Study of ecologo-biological reactions of common flax to finely dispersed metallurgical wastes

O Zakharova 1, A Gusev 1,2, E Skripnikova 3, M Skripnikova 3, Yu Krutyakov 4,5, A Kudrinsky 4,5, I Mikhailov 2, S Senatova 2, C Chuprunov 2, D Kuznetsov 2

1G.R. Derzhavin Tambov State University, 33, Internatsionalnaya street, Tambov, 392000, Russia
2National Research Technological University "MISIS", 2, Leninsky ave., Moscow, 119049, Russia
3Michurinsk State Agrarian University, 101, Internatsionalnaya street, Michurinsk Tambov reg., 393760, Russia
4M.V. Lomonosov Moscow State University, Department of Chemistry, 1-3 Lenin Hills, Moscow, 119991, Russia
5National Research Center “Kurchatov Institute”, 1, Academician Kurchatov Square, Moscow, 123182, Russia

E-mail: olgazakharova1@mail.ru

Abstract. Study was carried out on the influence of metallurgic industrial sludge on morphometric and biochemical indicators as well as productivity of common flax under laboratory and field conditions. In laboratory settings negative influence on seed germinating ability and positive influence on sprouts biomass production in water medium were observed. In sand medium suppression of biological productivity under the influence of sludge together with photosynthetic system II (FS II) activity stimulation were registered. Biochemical study showed peroxidase activity decrease in laboratory, while activity of polyphenol oxidase, superoxide dismutase and catalase were given a mild boost under the influence of sludge. In the field trial, positive influence of sludge on flax photosynthetic apparatus was shown. Positive influence of sludge on vegetation and yield indicators was observed. The analysis of heavy metals content showed excess over maximum allowable concentration (MAC) of copper and zinc in control plants, it may point to the background soil pollution. In the plants from the trial groups receiving 0.5 and 2 ton/ha heavy metals content below the control values was registered. Application of 4 ton/ha led to the maximum content of copper and zinc in the plants among the trial groups. The analysis of soils from the test plots indicated no excess over maximum allowable concentrations of heavy metals. Thus, further study of possibilities of using metallurgic industrial sludge as a soil stimulator in flax cultivation at the application rate of 0.5 t/ha seems promising.

1. Introduction
The problem of production and recycling of finely dispersed industry-related wastes is one of the crucial scientific issues of the 21st century lacking thorough study. In Russian Federation about 7 bln ton of industrial wastes is produced yearly, while no more than 28% (2 bln ton) of the volume is recycled. Metallurgical wastes comprise major part of all the unusable wastes.
At the present time in RF there are no effective industrial technologies for utilizing finely dispersed ashes and sludges from electrometallurgical and blast-furnace processes containing high amounts of iron and heavy metals such as zinc, arsenic, lead, cadmium, nickel, copper, etc. In spite of high concentration of iron in such wastes amounting up to 40-50%, the presence of heavy metals, especially zinc, in concentrations higher than 0.5%, makes it impossible to use them for agglomeration and blast-furnace production with further iron and steel smelting. As a result, most of the metallurgical companies in Russia utilize such wastes by dumping in disposal areas [1]. The present work suggests a prospective way to utilize the stocks of finely dispersed metallurgical wastes accumulated by the traditional storage practices in Russia. The novelty of the suggested approach is in the attempt to simultaneously solve two problems: ecologically safe bioutilization of metal-containing sludge and development of effective and cheap agricultural fertilizers. For instance, iron is found in a number of plant enzymes, it is also involved in chlorophyll synthesis, in breathing and metabolic processes; zinc plays an important role in protein, carbohydrate and phosphoric metabolism as well as in vitamins and growth substances (auxins) synthesis. In patent literature a method is suggested for ecologically safe bioconversion of finely dispersed metallurgical wastes containing heavy metals [2], but it considers employment of sludge as a component for artificial soil, which is unacceptable for a large number of popular industrial crops cultivated in the open.

