

Influence of increasing nickel content in soil on *Miscanthus × giganteus* Greef and Deu. Yielding and on the content of nickel in above-ground biomass

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Abstract: The aim of the research conducted in a 2-year pot experiment in an unheated plastic tunnel was to determine suitability of *Miscanthus × giganteus* for phytoextraction of nickel from soil as well as to assess tolerance of this species on increasing concentrations of this metal in soil. Pots were filled with mineral soil (sand) and a mixture of soil with high-moor peat and three levels of nickel were introduced, i.e. 75 mg dm⁻³, 150 mg dm⁻³ and 600 mg dm⁻³ and the control combinations used substrates without the addition of nickel. Nickel was introduced only in the first year of the experiment in the form of nickel sulfate (NiSO₄ · 6H₂O). *Miscanthus × giganteus* accumulated a considerable amount of nickel in biomass. *Miscanthus × giganteus* growing in contaminated mineral soil turned out to be a species tolerant to high nickel concentrations.

Introduction

Continuous phytoextraction is a cheap and cost-effective method used in the removal of heavy metals from soil (Ros et al. 1992, Boyajian and Sumner 1997, McGrath and Zhao 2003, Nadgórska-Socha and Ciepał 2009). Effectiveness of continuous phytoextraction depends on the selection of an appropriate plant species (Żurek and Majtkowski 2009, Bosiacki and Zieleziński 2011, Bosiacki and Wojciechowska 2012). Plants used in this method have to accumulate considerable amounts of heavy metals in their aboveground parts. They should produce high yields of biomass within a short period of time, be easy to harvest, have a deep root system (collecting heavy metals not only from a depth of 0–20 cm) and exhibit resistance to diseases and adverse environmental conditions (tolerance to high heavy metal concentrations).

Renewable energy sources play an increasingly important role in energy policy of European countries. These sources are divided into annual (e.g. cereals, rape) and perennial (e.g. willow, poplar and *Miscanthus × giganteus*) (Lewandowski et al. 2000). The most significant parameters of these plants include high annual increment of biomass and its high calorific value (Pulford and Watson 2003, Szempliński and Dubis 2011). Hadde et al. (2013) conducted research aiming to assess the impact of *Miscanthus × giganteus* biomass crop on invertebrate communities in contaminated

agricultural soils. A stimulus for the development of energy crop plantations, including *Miscanthus*, may be connected with phytoextraction of chemically contaminated soils. *Miscanthus × giganteus* Greef and Deu. is grown mainly in western Europe. In Poland it is still not a popular crop. In the nearest future interest in this plant cultivation in Poland will be increasing. *Miscanthus × giganteus* is a triploid species belonging to the *Poaceae* family (Greef and Deuter 1993). Plants of this species grow to a height of 3–4 m, they do not produce seeds and are propagated vegetatively. From 1 hectare of crop the annual yield may reach as much as 20 up to 35 tons of dry matter (Pyter et al. 2009). *Miscanthus × giganteus* exhibits resistance to drought and very high temperatures. Success of its cultivation results from its low environmental requirements, possibility to mechanize field operations both when establishing plantations and at harvest, as well as the plant resistance to diseases and pests. Species of energy crops, with a high yielding potential, are particularly recommended to be grown in areas exposed to erosion and in contaminated areas for phytoremediation purposes.

The aim of the conducted studies was to determine the effect of increasing doses of nickel introduced to mineral soil (sand) and to mineral soil with an addition of high-moor peat (at a 1:1 ratio, v/v), on the yield of *Miscanthus × giganteus* and to estimate what amounts of nickel are transported to above-ground parts of this plant.

Material and methods

The vegetation experiment was conducted in an unheated plastic tunnel with suspended sides of 6 × 30 m in size, at the Marcellin Experimental Station of the Poznan University of Life Sciences.

Seedlings of *Miscanthus × giganteus* were produced at a tissue culture laboratory of Vitroflora. Plants were planted at the beginning of May in pots filled with previously prepared substrate. The experiment comprised 8 combinations (in each year of the study) and each combination consisted of six replications. A replication comprised one plant growing in a drainless container of 7 dm³.

