*Letters to Editor*

# Phytoextraction of Heavy Metals by Hemp during Anaerobic Sewage Sludge Management in the Non-Industrial Sites

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*Received: 12 July, 2002*
*Accepted: 7 February, 2003*

**Abstract**

The aim of our research was to estimate the efficiency of monoaecian fibrous hemp (*Cannabis sativa L.*, "Benico" variety) for heavy metals phytoextraction from soil-sludge substratum during anaerobic sewage sludge management in non-industrial sites. Below are the results from a pot experiment with hemp as a plant which phytoextracts zinc, copper and nickel. On the basis of heavy metals analysis in substratum it was ascertained that an increase of hemp biomass caused thirtyfold reduction of zinc, thirty-fivefold reduction of copper and tenfold reduction of nickel in light soil of sewage sludge fertilized in comparison with their concentration at the beginning of the pot experiment.

**Keywords:** dewatered anaerobic sewage sludge, hemp, zinc, copper, nickel, biocumulation

## Introduction

Trials of natural uses of sewage sludge have been conducted in Poland for a long time. Each method of natural use requires appropriate physical, chemical and biological treatments of sewage sludge properties. In this way, a transformation of waste to ecological-productive use raw materials takes place. Fermentation and dewatering are basic agents of sewage sludge treatment for their natural use. The fermentation modifies physical, chemical and biological properties of sewage sludge, reduces pathogenic threat and offensive smell. It also regulates organic carbon to nitrogen quantity ratio and simplifies dewatering of sewage sludge [1, 2]. Proper treatment and cultivation make sewage sludge a useful product. The inordinate content of heavy metals is a barrier in natural use of the sewage sludge from many wastewater treatment plants. Very expensive technologies are needed for heavy metals removal from sewage sludge and this is why they are not used in practice [3, 4]. Heavy metals removal using phytoextraction has recently gained popularity. Phytoextraction has been used on a large scale to organic and inorganic pollutants from ground removal for over 30 years. It uses the ability of plants to phytosorb and biocumulate considerable quantities of heavy metals in tissues. The phytoextraction is a biological method friendly to the environment which reduces concentrations of contaminators and does not cause the side-effects connected with chemical technologies [5]. The effect of application of these types of aggressive methods is devastating to all aspects of biological life in purificated ground. The efficiency of phytoextraction most of all depends on plant choice, which is used with the object of pollutant phytoextracts from the ground to their overground portions. The efficiency of method mentioned above is conected not only with the quantity of metals cumulated in tissues of plants but also with the yield of hemp. The greater biomass, the more heavy metals can be removed with the yield of hemp [6].

Therefore, during research of the influence of anaerobic sewage sludge on fertilization of light soil at organic

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matter and by extension on the value of hemp yield, the efficiency of three heavy metals (Zn, Cu, Ni) phytoextraction by this plant have been described. The content of heavy metals in anaerobic sewage sludge and in soil was low and not overstepped. The aim of our research was to gauge the efficiency of hemp in removing these low values of heavy metals. In order to attain this organic carbon and nitrogen quantity and proportions between these two, macroelements at the beginning and at the end of the pot experiment were compared. The values of hemp yield and content of zinc, copper and nickel in the substratum and in hemp (root, stem and leaf) have been designated.

## Materials and Methods

The research material was dewatered anaerobic sewage sludge, moisture 62%, after mezophilic fermentation from the sugar industry's wastewater treatment biological plant in Kościan. The sewage sludge was dewatered by help of polyelectrolyte F-410 using DRAIMAD-TEKNOBAG dewatered system type 06BCAVPK in the sewage sludge dewatering station near Leszno. Hemp was chosen because of its efficiency in removing organic and inorganic pollutants from the substratum [7]. Research with the application of hemp was conducted as a pot experiment in a greenhouse in 2000 and 2001 in the experimental station of the Institute of Natural Fibres in Pętkowo, near Środa Wielkopolska. The hemp was seeded each year, when the average ambient temperature was 8-10$^{o}$C. The harvest took place each year about two weeks after blooming, which gives strong, high quality fibres. The pot experiment was conducted in twenty-four plastic pots especially for hemp growing, with U-shape and hole aeration system and capacity of 17 dm$^3$. The pots were filled a week before sowing by 10 kg of air-dried soil composed of poor clayey soil (later called light soil) thoroughly mixed with organic additions (organic manure and wheat straw) at appropriate mass proportions. There were two doses of sewage sludge appropriately to nitrogen content in pot ant to hemp manurial needs of nitrogen.

a) low dose - 5% d.m. sewage sludge /10 kg d.m. soil, and

b) high dose -10% d.m. sewage sludge /10 kg d.m. soil.