2. Research target and methods

Research target. For the research a sample of finely dispersed metallurgical waste represented by blast-furnace gas cleaner sludge was used. Blast-furnace sludges contain a number of elements, beside iron, such as zinc, copper, calcium, aluminum, lead, cadmium, etc. Zinc content is one of the main factors limiting recycling of such sludges. A sample of metallurgical sludge (blast-furnace gas cleaner dust) was collected from blast-furnace gas cleaner discharge water, it was filtered by a vacuum filter and air dried at room temperature for 48 hours. The dried sludge was homogenized by means of a mechanic triturating machine (Fritsch Pulverisette 2, no on-bottom weight applied, mixing time 15 min.). The prepared sample of dried sludge was stored in an air-tight polyethylene bag at room temperature, the sample’s humidity was no higher than 0.8 %. Detailed chemical examination of the sample was carried out according to standard methods, the analysis results are summarized in table 1.

| Analyte denomination                  | Measurement result, % | Specified uncertainty, % | Measurement procedure         |
|--------------------------------------|-----------------------|--------------------------|-------------------------------|
| Hygroscopic moisture                 | 0.68 ±0.20            |                          | GOST 23 581.1-79 [3]         |
| Metallic iron                        | <1                    | –                        | GOST 26 482-90 [4]           |
| Total iron                           | 38.4 ±9.1             |                          | PND F 16.3.24-2000 [5]       |
| Manganese                            | 0.086 ±0.029          |                          | PND F 16.3.24-2000 [5]       |
| Copper                               | <0.025                | –                        | PND F 16.3.24-2000 [5]       |
| Oil derivatives                      | 0.028 ±0.011          |                          | PND F 16.1.41-04 [6]         |
| Nickel                               | <0.05                 | –                        | PND F 16.3.24-2000 [5]       |
| Aluminum oxide                       | 1.38 ±0.39            |                          | GOST 23 581.17-81 [7]        |
| Iron oxide (II)                      | 3.44 ±0.17            |                          | GOST R 53657-2009 [8]        |
| Calcium oxide                        | 4.08 ±0.59            |                          | GOST 23 581.16-81 [9]        |
| Silicon oxide (IV)                   | 5.87 ±0.59            |                          | GOST 23 581.15-81 [10]       |
| Magnesium oxide                      | 1.67 ±0.29            |                          | GOST 23 581.16-81 [9]        |
| Losses on ignition                   | 39.3 ±0.7             |                          | GOST 23 581.13-79 [11]       |
| Sulfur                               | 0.42 ±0.1             |                          | GOST 23 581.20-81 [12]       |
| Phosphorus                           | 0.043 ±0.098          |                          | GOST 23 581.19-91 [13]       |
| Chrome                               | 0.117 ±0.018          |                          | PND F 16.3.24-2000 [5]       |
| Zinc                                 | 8.30 ±0.72            |                          | PND F 16.3.24-2000 [5]       |
The sludge chosen for the research is characterized by total iron content of 38.4% w/w. While zinc content is the highest among heavy metals amounting to 8.3% w/w.

Phase composition examination was carried out by means of qualitative X-ray phase analysis using a desktop X-ray diffractometer Difray 401 (ZAO ‘Scientific Instruments’, St. Petersburg). A chromium anode tube (Cr-Kα) was used as an X-rays source; with operating current of 4mA and voltage of 25 kV. Focus control was carried out according to Bragg-Brentano X-ray optics. The obtained diffraction pattern is represented in figure 1.

![Figure 1. An X-ray diffraction pattern of a metallurgical sludge sample](image)

Quantitative phase study was not carried out due to low amount of phases (except hematite Fe$_2$O$_3$, wurtzite ZnS and zinc ferrite Fe$_2$ZnO$_4$) in the sample. Nevertheless, the obtained diffractometric data were processed (software for X-ray phase analysis Match! v.2.0) and evaluation of content ratio of the main phases was carried out, the results are presented in table 2.

| Phases            | Content, % |
|-------------------|------------|
| Fe$_2$ZnO$_4$     | 12         |
| Fe$_2$O$_3$       | 84         |
| ZnS               | 4          |

As one can see from Figure1 and Table 2, the major part of iron in the sample is represented by Fe$_2$O$_3$ and Fe$_2$ZnO$_4$, a quantity of zinc is represented as wurtzite ZnS phase.