Phytoremediation of nickel by *Miscanthus × giganteus* was investigated in two years of growth with the plants grown in two substrates, at four levels of metal contents. Since light soils with low contents of organic matter predominate in Poland, such soil was selected for the experiment. Another substrate was a mixture of this mineral soil with high-moor peat. High-moor peat was added to increase the amount of organic matter in the mineral soil.

1. Substrates
 - a. Mineral soil (sand)
 - b. Mineral soil with high-moor peat (1:1 v/v).
2. Doses of nickel: control (nickel contents in analyzed substrates after liming amounted to: in mineral soil 2.88 mg dm⁻³ and mineral soil with high-moor peat 1.12 mg dm⁻³), 75, 150 and 600 mg dm⁻³)

Prior to the establishment of the experiment, Corg content in mineral soil was determined according to the Tiurin method (Golcz and Bosiacki 2011).

In the substrate composed of a mixture of mineral soil with high-moor peat (1:1 v/v) the percentage of organic substance was determined by loss on ignition of the substrate by the direct method at high temperature in the presence of oxygen, under the influence of which organic substance is decomposed (carbon is released in the form of CO₂, hydrogen in the form of H₂O and nitrogen as N₂, while the other elements remain in ash).

The content of organic carbon in sand (the Tiurin method) was 0.55% (0.95% humus), while the percentage of organic matter in the mixture of sand and high-moor peat (from loss on ignition) was 10.05%.

In mineral soil the method of Mocek and Drzymała (2010) was used to determine bulk density (1.62 g cm⁻³). Total porosity of mineral soil was 38.9%. Moreover, grain size distribution of mineral soil was determined by the areometric method according to Prószyński (Mocek and Drzymała 2010). On the basis of the percentage of fractions the grain size class of soil was identified as sand, according to the guidelines of the Polish Society of Soil Science and United States Department of Agriculture standard (PTG 2009).

Experiments were conducted using high-moor peat produced by Hartmann (sphagnum, ground, fractional peat), with acidity of pH 4.50. This peat has a high water capacity, at the same time retaining its elastic structure. The weight of 1 dm³ of peat was 490 grams.

In order to obtain an appropriate pH for growing of *Miscanthus × giganteus* a neutralization curve was plotted for the analyzed substrates. On its basis the dose of CaCO₃ required for the maintenance of pH within the range of

6.5–7.0 was established. The reaction of the substrate (mineral soil + high-moor peat) was regulated using 3 g dm⁻³ CaCO₃ (chemically pure reagent). The substrate composed of mineral soil did not need any pH regulation. Despite that fact 1 g dm⁻³ CaCO₃ was applied in order to maintain pH at 6.5–7.0. An adequate amount of calcium carbonate was introduced to each experimental container with the substrate. Two weeks after liming, nutrients and nickel were introduced to the substrate. Nickel was introduced only in the first year of the experiment in the form of chemically pure reagents (C.P.): nickel sulfate (NiSO₄·6H₂O). Pre-vegetation fertilization (in the first year) with macro- and micronutrients was determined taking into consideration initial nutrient contents in substrates, after liming reaching the following levels (in mg dm⁻³): N 200, P 120, K 250, Mg 100, Fe 50, Mn 20, B 1.5 and Mo 1.5. All macro- and micronutrients were introduced in the form of solutions using chemically pure reagents (potassium mono-phosphate, potassium nitrate, ammonium nitrate, magnesium saltpeter, magnesium sulfate, iron sulfate, copper sulfate, zinc sulfate, manganese sulfate, ammonium molybdate, borax). In the second year of the experiment, an identical experimental design was used as in the first year. After plant cutting in the first year of the experiment the containers with polluted substrates were stored in an unheated tunnel to the next vegetation year (the second year of the study). In the second year of the study, in March, prior to the beginning of vegetation substrate samples were collected and chemical analyses were performed to determine nutrient contents. On this basis nutrient fertilization was established (leading to nutrient contents at the same levels, which were applied in the first year of the experiment). Nutrients in substrates were determined using the "Universal" method (Kozik and Golcz 2011) in CH₃COOH extraction solution at a concentration of 0.03 mol dm⁻³, pH in water was determined by the potentiometric method (the substrate to water ratio of 1:2), while the conductivity method was applied to determine EC (mS cm⁻¹), (the substrate to water ratio of 1:2) (Golcz 2011).

