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Original Article

Agronomic Biofortification of Cereal Crop Plants with Fe, Zn, and Se, by the Utilization of Coal Fly Ash as Plant Growth Media

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ABSTRACT
The implementation of agronomic biofortification of cereal crops with Fe, Zn, and Se appears to be a rapid and simple solution to the deficiency of these elements in soils and plants. These deficiencies are a reason for serious public health concerns. Low levels of Fe, Zn, and Se are important soil constraints to crop production, especially in the developing world. In our study we planted six cereal crops on soil control and different coal combustion residues, naturally rich in these micronutrients. Plants were harvested AND chemically analyzed for Fe, Zn, and Se concentration using ICP.Six plant species have been tested including barley (Hordeumvulgare), Jerry oats (Avena sativa), rye (Secalecereale), wheat (Triticumaestivum), perennial ryegrass (Loliummultiflorum), and ReGreen (wheat x wheatgrass hybrid (Triticumaestivum x Thinopyrumintermedium).All tested plants were able to establish growth on coal ash based growth media, and accumulated significant amounts of Fe, Zn, and Se. It supported our hypothesis, that phytoremediation of coal ash piles may serve also as agronomicbiofortificationof plants, especially cereal crops cultivated on coal fly ash.
***Key words**: agronomic biofortification, cereal crops, coal fly ash, Fe, Zn, Se.*

INTRODUCTION

Iron (Fe), zinc (Zn), and selenium (Se) deficiencies are serious public health issues and important soil constraints to crop production, particularly in the developing world [1, 2].

Iron deficiency is considered to be the common worldwide nutritional deficiency that affects approximately 20% of the world population. Women and children are especially at risk [3].It is one of the most prevalent and most serious nutrient deficiencies threateninghuman health in the world, affecting approximately two billion people [4]. Various physiological diseases, such as anemia and some neurodegenerativediseases are triggered by Fe deficiency [5]. Especially those countries areaffected by Fe deficiency diseases, where people have low meat intake and the diets aremostly based on staple crops. Human health problems caused byFe deficiency can be prevented by specific attention to food composition and by choosing abalanced diet with sufficient Fe concentration.

Zinc is one of the most important micronutrients in biological systems, and plays critical role in protein synthesis and metabolism. Several of Zn-binding proteins are transcription factors necessary for gene regulation and necessary for more than a half of enzymes and proteins involved in ion transport [6]. Any decrease in Zn concentration in human body may result in number of cellular disfunctions, including a high susceptibility to infectious diseases, retardation of mental development, and stunted growth of children [7]. Zinc deficiency is considered to be one of major causes of children death in the world. It is responsible for more than 4% of the deaths of children less than 5 years of age [7].

High consumption of cereal-based foods with low concentration of Zn is the major reason of Zn deficiency in human populations, especially in developing world. This problem is aggravated by growing cereal crops on potentially Zn deficient soils A widespread deficiency of Zn in humans occurs mainly in the regions where soils have Zn deficiency problem and cereals are major source of daily calorie intake [1].

Selenium (Se) is an essential micronutrient for normal mammalian cell function, with antioxidant, anti-cancer and anti-viral properties, and cereal crops are major dietary sources of Se [2, 3].

Se deficiency is widespread and likely manifested in populations as increased risk of thyroid and immune dysfunction, viral infections, cancer and inflammatory conditions [2]. There is strong evidence suggesting that supranutritional Se intake provides protection against cancer [8, 9]. In many soils Se is poorly available to plants, and of concern is a trend toward a reduction of Se in a global food chain, possibly caused by fossil fuel burning (with release of sulphur, a Se antagonist), acid rain, soil acidification and use of high-S fertilizers [2]. As cereal crops are major dietary sources of Se, it is important to modify agronomic practices, mainly through plant fertilization, that may influence Se concentration in these crops in order to maintain, if not increase, the concentration of selenium in plants [2].

Exploiting the genetic variability and biotechnological approach to the development of plants with high Fe, Zn, and Se content may be an effective method to improve the human nutrition, but, unfortunately, it is not very cost effective and requires significant amount of time. Agronomic approaches such as application of Fe, Zn, and Se to plant growth media, called "agronomic biofortification" [10], seems to be a very cost-effective, fast and practical approach to improve Fe, Zn, and Se concentration in cereal crops.