The data on size distribution of metallurgical sludge particles (grain size distribution analysis) were obtained by means of laser diffraction analysis carried out using a Fritsch Analysette 22 NanoTec device in submicro- and micro-range analysis mode. The analysis results in the form of a histogram and a grain size distribution curve are represented in figure 2.
Figure 2. Grain size distribution analysis of a metallurgical sludge sample

It was observed that the sludge particles sizes range from 0.1 to 100 μm. Grain size distribution is close to bimodal distribution by its character with distribution median d50 value of about 14 μm.

Morphology of the particle aggregates in the studied sample was examined by means of scanning electron microscopy (HitachiTM-1000 equipment) and the images are represented below in figure 3.

![Figure 3. Electron microphotography of a sludge sample (with magnification: a – 100, b – 500, c – 1000, d – 5000)](image)

As one can see from the presented images, a metallurgical sludge sample features finely dispersed powder consisting both of agglomerates and separate particles with irregular morphology (their shape
is mostly irregular) and varying dispersiveness. The particle size varies within the range of values from below 1 μm to about 80 μm (for the largest agglomerates).

**Test object.** Considering possible accumulation of heavy metals in plants, common flax (*Linum usitatissimum L., 1753*), being a widely cultivated commercial crop, was chosen as a test object.

**Methods of research.** Germination is a critical stage of plant ontogenesis, that is why this stage attracted special attention during the study. Laboratory experiments to evaluate sludge influence on seeds germination were set in water media and in sand soil.

For the experiment in water media sludge suspensions in distilled water (10…0.001%) were prepared. For this purpose batches of metallurgical sludge (100…0.01g = 10…0.001%) were weighed using analytical scales ViBRA HT (Shinko Denshi, Japan, (precision ±0.0001 g)), poured into prepared reservoir containing 1l of water and stirred for 20 sec. with a glass stick. After that the suspension was dispersed by ultrasound with power of 300W and frequency of 23,740 kHz.

For germinations the seeds were placed in humid cameras on paper filters moistened with the liquid (10…0.001%) corresponding the variant. Seeds on filters were placed in Petrie dishes. Each variant of the experiment was produced in 4 replications. In each replication 100 seeds were used. The experiment was divided into two periods. Thus, totally 800 seeds were germinated for each variant. Germination was carried out at 20°C.

The seeds were observed every 24 hours, filters with seeds were sprayed with distilled water.

Germinating energy and germinating ability were measured according to GOST 12038-84 [14] regulations on the 3rd and 7th day accordingly. After that the aboveground and underground parts of the plants were measured, dried for 1 hour at 105°C and weighed.

To discover the peculiarities of seed germination and seedlings development in solid medium a substrate was prepared consisting of sand and sludge in the ratio of 9 weight parts of sand to 1 weight part of sludge (equal to the maximum sludge concentration in water). Triple-washed fluvial sand was used as control substrate.

500 ml plastic containers were used for the experiment. Each one contained either 440g of fluvial sand (control) or 400g of sand and 40g of sludge (experiment), the components of the experimental substrate were mixed thoroughly and moistened till complete wetting. After that the seeds were sown on the surface and the containers were covered with lids to create humid camera conditions. The containers were initially placed in darkness, they were placed in the light when the seedlings appeared. Biological productivity of the plantings was evaluated on the 7th and 15th day. For this purpose samples of plants with the substrate were taken with a probe of 1 sm² in diameter. The substrate was washed off, excess of water was removed from the plants by filter paper and the plants weight was measured (first wet weight was measured and then, after drying in boxes at 105°C, dry weight was measured).

Besides, activity of antioxidant system enzymes, such as peroxidase, polyphenol oxidase, superoxide dismutase and catalase was analyzed in seedlings grown in solid medium [15-17].

