parts are Cd, Cu and Zn.

Discussions

Zinc and copper are essential elements for humans and among the micro-metallic elements they are normally the most abundant in plants (Singh et al. 2010; Bini et al. 2013; Grembecka and Szefer 2013; Zhang et al. 2014; Madejón et al. 2015; Pirsaheb et al. 2016). Measured concentration of Zn and Cu in seeds (40 and 7.4 $mg*kg^{-1}$ respectively) are in agreement with the data obtained by Bianchini et al. (2012) on cereals grown in the same area (Fig. 2C). Zinc and copper concentrations measured by Murillo et al. (1999) in sorghum samples grown in a soil polluted by mining activities are 32.7 and 7.5 $mg*kg^{-1}$, respectively. The latter is in line with the data of this study, while the Zn concentration is slightly lower. Despite the higher Zn contents observed when comparing our samples with those from a polluted soil, this does not allow us to state the harmfulness of the Italian sorghum. In order to assess the potential risks that sorghum can have on consumers we have to evaluate the needed dietary intakes of heavy metals. The estimated mean dietary Zn and Cu intakes are 11 and 1.4 $mg*day^{-1}$, respectively (Trumbo et al. 2001). The Tolerable Upper Intake (TUI) level recommended by the European Commission is 25 $mg*day^{-1}$ for Zn and 5 $mg*day^{-1}$ for Cu (EFSA 2006). According to the presented data, consumption of 100 g of sorghum cultivated on studied soils would correspond to a daily intake of 4 $mg*day^{-1}$ of Zn and 0.74 $mg*day^{-1}$ of Cu. These concentrations are well below the limits and therefore harmless if compared with the tolerable dietary intakes reported from literature.

Nickel is not an essential element for humans, even if according to Andrews et al. (1988) and Kim et al. (1991) it may serve as a cofactor, or structural component, of some biological functions. In the study area, very high Ni concentrations were found in the soil, but the TF soil to seed is very low (0.01). Total Ni content in seeds is higher than that detected by Bianchini et al. (2012) (Fig. 2C), but the TUI level (0.1 $mg*day^{-1}$) does not exceed the limits (1 $mg*day^{-1}$) reported by Institute of Medicine-USA (2001).

Arsenic is poisonous to humans (Institute of Medicine-USA 2001) and its biological effects depend strictly on its chemical form. In fact, inorganic compounds are more toxic than most organic ones (WHO 1996). In this study As in soil presents mean level below those found in literature (Kabata-Pendias 2011) and below the limits reported in the Italian regulatory guidelines. This is reflected in low concentrations also in the seeds (0.9 mg*kg^{-1}) and in the low TF soil to seed (0.2).

Chromium is an essential element for humans and has been related to nutrient metabolism (Berdanier et al. 2008). In this study, Cr was detected in high concentration in all soil samples, but as already pointed out by Bini et al. (2013) it is slightly mobile and is usually found in low concentrations in seeds. This is also supported by the very low TF soil to seed (0.003). As already seen for Ni, also the Cr values in the seeds are higher than those found in other cereals by Bianchini et al. (2012) (Fig. 2C). Estimating a possible daily intake of 71 µg*day^{-1} of Cr, the consumption of 100 g*day^{-1} of sorghum does not exceed the TUI level of 250 µg*day^{-1} fixed by (WHO, 1996).

Exposure of humans to Cd affects health, particularly in older age groups (Friberg et al. 1985). The maximum content of Cd in cereals admitted by the European Commission is 0.1 mg*kg^{-1}, while the Joint FAO/WHO Expert Committee on Food Additives (WHO, 1996) recommends a dietary intake of 65 µg*day^{-1}. Although Cd is present in low concentrations in our studied soil (<2 mg*kg^{-1}), it is moderately concentrated in the seeds (0.2 mg*kg^{-1}). Fortunately, estimating a possible daily intake of 20 µg*day^{-1} of Cd, the consumption of 100 g/day of sorghum would not represent a hazard for human health.

The analyses carried out on sorghum highlight that heavy metals concentration in crops decrease in time. This trend can be explained as due to the scarce ability of sorghum to absorb and translocate metals from soil to the tissues. This feature is typical of excluders plants according to the classification proposed by Baker and Walker (1990).

Conclusion

In this contribution, ICP-MS analyses carried out on agricultural soils from the easternmost part of the Padanian Plain (Codigoro town) and on some distinct parts of *Sorghum Vulgare* plants in two different vegetative periods were presented. The results provide for the first time phyto-availability of heavy metals of a neutral saline sulphate soil of the easternmost Po River Plain.

On the whole, the measured heavy metal concentrations show the same range of the local natural geochemical background concentration levels from Di Giuseppe et al. (2014b), though some elements (Cr and Ni) overcome the limits imposed by the Italian law.

Anomalies in the Cr and Ni concentrations in the studied soils are mainly due to the weathering of mafic and ultramafic lithologies outcropping in the Po River catchment. These elements are transported through the rivers to the southeastern areas of the Po River Plain. The other elements (Zn, As, Cd and Cu) fall within the limits of the natural background.

The most abundant heavy metals in the studied soils (Cr and Ni) are not the same prevailing in the seeds (micronutrients such as Zn and Cu). Metals classified as non-essential for humans (As, Cd, Cr and Ni) are scarcely absorbed by the plant and their average concentrations decreased according to this pathway: soil > rhizosphere > root > aerial parts > seed. There are exceptions for Cd, Zn and As which that have a tendency to be partitioned in the rhizosphere with respect to the bulk soil.

Heavy metals concentrations in seeds are consistent with normal ranges reported in literature (Kabata-Pendias 2011; Bianchini et al. 2012) except for Cd slightly exceeding the legislative limits.

In conclusion, it is possible to state that there is no evidence of heavy metals contamination in the soils belonging to the eastern part of the Padania Plain, and the heavy metals' uptake from the plants can be considered harmfulness for human health. Bioaccumulation processes are responsible for Zn, Cu and Cd accumulations, but further investigations are required to test the Cd accumulation in the sorghum.

Finally as by-product of this study, the observed soil/plant relationships could be used for the definition of a territoriality marker (provenance fingerprint) for the local agricultural products.

Obviously, this study is preliminary, and considering that metal transport from soils to plants are due to the metal concentrations as well as the soil properties, further studies will be necessary to constrain thoroughly specific translocation processes occurring in the Po River Delta Plain.

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Figure captions

Figure 1: (**A**) Easternmost part of the Padanian Plain with the different types of sediments recognized in this area. (**B**) Evolution of the study area since the 1860 due to the wetlands reclamation.

Figure 2: Heavy metal concentration measured on the bulk soils from Codigoro, compared with the data of (Di Giuseppe et al. 2014b) and the Italian legislation (ILD 152/2006) (**A**); heavy metal concentration in the rhizosphere samples (**B**); heavy metal concentrations in the seeds compared with those of the maize and wheat cultivation from Bianchini et al. (2012) (**C**).

Figure 3: Heavy metal concentrations measured on the roots and aerial parts (AP) of the *Sorghum vulgare* plants the 13/06/2013 (**A** and **B**) and 16/07/2013 (**C** and **D**).

Table captions

Table 1: Bulk soil heavy metal concentrations. These values are compared with data from literature (Di Giuseppe et al. 2014b) and from the Italian legislation (ILD 152/2006).

Table 2: Heavy metal concentrations measured in the rhizosphere, and average values of each analysed metal.

Table 3: Heavy metal concentrations measured in the seeds, and average values of each analysed metal.

Table 4: Heavy metal concentrations and average values of each analysed metal measured in the roots and aerial parts during two different vegetative phases of the plants.