It is important to highlight, that the use of agronomical biofortification might be especially desirable in various developing countries. Most of farmers in such countries (e.g. in Africa) cannot afford application of mineral fertilizers, and it would be desirable find a solution through the application of some cheap materials which would serve as potential fertilizers, would contain a variety of micronutrients, and would be considered as non-toxic from environmental heath point of view.

Coal combustion residues seem to meet these criteria [11, 12].Coal will remain the primary fuel used to generate electricity worldwide – coal combustion generates 39% of the world's electricity [11, 13]. Coal residues are produced during the combustion of solid fuel and can be carried with the flue gas, which is called fly ash (FA), or deposited as a bottom ash, having similar characteristics as a FA [14, 15]. During an average coal combustion process, about 12% of the combusted fuel becomes ash [16].

It is widely known that FA particles emitted from coal-fired plants may contain several toxic trace metals (13).On the other hand, due to the availability of large quantity of FA and the presence of high concentrations of Ca, Mg, Zn, and Fe in most FA sources, FA appears to be a suitable soil amendment for liming purposes and to enhance Ca, Mg and micronutrients contents in the soil [17]. Since FA is composed of mostly silt size particles, addition of fly ash to sandy soils could permanently alter soil texture, increase micro porosity and improve water retention capacity (18).

Despite some objections from the public, The Environmental Protection Agency (EPA) considers coal ash as being safe to use as fertilizer [19]. Studies on the effects of coal fly ash on growth and mineral nutrition of various plants have shown the potential benefit of different soil-FA mixtures on plant germination rates and productivity [12, 20].

The results of the research on the utilization of FA as soil amendment [21, 22] indicate that the increase of elemental concentration as a result of FA addition to growth media is not consistent and differs widely among plant species. The concentrations of Fe, Zn, and Se however, are usually greater in plants grown on FA amended soil as compared to the soil. This regularity should be taken into the consideration in all attempts to use FA as a component of growth media for plants, including attempts to treat FA as a useful alternative for agronomical biofortification purposes. In addition, covering coal ash piles with cereal crops grown on these piles, would create a bridge between two extremely important issues: biofortification and phytoremediation [2]).

MATERIAL AND METHODS

As a plant growth media, soil control (Fargo clay), and two coal FA, one from Montana semi-bituminous coal and another from North Dakota lignite alone or in combination with BA from Montana semi-bituminous coal were used.

Experimental treatments consisted of following growth media:

1. Soil (Fargo Clay) as a control
2. FA from North Dakota lignite coal (FA1)
3. FA from semi-bituminous coal from Montana (FA2)

4. BA from the same semi-bituminous coal from Montana
5. FA/BA (1:1 weight based) from semi-bituminous coal from Montana

Six plant species have been tested including barley (*Hordeumvulgare*), Jerry oats (*Avena sativa*), rye (*Secalecereale*), wheat (*Triticumaestivum*), perennial ryegrass (*Loliummultiflorum*), and ReGreen (wheat x wheatgrass hybrid (*Triticumaestivum x Thinopyrumintermedium*).

The rationale for choosing listed above plant species was: 1) the diversity of its nutritional requirements, 2) resistance to unfavorable environmental conditions, and 3) these plants belong to popular cereal crops.

For each plant, 30 seeds were planted and covered with thin layer of growth media in 10 cm Petri dish, and watered to approximate field capacity of growth media 3 replications.

Plants were grown for 14-21 days (depending on the time of seedlings germination and growth), harvested, dried, and weighed. Experiments have been replicated three times. The concentration ofZn, Fe, and Se in growth media and digested young plants was determined. Before digestion plant samples were washed in order to remove possible adhering FA particles, oven dried at 60º C to constant weight, and grounded to pass a 0.841 screen. Plant samples were wet-digested in a nitric-perchloric acid mixture prior to analysis of elements .

Chemical analysis was performed using inductively coupled plasma (ICP) emission spectrophotometry (11). The data were analyzed statistically using ANOVA and Statistical Analysis System (23).

RESULTS

All tested plants germinated (Fig. 1) and were able to grow (Fig. 2) in media with FA and /or BA.