The following nutrient determination techniques were applied: N – NH₄ and N – NO₃ by micro-distillation (Bremner modified by Starck), P by colorimetry using the vanadium-molybdenum method, K, Ca and Na by flame photometry, Mg by atomic absorption (AAS), Cl and S – SO₄ by nephelometry (Kozik and Golcz 2011).

In October in each year of the studies prior to harvest, plant height was measured. Dry weight of plants was recorded and samples of plant material were collected for analyses.

Harvested plant material (entire aboveground mass) was dried in an extraction drier at a temperature of 105°C for 48 h. Next the material was ground and at the amount of 2.5 g taken from each sample it was digested in a mixture of concentrated HNO₃ (ultra pure) and HClO₄ (analytically pure) at a 3:1 ratio (Bosiacki and Roszyk 2010). Content of nickel in the plant material was determined by flame atomic absorption spectrophotometry (FAAS), using AAS-3 spectrophotometer by Zeiss. Moreover, the content of nickel in the reference material (*Pseudevernia furfuracea* BCR®-482/2009, certified by the Institute for Reference Materials and Measurements in Belgium), was determined as well (Table 1).

Table 1. Contents of nickel in reference materials *Pseudevernia furfuracea* (mg kg⁻¹ dry weight)

Metal	Reference material certified content		Digestion			
	mg kg ⁻¹	+/-	mg kg ⁻¹	recovery (%)	difference (mg·kg ⁻¹)	difference (%)
Ni	2.47	0.07	2.37	95.95	-0.10	-4.05

In the first and second year of the studies samples of the substrate were collected after harvest. Nickel was extracted from them using modified Lindsay's solution containing 5 g EDTA (ethylenediaminetetraacetic acid), 9 cm³ 25% NH₄OH solution, 4 g citric acid and 2 g Ca(CH₃COO)₂·2H₂O in 1 dm³ (Nowosielski 1988). The ratio of the soil to the extraction solution was 1:4 (50 cm³ : 200 cm³). Next this metal was determined by flame atomic absorption spectrophotometry (FAAS) in AAS-3 spectrophotometer by Zeiss.

Results of the nickel content in substrates and aboveground parts of *Miscanthus × giganteus* and results of plant dry weight, and height were elaborated statistically in the Statobl program applying a one-way analysis of variance for orthogonal factorial experiments, with differences between means determined at a significance level $p < 0.05$.

Results and discussion

Nickel is characterized by high mobility in ecosystems and it is generally readily absorbed by plants, typically in proportion to its content in soil (Kabata-Pendias and Pendias 1999). According to Antoniewicz and Jasiewicz (2002) the translocation of nickel to aboveground parts is limited, while Drażkiewicz (1994) claimed that this metal penetrating to the soil as an industrial or agricultural pollutant is readily absorbed by the root system and transported to stems and leaves with xylem sap. In the opinion of Drażkiewicz (1994), nickel in plants is found in complexes with organic compounds, while at excess Ni²⁺ it may be found in

plants in the cation form (Knypl 1980) inhibiting plants growth (Reeves and Baker 2000, Nadgórska-Socha and Ciepał (2009), as well as their metabolism (Sheoran et al. 1990, Van Assche and Clijsters 1990).

In the conducted investigations mineral soil polluted with increasing doses of nickel did not have a significant effect on plant dry weight in the first year of growth (Table 2). In mineral soil with an addition of peat, the lowest weight was found at a nickel dose of 600 mg dm⁻³, while it was highest at the 150 mg dm⁻³ dose of nickel, which did not differ significantly from the weight obtained in the control substrate (with no Ni added).

In the first year of growth, in the mineral soil, the lowest plants were reported at a nickel dose of 150 mg dm⁻³, while doses of 75 and 600 mg Ni dm⁻³ did not have a significant effect on plant growth in comparison to plants growing in the soil, to which no nickel was introduced (Table 2). In a mixture of soil with peat, nickel introduced at 600 mg dm⁻³ caused the production of the lowest plants, which did not differ significantly from those growing in this substrate contaminated with 75 mg Ni dm⁻³.

In the second year of growth, in mineral soil, a dose of 600 mg Ni dm⁻³ had a significant effect on the production of the greatest dry matter in plants, while in a mixture of soil with peat, both the lowest dry matter content and the lowest plants were obtained at this dose (Table 3). No effect of increasing nickel doses introduced to mineral soil was found on plant height in the second year of growth (Table 3).