For the study of metallurgical sludge influence on biological and industrial productivity of flax in the field conditions the following doses of sludge were used: 0.5 t/ha, 2 t/ha and 4 t/ha. During the field experiment the following indicators were measured: main photosynthetic characteristics of flax plantings [18], main biometric and yield characteristics [19]. After the experiment was accomplished, analysis of heavy metals content in the plant tissues and in the soil was carried out [20-24].

3. **Results and discussion**

**Water medium.** It was ascertained that sludge has negative influence on germinating energy and germinating ability of flax seeds (up to 20% decrease was observed) (figure 4).
Besides, decrease of the root length was observed by 30-40% in the whole, though the value of average weight of the seedlings root part displayed no difference from the control group, while at 0.01% concentration considerable, more than twofold, increase in root weight was registered (figure 5). Average stalk length underwent no considerable alteration under the influence of sludge, while average stalk weight exceeded the control values by 1.5-2 times at some concentrations.

**Figure 4.** Influence of metallurgical sludge in water medium on germinating energy and germinating ability of common flax

**Figure 5.** Influence of metallurgical sludge in water medium on morphometric characteristics of common flax seedlings

*Sand medium.* Comparison of biological productivity of the plantings in sand medium on the 7th day showed that high doses of sludge (10% by weight) have negative influence on the studied characteristic (figure 6 (a)). On the 15th day after germination the characteristics of slow chlorophyll fluorescence induction differed in the test and control variants, growing power coefficient being higher in the experimental variant [18]. It was accompanied by increase in the plants’ desiccation resistance [25]. After 3-day incomplete drying the experimental seedlings totally recovered their turgor within an hour, while only 80% of the control plants recovered their turgor within 24 hours after being placed in humid cameras.
At the same time, sludge application to the sand substrate stimulates photosynthetic system II (PS II) activity which may lead to the plantings bioproductivity increase (figure 6 (b)).

![Graph](image1.png)

**Figure 6.** Influence of metallurgical sludge on biological productivity of common flax in sand medium: a) 7th day; b) 15th day

Seed germination is accompanied by a variety of biochemical processes and performance of oxidation-reduction reaction enzymes is the most important one. In the process of seed germination active lipids oxidation can lead to decrease in germination rate and in growth-regulating activity of seedlings. The study showed that in flax peroxidase activity was decreased in the experimental plants, while polyphenol oxidase, superoxide dismutase and catalase activity slightly increased under the influence of sludge (table 3). Association of enzyme activity in roots with seedlings growth activity proves the crucial role of these enzymes in formation of protective mechanisms, taking place in a plant undergoing influence from external factors which can stimulate the activity of oxidation processes in a cell.

**Table 3.** Influence of metallurgical sludge on enzyme activity in seedlings roots on the 7th day

| Variant    | Peroxidase, umol/min g d.w. | Polyphenol oxidase, rel. act. unit/min | Superoxide dismutase, rel. act. unit | Catalase, umol/min g |
|------------|-----------------------------|----------------------------------------|--------------------------------------|----------------------|
| Control    | 264.0±16.8                  | 13.4±1.7                               | 15.8±1.1                            | 0.79±0.02            |
| Experiment | 249.0±9.9                   | 13.8±1.5                               | 16.4±1.0                            | 0.84±0.01            |

*Field experiment:* Assessment of the impact of finely dispersed sludge wastes on photosynthetic apparatus of common flax grown in the field showed that flax plants receiving 0.5 t/ha of sludge formed the largest assimilating surface amounting to $20.76 \times 10^3$ m²/ha. The minimal assimilating surface area, amounting to $18.07 \times 10^3$ m²/ha was registered in plants from the control group (figure 7). Photosynthetic characteristics (PSC) varied accordingly (table 4).
**Figure 7.** Foliage surface of common flax plants depending on the amount of applied product