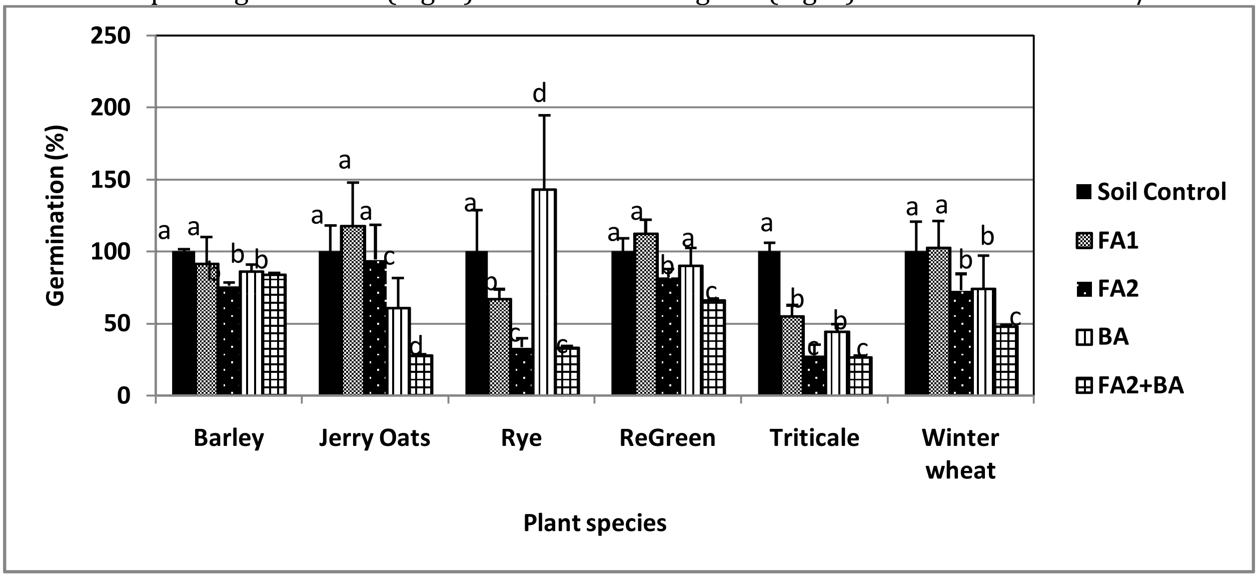

Fig. 1. Germination of plants (%; expressed as percentage of control soil) grown in media consisting of soil control, FA and/or BA(mean±SEM); [a,b,c,d]P<0.05 values with different superscripts differ within a species.

Germination of tested plants was decreased in media containing FA and/or BA compared to soil control (Fig. 1). Compared to soil control, germination of rye was lower (P<0.05) in media consisting of FA1, FA2 or FA2+BA, but higher in media consisting of BA; ReGreen was lower (P<0.05) in media consisting of FA2 or FA2+BA; triticale was lower (P<0.05) in media consisting of FA1, FA2, BA or FA2+BA; barley and jerry winter wheat germination was lower (P<0.05) in media consisting of FA2, BA or FA2+BA; and jerry oats was lower in media consisting of BA or FA2+BA (Fig. 1).

Weight of plants was affected (P<0.05) by media consisting of FA and/or BA (Fig. 2). Barley and ReGreen outperformed other plants, showing higher weight while growing on some of coal ashes, than on soil control. In fact, all tested plants expressed some level of adaptability to grow on at least one of coal ashes used in our study.