Both low and high doses were encompassed from three combinations of the substratum:

- The first - raw anaerobic or aerobic sewage sludge.
- The second - anaerobic or aerobic sewage sludge with wheat straw.
- The third - anaerobic or aerobic sewage sludge with organic manure.

Each combination was repeated three times.

There were also soil-control pots (repeated three times) for each part of the experiment (low and high doses). The soil-control pots were supplemented fertilizers taking into account annual doses of fertilizers in ploughland and hemp manurial needs of nitrogen, phosphorus and potassium.

a) N in ammonium nitrate ($NH_4NO_3$) -0.3 g/kg soil

b P in sodium dihydrogen phosphate (Na $H_2PO_4 \cdot 2H_2O$) - 0.1 g/kg soil

c) K in potassium sulphate ($K_2SO_4$) - 0.3 g/kg soil.

During vegetation the hemp was watered to 55-60% of water capacity. After the harvest the biomass (weight and height) of hemps was indicated.

The heavy metals were indicated at a low dose of pot experiment. The content of mean zinc, copper and nickel in raw materials used in the experiment, in pot's samples at the beginning and at the end of the experiment and also in hemp (roots, leaves and stems) were determined using atomic absorptivity spectrophotometry (ASA). The samples were mineralized and burned using a mixture of spectra pure concentrated nitric acid and perchloric acid (1:1 v/v, Merck). The contents of Zn, Cu and Ni in the mineralized samples were determined using flame atomic absorption spectrometry (SpectrAA 250 Plus, Varian).

### Chemical Analyses of Pot Contents

The analyses were made at the beginning and at the end of the pot experiment. The analyses included:

- total nitrogen by Kjeldahl method,
- total carbon by Tiurin method,
- assimilable phosphorus and potassium by Egner-Riehm,
- calcium by flame spectrometry,
- class and category of soil by Bouyoucosa in modification of Cassagrande and Prószyński method.

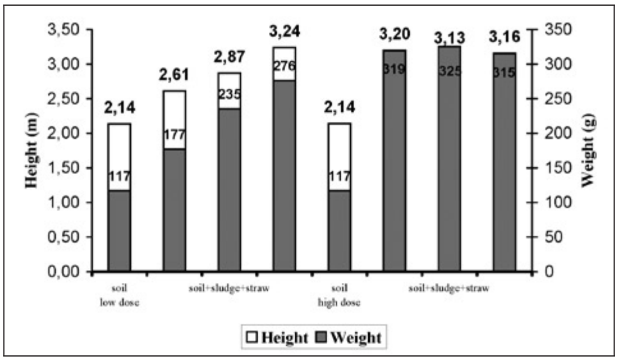


Fig. 1. Height and weight of hemp on the basis of anaerobic sewage sludge.

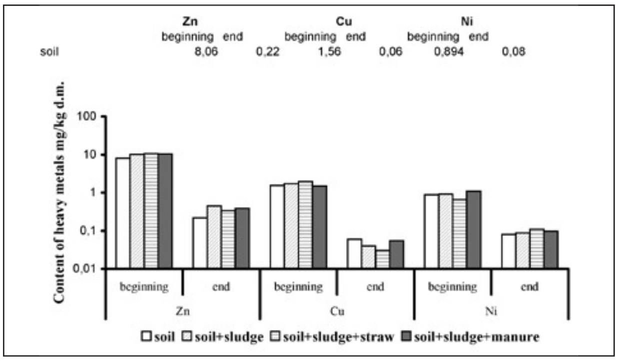


Fig. 2. Changes of heavy metals in substratum on the basis of anaerobic sewage sludge.

Table 1 Changes of heavy metals (mg·kg$^{-1}$ d.m.) in substratum on the basis of anaerobic sewage sludge.

| Metals | soil | | soil + sludge | | soil + sludge + straw | | soil + sludge + manure | |
|---|---|---|---|---|---|---|---|---|
| | beginning | end | beginning | end | beginning | end | beginning | end |
| Zinc (Zn) | 8.06 ± 1.69 | 0.28 ± 0.12 | 30.15 ± 8.36 | 1.124 ± 0.87 | 26.18 ± 6.17 | 0.74 ± 0.47 | 28.91 ± 18.16 | 1.18 ± 1.14 |
| Copper (Cu) | 1.56 ± 0.39 | 0.02 ± 0.01 | 5.64 ± 2.11 | 0.19 ± 0.10 | 5.69 ± 1.47 | 0.16 ± 0.10 | 5.41 ± 0.17 | 0.17 ± 0.08 |
| Nickel (Ni) | 0.89 ± 0.25 | 0.06 ± 0.04 | 2.49 ± 0.84 | 0.18 ± 0.14 | 2.34 ± 0.95 | 0.29 ± 0.22 | 2.66 ± 1.22 | 0.29 ± 0.14 |