**Table 4.** Main photosynthetic characteristics of common flax plantings (flowering period)

| Variant | PSC, 10^3 m² day/ha | Dry matter yield, t/ha | Net primary productivity, g/(m² day) |
|---------|----------------------|-------------------------|-------------------------------------|
| Control | 371±8                | 2.65±0.11               | 7.43±0.19                           |
| 0.5 t/ha| 438±9                | 3.71±0.18               | 8.33±0.21                           |
| 2 t/ha  | 407±10               | 3.38±0.14               | 8.12±0.16                           |
| 4 t/ha  | 379±9                | 2.95±0.11               | 7.86±0.17                           |
| LCDₐₕ | 12.6                 | 0.27                    | 0.36                                |

Increase in foliage surface affects photosynthetic potential changing it from 371×10³ m²/day/ha on the control plot to 438×10³ m²/day/ha on the plot with 0.5 t/ha amount of introduced slurry. Increase in photosynthetic potential has been observed at the plots with 2 t/ha or even 4 t/ha sludge.

Maximal photosynthesis net yield 8.33 g/m²/day is recorded with 0.5 sludge, while minimal yield 7.43 g/m²/day is recorded on the control plot.

On the plots with 0.5 t/ha and 2 t/ha sludge growth acceleration has been observed at all stages. Seedlings appear 3-4 days earlier than on the control plot, and blooming and seed ripening is intensified (figure 8).

**Figure 8.** Flowering and bearing of common flax: (a), (b) – control, (c), (d) – 2 t/ha
Sludge application leads to plant height increase by 17% for 4 t/ha solution amount and 25% for 0.5 t/ha solution amount (figure 9).

![Figure 9. Common flax plant height depending on the amount of applied sludge](image)

Flax yield is constituted by total number of plants per unit area, the quantity of seeds per plant and seed weight. Flax yield component analysis shows different impact of the sludge depending on applied amount and specific yield component (figure 10).

![Figure 10. Flax crop yield depending on the amount of applied sludge: a) seed heads per 1 flax plant (LCD\(_{0.05}\) = 2.32); b) average weight of 1000 seeds (LCD\(_{0.05}\) = 0.45)](image)

The largest number of seed capsules per plant has been recorded on the lot with 0.5 t/ha sludge and equals 17.4, while the lowest number equals 6.4 and was found on the control plot (figure 10a). The heaviest seeds have been collected on 0.5 t/ha sludge lot also. Mass of 1000 of those seeds equals 16.46 g, while the minimal mass equals 14.7 and is recorded on 4 t/ha plot (figure 10b).

As could be seen from the above, to increase flax yield and plant quality, i.e. its height, the optimal amount of introduced sludge is 0.5 t/ha. Besides, reliable positive impact of higher sludge amount on flax growth has been seen also, but particular attention should be paid to ecological safety.

Accounting for high heavy metals concentration in the sludge, control and test plants have been tested on content of heavy metals which are known to be in the sludge and regulated by Russian...
sanitary standard SanRaN 42-123-4089-86 [26] (table 5). Content of the heavy metals which MAC in soil is regulated by [27] has been monitored also (table 6).

### Table 5. Heavy metal content in mg/kg depending on sludge amount in t/ha.

| Element | Control | 0.5 t/ha slurry introduced | 2 t/ha slurry introduced | 4 t/ha slurry introduced | MAC in soil* |
|---------|---------|----------------------------|--------------------------|--------------------------|--------------|
| Pb      | 0.31    | 0.39                       | 0.41                     | 0.43                     | 0.16         |
| Cd      | 0.05    | 0.051                      | 0.064                    | 0.075                    | 0.04         |
| Cu      | 7.90    | 11.52                      | 13.91                    | 9.62                     | 4.72         |
| Zn      | 7.10    | 25.46                      | 30.63                    | 22.50                    | 26.1         |
| Fe      | 388.    | 411.3                      | 1132.                    | 1240.                    | 91.6         |

*SanRaN RF 42-123-4089-86 [26]

As could be seen from table 5, roots and seeds heavy metals content exceed MAC on zinc and copper, moreover MAC is exceeded in seeds more than three times in control that could be an evidence of soil background contamination. In case of plants from the 0.5 and 2 t/ha sludge amount plots, a minor excess was observed, but 4 t/ha amount result in maximal copper and zinc content among tested groups. Maximal iron content is recorded at plant roots from 4 t/ha lot.