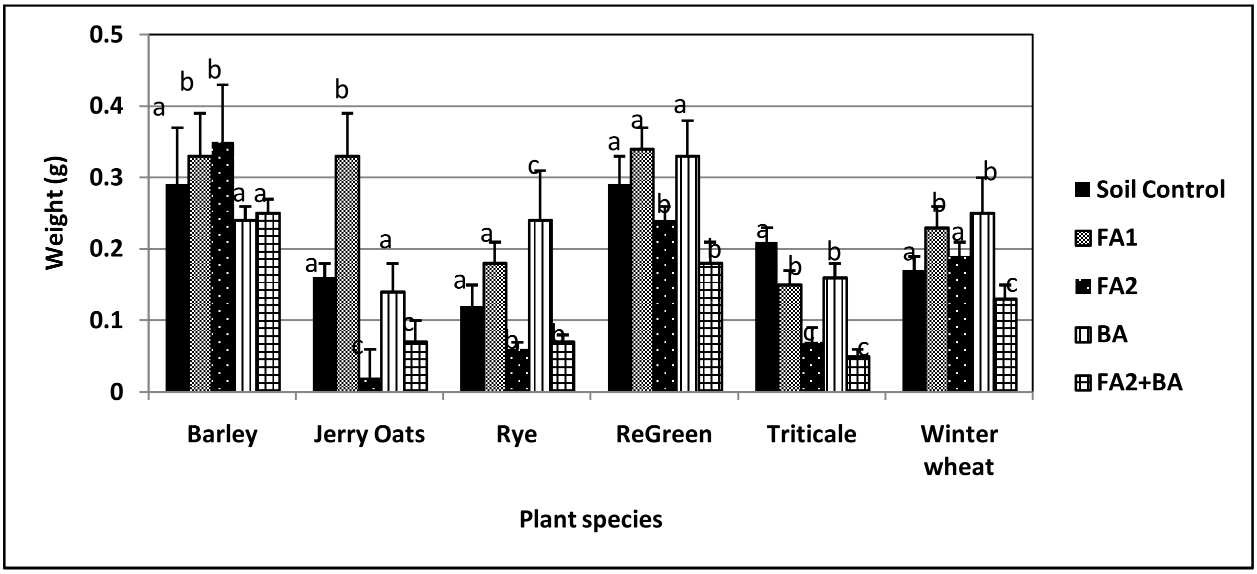

Fig. 2. Weight (g) of plants grown on media consisting of soil control, FA and/or BA harvested on day 14-21 (mean±SEM); ^{a,b,c,d}P<0.05 values differ within a species.

Results of plan germination and seedlings weight indicate a great level of diversity in terms of tolerance to the growth on different ashes. All tested cereal crops species were very diverse in their growth and expressed very selective adaptability to growth media.

Table 1. The concentration of Fe in growth media (%) and in plants (mg x kg^{-1}) grown on media consisting of soil control, FA1, FA2, BA or FA2+BA.

Media	**Growth medium**	**Barley**	**Jerry oats**	**Rye**	**Regreen**	**Triticale**	**Winter wheat**
Soil	0.87± 0.04^{a}	175± 29^{a}	138± 23^{a}	99± 11^{a}	113± 18^{a}	103± 21^{a}	102± 13^{a}
FA1	4.32± 1.0^{b}	834± 49^{b}	834± 62^{b}	1621± 109^{b}	638± 74^{b}	397± 50^{b}	435± 59^{b}
FA2	3.8±0.63^{b}	524± 44^{c}	612± 55^{c}	1120± 104^{c}	650± 71^{c}	401± 51^{b}	441± 40^{b}
BA	1.26±0.17^{c}	362±51^{d}	358± 27^{d}	349± 32^{d}	295± 37^{d}	199± 28^{c}	219± 26^{c}
FA2+BA	2.08±0.37^{d}	407± 37^{e}	452± 39^{e}	405± 47^{e}	362± 43^{e}	203± 21^{d}	247± 37^{d}

The concentration of Fe in all media consisting of coal ash was significantly greater (P<0.05) than in soil control, and was greater in media consisting of FA1 or FA2 (no significant differences between these coal ashes) than in bottom ash (BA) or in a medium combined of bottom ash (BA) and one of fly ashes (FA2). The highest concentrations of Fe have been noticed in rye seedlings grown on coal fly ash based media, and these concentrations exceeded by up to 17 fold the concentration of Fe in plants grown on soil.
The uptake of Fe by all tested plants was much greater by plants grown on ashes based media, and the amount of Fe in plant tissues has been correlated with the level of Fe concentration in growth media. All cereal crop plant seedlings in our experiment expressed at least some level of Fe hyper accumulation ability. Rye showed the highest level of iron accumulation out of all species in our study.

Table 2. The concentration of Zn in growth media and in plants (mg x kg^{-1}) grown on media consisting of soil control, FA1, FA2, BA or FA2+BA.