Table 2. Content of heavy metals (mg/kg d.m.) in particular organs of hemp.

| Combination | Organ | Zn | Cu | Ni |
|---|---|---|---|---|
| Soil | Root | 8.42 | 1.23 | 0.32 |
| | Stem | 1.08 | 0.74 | 0.75 |
| | Leaf | 6.35 | 0.93 | 0.21 |
| Soil + sludge | Root | 16.8 | 2.08 | 0.75 |
| | Stem | 2.22 | 0.80 | 0.20 |
| | Leaf | 10.24 | 2.45 | 0.51 |
| Soil + sludge + straw | Root | 25.91 | 2.35 | 1.33 |
| | Stem | 3.73 | 1.07 | 0.35 |
| | Leaf | 12.35 | 4.23 | 0.54 |
| Soil + sludge + manure | Root | 36.74 | 4.63 | 2.56 |
| | Stem | 9.40 | 1.61 | 0.48 |
| | Leaf | 16.32 | 6.82 | 1.10 |

## Accumulation and Translocation Indicators (%) of Zn, Cu and Ni in Hemp

- indicator of accumulation counted as a relation of average content of metal in plant from all combinations to its concentration in control plant,
- indicator of translocation in plant counted by accepting the metal concentration in root as 100%, and its concentration in other organs as a % of this value,
- indicator of translocation soil-plant counted by accepted the metal concentration in soil as 100%, and its concentration in plant organs as a % of this value.

## Statistical Methods

Statistical evaluation of the data has been made by analysis of variance, Levene's test, Kruskall-Wallis test, LSD test. Calculates were made using program Statistica 5.0.

## **Results and Discussion**

In the anaerobic sewage sludge which was the research material the content of organic carbon was 9.8%. The total contents of macronutrients in this material were: N-2.24%, P-0.5%, K-0.5% and Ca-20.8%. The organic carbon to total nitrogen ratio was 11:1. Whereas contents of assimilable macronutrients in sewage sludge were: P - 0.097% (10% HCl),

K - 0.06% (10% HCl), Ca - 6.5% (10% HCl). The pH was close to neutral at 6.5.

The soil examined in the pot experiment was lightly acid poor clayed soil, with pH 5.6 in 1 M KCl and consisted of 66% sand, 21% dust and 13% floatable parts (in this 3% of colloidal clay).

The content of organic carbon in soil used in the experiment was 0.9% and total nitrogen 0.11%. Concentrations of phosphorus and potassium were very high: $P_2O_5$ - 25.6 mg/100 g a.d.m. and $K_2O$ - 20.7 mg/100 g a.d.m. In analyzed soil organic carbon to total nitrogen proportion was 8:1. To obtain optimum conditions of hemp growth the materials, which composed of substratum in pots in each combination and were taken based on the required organic carbon-to-total-nitrogen proportion. The carbon-to-nitrogen ratios in particular combinations were changed from 8.8-10.4 to 10.2-11.8 at the end of the pot experiment, which was caused by taking more nitrogen towards carbon ($p<0.005$) (Table 4). In relatively short

Table 3. Accumulation and translocation indicators (%) of Zn, Cu and Ni in hemp.

| Organ | *Indicator of accumulation | | | **Indicator of translocation in plant | | | ***Indicator of translocation soil-plant | | |
|---|---|---|---|---|---|---|---|---|---|
| | Zn | Cu | Ni | Zn | Cu | Ni | Zn | Cu | Ni |
| Root | 3.15 | 2.46 | 4.83 | 100 | 100 | 100 | 223 | 153 | 139 |
| Stem | 8.7 | 2.18 | 0.64 | 18.70 | 41.00 | 35.89 | 42 | 63 | 50 |
| Leaf | 1.48 | 1.73 | 2.29 | 51.51 | 14.02 | 47.58 | 115 | 214 | 166 |

Table 4. Organic carbon to total nitrogen proportion in each combination of pot experiment.