Table 2. Dry weight of above-ground parts of *Miscanthus × giganteus* (g plant⁻¹) and height of plants (cm) in the first year of growth

	Dose of Ni mg dm ⁻³	Substrates							
		Mineral soil				Mineral soil + high-moor peat			
		min.-max. range	Range R	Standard deviation	Mean	min. max. range	Range R	Standard deviation	Mean
Dry weight	control	69–89	20	7.0	77.0 a	125–175	50	16.5	148.8 c
	75	39–85	46	15.2	65.2 a	105–145	40	16.6	126.8 b
	150	67–80	13	5.3	72.8 a	125–175	50	16.4	149.0 c
	600	69–89	20	7.8	79.0 a	60–90	30	12.1	78.3 a
Mean		73.5 a				125.7 b			
Height	control	44–48	4	1.4	45.7 b	60–66	6	2.3	63.2 e
	75	35–54	19	7.3	49.5 bc	54–65	11	3.5	59.2 de
	150	23–49	26	9.6	35.5 a	60–66	6	2.2	62.7 e
	600	44–48	4	1.6	46.3 b	47–72	25	9.2	55.7 cd
Mean		44.2 a				60.2 b			

* homogeneous groups were identified with the Duncan test, $p < 0.05$ (values denoted with identical letters do not differ significantly)

Irrespective of nickel doses, both in the first and second year of growth of *Miscanthus × giganteus* a greater dry matter and plant height were recorded in a mixture of the soil with peat.

The effect of nickel on the growth of ornamental plants was investigated by many researchers. Adhikari (2012) observed a growth inhibition in *Ricinus communis* only at a dose of 250 mg Ni dm⁻³. In turn, Ahmad et al. (2011) recorded a toxic effect of nickel on *Helianthus annuus* already at 10, 20, 30 and 40 mg Ni dm⁻³. Bosiacki and Wojciechowska (2012) obtained a lower total yield of above-ground parts in *Tagetes erecta* at all the applied nickel doses (from 25 to 300 mg dm⁻³). The same authors observed a stimulatory effect of nickel on the total yield of above-ground parts in *Amaranthus caudatus*, recorded in a substrate to which 150 mg Ni dm⁻³ were introduced.

Phytoextraction assumes the use of the so-called hyperaccumulators, i.e. plants, which are genetically and physiologically capable of accumulating considerable amounts of heavy metals with no symptoms of their toxicity (Boyd and Martens 1994, Boyd 1998). The term hyper-accumulator of Ni was devised by many authors (Jaffré et al. 1976, Reeves 1992, Baker et al. 2000, Reeves and Baker 2000, McGrath and Zhao 2003). They claimed that a hyper-accumulator of Ni is a plant in which a Ni concentration of at least 1 000 µg·g⁻¹ (1 000 mg·kg⁻¹) has been recorded in the dry matter of any above-ground tissue in at least one specimen growing in its natural habitat. According to Van der Ent et al. (2013) only plant leaves (or fronds) are to be considered in establishing hyper-accumulator status.

Miscanthus sp. is a plant having the ability to accumulate large amounts of heavy metals in contaminated soil (Pogrzeba et al. 2011). Higher content of Cd, Pb and Ni in triploid than diploid *Miscanthus* biomass was found (Kalembasa and Malinowska 2009a). Kalembasa and Malinowska (2009a) also claimed that mineral fertilization (NPK) influences cadmium content in biomass of diploid genotypes and nickel in biomass of diploid and triploid genotypes, while the contents of Cd, Pb and Ni were dependent on a harvest date. The highest

nickel concentration was recorded in *Miscanthus* biomass at the beginning of July.

In the conducted investigations, the highest content of nickel was observed in the second year of growth, in plants growing in mineral soil contaminated with the highest levels of this metal (Table 4). When comparing the content of nickel in above-ground parts of plants in the first and second year of growth significant differences in Ni contents were recorded only in plants growing in substrates with the highest nickel content (600 mg dm⁻³). In the above-ground parts of *Miscanthus × giganteus* growing in mineral soil contaminated with 600 mg Ni dm⁻³ a significantly higher content of nickel was detected in the second year of growth. The same dependence was found for nickel contents in above-ground parts of *Miscanthus × giganteus* growing in a mixture of soil and peat. Both in the first and second year of *Miscanthus × giganteus* growth higher nickel contents were observed in plants growing in the mineral soil.