Media	**Growth medium**	**Barley**	**Jerry oats**	**Rye**	**Regreen**	**Triticale**	**Winter wheat**
Soil	15.2± 0.4^{a}	17.5± 3.3^{a}	33.4± 6.2^{a}	27.0± 4.1^{a}	21.9± 2.8^{a}	31.1± 3.4^{a}	18.0± 2.4^{a}
FA1	51.1± 7.4^{b}	92.8± 19.1^{b}	133.4± 22.2^{b}	87.8± 8.9^{b}	93.5± 11.4^{b}	78.0± 7.9^{b}	192.2± 29.8^{b}
FA2	46.9± 8.3^{b}	89.4± 14.0^{b}	134.2± 25.5^{b}	93.1± 9.4^{b}	89.7± 12.1^{b}	74.2± 8.1^{b}	167.6± 24.2^{b}
BA	21.7±3.7^{c}	51.4±7.7^{c}	93.6± 17.0^{c}	53.3± 5.2^{c}	71.7± 9.7^{c}	53.8± 6.8^{c}	76.5± 16.0^{c}
FA2+BA	31.1± 5.7^{c}	50.2± 6.7^{c}	84.8± 19.3^{d}	60.8± 7.7^{c}	74.3± 9.3^{c}	47.9± 5.1^{c}	73.6± 17.3^{c}

The concentration of Zn in all media consisting of coal ash was significantly greater ($P<0.05$) than in soil control, although the differences between Zn concentrations in the soil and coal ashes were much smaller than in the case of Fe. The concentration of Znin plants grown on coal fly ashes (FA1 and FA2) was higher than in plants grown on bottom ash (BA) and on a media where fly ash has been mixed with a bottom ash (FA2+BA2). Winter wheat accumulated the highest levels of Zinc in their tissues, followed by the oats. All plants grown on coal fly ashes accumulated at least 3 fold more Zn than plants grown on the soil. All plant species in our experiment expressed some level of zinc hyper accumulation ability, with the highest accumulation by winter wheat and jerry oats.

Table 3. The concentration of Sein growth media (mg/kg) and in plants (μg/kg) grown on media consisting of soil control, FA1, FA2, BA or FA2+BA.

Media	Growth medium	Barley	Jerry oats	Rye	Regreen	Triticale	Winter wheat
Soil	0.3± 0.02^{a}	0.00^{a}	34± 6.2^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
FA1	9.07± 0.74^{b}	0.44± 0.09^{b}	133.4± 22.2^{b}	1.64± 0.19^{b}	0.03± 0.002^{b}	0.39± 0.0.4^{b}	0.18± 0.02^{b}
FA2	32.4± 4.3^{c}	3.47± 0.45^{c}	134.2± 25.5^{b}	18.7± 1.94^{c}	1.01± 0.01^{c}	0.87± 0.08^{c}	0.37± 0.05^{c}
BA	3.23±0.37^{d}	0.14±0.07^{d}	93.6± 17.0^{c}	0.00^{a}	0.00^{a}	0.16± 0.006^{d}	0.00^{d}
FA2+BA	7.29± 0.67^{e}	0.14± 0.05^{d}	84.8± 19.3^{d}	0.97± 0.07^{d}	0.3± 0.002^{b}	0.14± 0.005^{d}	0.35± 0.05^{c}

The concentration of selenium (Se) in all plants growth media composed of coal ashes was ~10-100-fold greater ($P<0.05$) than in soil control, with the highest concentration of Se in a coal ash obtained from semi-bituminous coal from Montana (FA2). Such great differences in selenium concentration in growth media translated into differences in Se concentration in the tissues of plants grown on these media. Only oats seedlings grown on the soil accumulated significant amount of selenium. Other plant seedlings didn't show any Se accumulation which would fit into a detectable level (>0.1 μg Se/kg). High concentration of Se in coal fly ash (FA2) resulted in significant accumulation of selenium by oats seedlings, followed by rye seedlings. Among tested plant species, oats and rye expressed the highest hyper accumulating abilities towards selenium uptake from growth media.

DISCUSSION

The results of our study demonstrated that cereal crop plants can germinate and grow in media consisting of coal ash, indicating a possibility to use these plants as a plant cover of piles of coal ashin order to prevent water and wind erosion of coal ash piles. Furthermore, since plants were able to grow in media consisting of coal ash only, coal ash might be used also as a soil supplement [12].