| Combination | Beginning of pot experiment | | | End of pot experiment | | |
|---|---|---|---|---|---|---|
| | C (%) | N (%) | C:N | C (%) | N (%) | C:N |
| Soil – control of low dose | 0.930 | 0.102 | 9.1 | 0.840 | 0.073 | 11.5 |
| Soil + sludge 5% | 1.280 | 0.137 | 9.3 | 1.090 | 0.092 | 11.8 |
| Soil + sludge 5% + straw (17 g/kg) | 1.420 | 0.148 | 9.6 | 1.070 | 0.098 | 10.9 |
| Soil + sludge 5% + manure (191 g/kg) | 1.280 | 0.142 | 9.0 | 1.130 | 0.095 | 11.9 |
| Soil – control of high dose | 0.930 | 0.102 | 9.1 | 0.840 | 0.073 | 11.5 |
| Soil + sludge 10% | 1.570 | 0.179 | 8.8 | 1.070 | 0.105 | 10.2 |
| Soil + sludge 10% + straw (34 g/kg) | 1.640 | 0.157 | 10.4 | 1.090 | 0.093 | 11.7 |
| Soil + sludge 10% + manure (381 g/kg) | 1.740 | 0.167 | 10.4 | 1.240 | 0.112 | 11.1 |

vegetative experiments only small parts of organic carbon are used by plants. The greatest amount of organic carbon is releasing and taking plants in the latter part of the vegetation season or in the case of many seasons growing in second or third vegetation season. Content of total nitrogen assimilated by plants already in the first months of vegetation is decidedly reduced [7].

After harvest hemp height and weight in particular combinations were designated (Fig. 1). In low doses the addition of organic matter (in particular sewage sludge) radically influenced hemp yield ($p<0.001$). In high dose radical differences in weight and height of hemp in particular combinations were not stated in the result of organic matter increase ($p>0.58$). On the basis of the results it can be stated, that in comparison with the yield of hemp from the minerally fertilized soil (NPK) ($p<0.01$) (height-2.14 m, weight-117 g), which was control, the addition of anaerobic sewage sludge in low doses radically influenced height and weight of hemp (height - 2.61 m and weight - 177 g). The greatest weight and height of hemp in case addition of sewage sludge and manure to soil at low doses was noticed. The addition of straw also has increased weight and height of plants in comparison with the addition of sewage sludge at low doses, but not so radically as the addition of manure ($p<0.01$). In the case of high doses of sewage sludge there were not radical differences in yield of hemp (average weight - 319.7 g, average height 3.16 m). The addition of manure and straw at this dose did not influence weight and height of hemp ($p>0.23$). These values were comparable with weight and height of plants fertilized with low doses of sewage sludge and manure ($p>0.10$). It can be explained

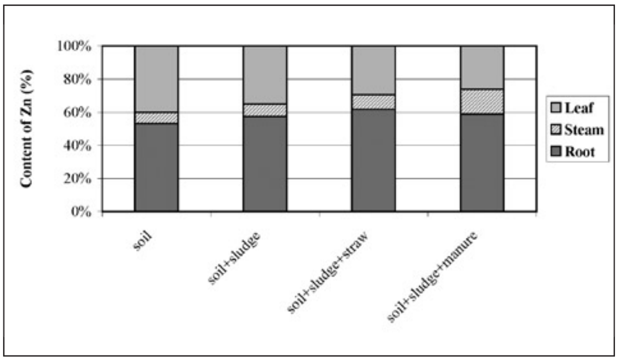


Fig. 3. The percentage content of zinc in hemp according to substratum type.

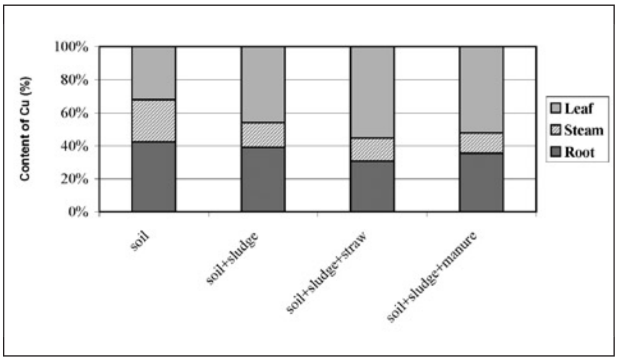


Fig. 4. The percentage content of cooper in hemp according to substratum type.