In the conducted analyses the nickel concentration index was calculated for above-ground parts of *Miscanthus × giganteus*. The metal concentration index was calculated from the formula

$$C = a : b$$

- a – content in a plant growing in a polluted substrate
- b – content in a plant growing in an unpolluted substrate (Bosiacki and Wojciechowska 2012).

Nickel concentration index in the above-ground parts of *Miscanthus × giganteus* of over 100 was recorded both in the first and second year of plant growth in mineral soil and in a mixture of soil with peat which was contaminated with nickel at 600 mg dm⁻³ (Table 5).

According to Kalembasa (2006) in ash of *Miscanthus sinensis* Thumb. the content of individual heavy metals ranks in the following decreasing levels: Zn>Cd>Pb>Ni>Cu>Cr. *Miscanthus × giganteus* is a more tolerant species to the total contamination of soil with Zn and Pb than mallow and

Table 3. Dry weight of above-ground parts of *Miscanthus × giganteus* (g plant⁻¹) and height of plants (cm) in the second year of growth

	Dose of Ni mg dm ⁻³	Substrates							
		Mineral soil				Mineral soil + high-moor peat			
		min.-max. range	Range R	Standard deviation	Mean	min.-max. range	Range R	Standard deviation	Mean
Dry weight	control	50–92	42	15.5	80.5 b	122–180	58	19.2	149.3 d
	75	72–114	42	15.5	100.0 b	116–147	31	11.9	138.0 cd
	150	60–111	51	21.4	86.7 b	115–170	55	19.9	143.0 cd
	600	117–135	18	7.4	124.5 c	21–83	62	20.2	56.3 a
Mean		97.9 a				121.7 b			
Height	control	51–68	17	6.8	59.8 a	67–90	23	7.7	76.5 b
	75	54–69	15	6.6	61.5 a	63–120	57	19.9	82.0 b
	150	38–63	25	9.0	55.0 a	60–96	36	12.0	76.5 b
	600	54–82	28	9.6	63.3 a	48–58	10	3.7	53.8 a
Mean		59.9 a				72.2 b			

* homogeneous groups were identified with the Duncan test, p < 0.05 (values denoted with identical letters do not differ significantly)

phytoextraction of Zn and Pb from the soils contaminated with these metals are much higher for *Miscanthus* than for Virginia mallow (Kocoń and Matyka 2012). As reported by Kabała et al. (2010), *Miscanthus* may be grown on soils weakly contaminated with heavy metals on condition they are provided with adequate abundance of nutrients and water. In turn, on soils with medium and strong heavy metal contamination selected clones of osier are recommended. In comparison to osier wood straw of *Miscanthus* grown on uncontaminated soils contains

greater amounts of macronutrients and lower amounts of heavy metals (Kalembasa and Malinowska 2009a, Kalembasa and Malinowska 2009b). Some researchers showed an effect of fertilization on changes in individual heavy metal content in the successive years of growth. In comparison to osier, *Miscanthus* is characterized by lower tolerance to high concentrations of heavy metals and as it is reported by Kabała et al. (2010), they need to be further tested in terms of phytoremediation capacity of individual cultivars of *Miscanthus*.

Table 4. Contents of Ni (mg kg⁻¹ dry weight) in above-ground parts of *Miscanthus × giganteus* growing in substrates polluted with nickel

Substrate	Dose of metal (mg dm ⁻³)	Year of cultivation							
		1st year				2nd year			
		min.-max. range	range R	SD	mean	min.-max. range	range R	SD	mean
Mineral soil	Control	3.2–4.0	0.8	0.3	3.5 a	3.3–4.8	1.5	0.6	4.0 a
	Ni 75	24.9–49.3	24.4	9.5	42.9 bc	45.6–52.5	6.9	2.7	50.9 c
	Ni 150	198.3–269.1	70.8	26.8	244.8 d	241.8–272.0	30.2	10.9	261.5 d
	Ni 600	389.2–467.3	78.1	27.9	426.0 f	423.6–494.2	70.6	26.8	476.0 g
Mineral soil + high-moor peat	Control	3.3–4.1	0.8	0.3	3.7 a	3.8–4.2	0.4	0.1	4.00a
	Ni 75	22.1–28.2	6.1	2.1	25.7 ab	19.3–29.4	10.1	3.4	23.8 ab
	Ni 150	212.3–280.6	68.3	26.7	265.9 d	198.8–301.3	102.5	35.2	265.2 d
	Ni 600	333.3–403.7	70.4	27.0	375.5 e	378.8–471.2	92.4	35.8	446.5 f