The concentration of Fe in all our growth media remained within the values considered to be the common range of Fe contents in soils –between 0.1 and 10% [24]. The appropriate content of Fe in plants, which is essential to both, the plant metabolismand the nutrient supply to humans and animals, has been reached in all plants grown in our experiment [25]. All cereal crops seedlings in our study were able to hyperaccumulatehigh amounts of Fe from coal ash based growth media, and it should be emphasized that none of these amounts (even 1120mg Fe/kg accumulated by rye seedlings) are considered to be unsafe to consumers of these plants (5). It indicates that the utilization of coal ashes as plant growth media may serve as means for safe Fe delivery, and the fortification of cereal plants, providing them significant amounts of iron. Most of Fe deficient soils occur under arid climates and are associated with calcareous, alkaline soils [12]. Since some of coal ashes are acidic [13], coal residues would be able not only to provide iron for plants grown on Fe deficient soils, but also to decrease a pH of Fe deficienf soils, thus facilitating better iron availability by plants [26].

Zinc is unevenly distributed in soils and its concentration ranges between 10 to 300 mg/kg, with a mean of about 50 mg/kg [27]. Zn deficiency in plants is generally observed when the plant contains less than 20mg Zn/kg, and toxic effects are expected when the concentration exceeds 300-400 mg Zn/kg (28). In our study, all plant species accumulated low levels of Zn while grown on soil control, and Zn concentration in barley and winter wheat seedlings falls even below the limits of "plant healthy" accumulation. The implementation of coal ashes as growth media caused significant increase in Zn concentration in all plant species in our study, but this concentration never exceeded mentioned above toxic levels in plant tissues. Significant increase of Zn accumulation in plants grown on coal ashes may suggest that these coal combustion residues might be treated as Zn fertilizers. As suggested by other studies [29], the application of Zn fertilizers (e.g., agronomic biofortification) seems to be a practical approach to improving Zn concentration in cereal cops.

Selenium contents of worldwide soils have been calculated at an average value of 0.44 mg/kg, but the range of its concentrations is very broad, from 0.005 to 3.5 mg/kg. The lowest amounts of selenium and selenium deficiency occur most frequently in sandy soils developed under humid climate [3]. Our soil control contained lower amount of Se than an average amount of Se calculated for worldwide soils. All coal ashes, especially fly ash from semi-bituminous coal from Montana, contained elevated amounts of Se as compared to soil control, and the highest concentrations in coal ashes in our study: 32.4mg Se/kg, exceededby almost 10-fold amounts spotted naturally in soils.

Most plants contain rather low Se levels, around 0.25μg /kg, and rarely exceed 10 μg /kg (3).It is notable, that in our study the concentration of Se in plants grown on soil control (except for Jerry oats) didn't accumulate enough Se to be detected by ICP analysis. It means, that the concentrations of selenium in these plants were below 0.01 μg/kg. It would indicate, that, except for oats seedlings accumulating 34 μg Se/kg while grown on the soil, other plants grown on soil in our experiment might be expected to suffer from inadequate supply of selenium. Such deficiency would be passed on animals or humans fed on plants with such low concentration of selenium.

On the other hand, a very high concentration of Se in coal ash based growth media didn't result in extremely high uptake of selenium by plants, which would make these plants unsuitable for feeding animals or humans.The highest concentration of Se in plant seedlings grown on coal fly ash from semi-bituminous coal from Montana reached 133 μg/kg. Such valuesby no means may indicate the uptake of excess selenium by consumers, primarily animals. Forage Se at a concentration of around 100 μg/kg is considered as optimum satisfactory concentration for most animals [30].

Our study demonstrated, that it is possible to establish cereal crops growth on growth media composed exclusively of coal combustion by-products, and that these crops are able to accumulate elevated amounts of Fe, Zn and Se, as compared to plants grown on the soil used as control. Accumulated levels of these elements may justify treating the process of cultivating cereal crops on coal ash piles not only as a phytoremediation process, but also as means of agronomic biofortification of planted crops [31].

However, in this study we terminated plant growth after 14-21 days. Therefore, additional studies should be undertaken to determine if these plants would grow on tested growth media till full maturity. In addition, we should be extremely aware of the necessity for close and continuous monitoring of coal the presence of potentially toxic micronutrients in coal ash. Such presence might be one of major limiting factors to the cultivation of cereal crops on coal combustion byproducts piles.

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