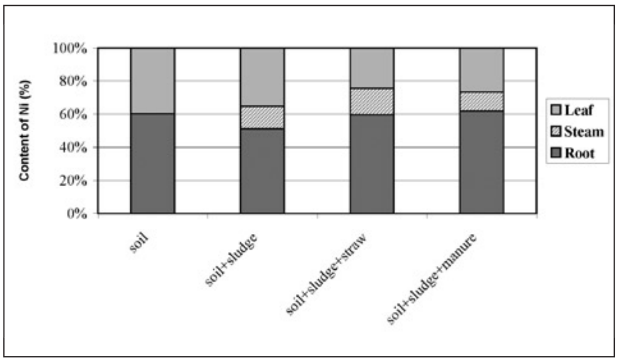


Fig. 5. The percentage content of nickel in hemp according to substratum type.

by sewage sludge excessively in relation to manurial needs of hemp [7]. The positive reactions on the addition of sewage sludge to soil, which cause an increase of field plants weight and height have been presented at many articles [8, 9]. On the basis of the results it can be stated, that the addition of sewage sludge became very efficient at the utilization aspect. This information about positive influence of sewage sludge from agricultural-food industry was confirmed by the research of industrial plant-hemp.

The total concentration of heavy metals in anaerobic sewage sludge was estimated at Zn - 406 mg/kg d.m. sewage sludge, Cu - 79 mg/kg d.m. sewage sludge and Ni - 31 mg/kg d.m. sewage sludge. The concentration of three heavy metals in soil was Zn - 8.06 mg/kg d.m. soil, Cu - 1.56 mg/kg d.m. soil and Ni - 0.89 mg/kg d.m. soil. The content of these three metals in soil is low and corresponded with soils of rustic lands [10]. The total contents of heavy metals in organic manure used in the experiment were: Zn - 26.97 mg/kg d.m. manure, Cu - 3.04 mg/kg d.m. manure and Ni - 0.06 mg/kg d.m. manure. In the wheat straw concentration of analyzed heavy metals was Zn - 23.54 mg/kg d.m. straw, Cu - 2.25 mg/kg d.m straw and Ni - 0.04 mg/kg d.m. straw.

At the beginning and at the end of pot experiment the heavy metals in soil-sludge substratum in combination with low sewage sludge dose were designated in purpose of hemps phytosorption efficiency estimation (Fig. 2). On the basis of statistical analysis ($\alpha=0.05$) the radical changes of all three analyzed heavy metals in substratum were Zn ($p<0.001$), Cu ($p<0.001$), Ni ($p<0.001$). The concentrations of Zn - 30.15 mg/dm$^3$, Cu - 5.6 mg/dm$^3$ and Ni - 2.49 mg/dm$^3$ mg/dm$^3$ can be removed from soil-sludge substratum by fibrous hemp growing and does not caused reduction of height and weight of hemp.

Zinc at the greatest degree in root and then in leaf of hemp in all combinations was accumulated (Fig. 3). The lowest zinc accumulation was in the stem.

The greatest sorption in case of copper took place by leaf, then by root and stem of hemp. The sorption of nickel at the greatest amount took place by root then by leaf and by stem of hemp (Figs. 4 and 5).

There was no radical influence of manure and straw addition on ability of heavy metals accumulation by hemp ($p>0.15$) (Table 1).

The greatest concentration of analyzed heavy metals was in roots and in leaves of plant (Tables 2 and 3). In conditions of heavy metals, particular concentrations in soils are cumulated in peripheral parts of plants. At the conditions of heavy metals very low concentrations in the substratum, as at presented experiment the most heavy metals are cumulated in roots.

In the case of greater concentrations of heavy metals in substratum, heavy metals cumulate in great amount also in hemp stem as it was in Bragato et al. research. In this way, after several hemp growing seasons concentration of heavy metals in substratum were reduced to satisfactory levels [11].

## Conclusions

1. The addition of anaerobic sewage sludge in high doses to pot experiments increased height one and a half and weight two-sevenfold of hemp.
2. At the conditions of heavy metals very low concentrations in the substratum, as at presented experiment the most heavy metals are cumulated in roots. The sorption of zinc and nickel at the greatest amount took place by root, copper at the greatest amount was accumulated in leaf.
3. The acquired results showed that it is advisable to dewater mesophilic anaerobic sewage sludge by fibrous hemp growing. The concentrations of Zn - 30 mg/dm$^3$, Cu - 5.6 mg/dm$^3$ and Ni - 2.5 mg/dm$^3$ mg/dm$^3$ can be removed from soil-sludge substratum by fibrous hemp growing and did not cause a reduction of hemp height and weight.

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