### Table 6. Heavy metal content in mg/kg in the soil after harvesting.

| Element | Control | 0.5 t/ha slurry introduced | 2 t/ha slurry introduced | 4 t/ha slurry introduced | MAC in soil* |
|---------|---------|----------------------------|--------------------------|--------------------------|--------------|
| Pb      | 0.4     | 0.91                       | 0.89                     | 0.93                     | 0.36         |
| Cu      | 0.1     | 0.11                       | 0.17                     | 0.44                     | 0.36         |
| Co      | 0.1     | 0.14                       | 0.16                     | 0.36                     | 0.36         |
| Zn      | 3.6     | 7.07                       | 12.2                     | 13.56                    | 5.0          |
| Cr      | 3.49    | 4.91                       | 4.54                     | 5.08                     | 4.0          |
| Ni      | 0.96    | 1.63                       | 1.82                     | 2.46                     | 4.0          |

**HS 2.1.7.2041-06 [27]**

As could be seen from table 6, MAC in soil has been never exceeded.

### 4. Conclusions

It is found in laboratory experiment that sludge presence suppresses flax germination and average root length in water environment, but average root mass remains the same and even increases twofold at 0.01% sludge concentration. Average stem length does not change significantly in the presence of sludge, while its average mass increases by 1.5 times at 0.001% and 1% sludge concentrations, and twofold at 0.01% and 10% sludge concentrations.

Biological productivity is found to be reduced by sludge application in sand soil, but viability and resistance to water deficiency increase with slurry introduction. Besides, it stimulates photosynthesis that could be explained by high zinc and iron content in the sludge that enhance ascorbic acid and chlorophyll synthesis thus affecting photosynthesis, plant growth, overheating resistance and protein metabolism [28]. Biochemical study educes reduction of peroxidase activity, slight increase in polyphenoloxidase, superoxidedismutase and catalase activities. It could be accounted for active oxygen including radical generation under unfavorable conditions resulting in diverse alterations of
enzymes activity depending on stress factor intensity and duration along with plant susceptibility and growth stage [29].

Evaluation of highly dispersed sludge impact on flax photosynthesis in the field shows the highest assimilating surface of 20.76×10^3 m²/ha has been recorded in case of 0.5 t/ha amount of sludge, while the lowest one of 18.07×10^3 m²/ha has been recorded at the control plot. Photosynthetic potential and photosynthesis net production increase with introduction of 0.5 t/ha sludge accordingly from 371×10^3 to 438×10^3 m²/day/ha for the former and from 7.43 to 8.33 g/m²/day for the latter. 0.5 and 2 t/ha applied amounts of sludge accelerate plant development and intensify blooming and seed ripening. Sludge application leads to increase of flax height up to 25% at 0.5 t/ha amount and yield increase at all tested amounts. Both maximal number of seed capsules and average seed mass has been registered at 0.5 t/ha sludge amount. Yield increase can be accounted for the presence of zinc and copper in the sludge. It is known that zinc is involved deeply into physiological and biochemical processes, and its deficit leads to metabolic disorder, plant productivity and quality reduction. Besides, zinc enhances plant resistance to unfavorable meteorological conditions such as excessive or deficient amounts of water or insolation, while copper reduces plant sensitivity to fungous and bacterial diseases [30].

Heavy metals content analysis discovers exceeds over MAC on zinc and copper, notably on the control lot it is found to be the highest and exceeds MAC by about three times that could be accounted for background soil contamination. In case of 0.5 and 2 t/ha of sludge applied those exceeds are insignificant, while 4 t/ha results in maximal copper and zinc content among the sludge treated plants. No MAC excess in soil examination on heavy metals content after harvesting have been found in any tested amount of introduced sludge.

Results of this work are in agreement with that of research [31], where sludge stimulating effect on rapeseed plant growth has been found. Thus, further studies of metallurgical sludge usage as a flax soil growth stimulator with introduced amount of about 0.5 t/ha seems to be of promising.

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