*homogeneous groups were identified using the Duncan test, p < 0.05 (values denoted with identical letters do not differ significantly)

Table 5. Metal concentration indexes in above-ground parts of *Miscanthus × giganteus*

Metal	Dose of metal (mg dm ⁻³)	Substrates			
		Mineral soil		Mineral soil + high-moor peat	
		The first year of growth	The second year of growth	The first year of growth	The second year of growth
Ni	75	12.3	12.7	6.9	5.9
	150	69.9	65.4	71.9	66.3
	600	121.7	119.0	101.5	111.6

Table 6. Contents of nickel (extracted with Lindsay solution) in substrates (in mg dm⁻³) after the completion of plant growth in the first and the second years of analyses

Type of pollution	Substrate	Year of growth	
		1st year	2nd year
Control (native content of Ni mg dm ⁻³)	mineral soil	2.4 c	1.5 b
	soil + peat	0.7 a	0.6 a
Weak pollution (Ni 75 mg dm ⁻³)	mineral soil	44.3 d	29.8 c
	soil + peat	25.6 b	20.7 a
Medium pollution (Ni 150 mg dm ⁻³)	mineral soil	87.3 c	64.6 b
	soil + peat	56.5 b	37.5 a
Strong pollution (Ni 600 mg dm ⁻³)	mineral soil	269.6 c	211.7 b
	soil + peat	193.4 ab	161.3 a

* homogeneous groups were identified with the Duncan test, p < 0.05 (values denoted with identical letters do not differ significantly)

Both in soil and in a mixture of soil with peat a lower nickel content was found after the second year of growth (Table 6). In all experimental combinations a lower nickel content was detected in the substrate being a mixture of soil and peat in comparison to that found in mineral soil. A lower content of soluble forms of nickel in that substrate results from the uptake of greater amounts of this metal in the biomass yield produced in that substrate. Moreover, as it was reported by Bosiacki and Tyksiński (2006) an addition of organic substance to soil results in a reduced availability of soluble heavy metal forms.

Conclusions

1. *Miscanthus × giganteus* growing in contaminated mineral soil turned out to be a species tolerant to high nickel concentrations.
2. *Miscanthus × giganteus* accumulated a considerable amount of nickel in biomass.
3. *Miscanthus × giganteus* needs to be further tested in terms of nickel phytoextraction capacity from contaminated soil in natural habitat.

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Wpływ wzrastającej zawartości niklu w glebie na plonowanie *Miscanthus × giganteus* Greef i Deu. i zawartość niklu w nadziemnej biomasie

Celem badań prowadzonych w dwuletnim doświadczeniu wazonowym w nieogrzewanym tunelu foliowym, było określenie przydatności *Miscanthus × giganteus* do fitoekstrakcji niklu z gleb, jak również ocena tolerancji tego gatunku na wzrastające stężenia tego metalu w glebie. Wazony wypełniono glebą mineralną (piasek słabo gliniasty) oraz mieszaniną gleby z torfem wysokim i wprowadzono do nich trzy poziomy zawartości niklu: $75 \text{ mg} \cdot \text{dm}^{-3}$, $150 \text{ mg} \cdot \text{dm}^{-3}$ oraz $600 \text{ mg} \cdot \text{dm}^{-3}$ a jako warianty kontrolne zastosowano podłoża bez jego dodatku. Nikiel został wprowadzony tylko w pierwszym roku badań, w postaci siarczanu niklu ($\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$). *Miscanthus × giganteus* kumuluje znaczącą ilość niklu w biomasie. *Miscanthus × giganteus* rosnący w zanieczyszczonej glebie mineralnej okazał się gatunkiem tolerancyjnym na wysokie stężenia niklu.