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Phytoremediation of Soil Polluted by Nickel Using Agricultural Crops

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ABSTRACT / Soil pollution due to heavy metals is widespread; on the world scale, it involves about 235 million hectares. The objectives of this research were to establish the uptake efficiency of nickel by some agricultural crops. In addition, we wanted to establish also in which part of plants the metal is stored for an eventual use of biomass or for recycling the metal. The experiments included seven herbaceous crops such as: barley (*Hordeum vulgaris*), cabbage (*Brassica juncea*), spinach (*Spinacea oleracea*), sorghum (*Sorgum vulgare*), bean (*Phaseolus vulgaris*), tomato (*Solanum lycopersicum*), and ricinus (*Ricinus communis*). We used three levels of treatment (150, 300, and 600 ppm) and one control. At the end of the biological cycle of the crops, the different parts of plants, i.e., roots, stems, leaves, fruits, or seeds, were separately collected, oven dried, weighed, milled, and separately analysed. The leaves and stems of spinach showed a very good nickel storage capacity. The ricinus too proved to be a very good nickel storer. The ability of spinach and ricinus to store nickel was observed also in the leaves of cabbage, even if with a lower storage capacity. The bean, barley, and tomato, in decreasing order of uptake and storage capacity, showed a high concentration of nickel in leaves and stems, whereas the sorghum evidenced a lesser capacity to uptake and store nickel in leaves and stems. The bean was the most efficient in storing nickel in fruits or grains. Tomato, sorghum, and barley have shown a storage capacity notably less than bean. The bean appeared to be the most efficient in accumulating nickel in the roots, followed in decreasing order by sorghum, ricinus, and tomato. With regard to the removal of nickel, spinach was the most efficient as it contains the highest level of this metal per gram of dry matter. The ricinus, cabbage, bean, sorghum, barley, and tomato evidenced a progressively decreasing efficiency in the removal of nickel.

The development of industry and services, the intensification of agriculture, the enlargment of urbanized area, the development of information technology, and the huge increase of transports has favoured human needs. On the other hand, this development also has caused a considerable increase of pollution with consequent damage to the ecosystems.

Pollution of soil, water, and air, in its various forms, is probably the most outstanding outcome of the evolution of our society. Soil pollution determines either a degradation of productivity of ecosystems or serious risks for human and animal health. At a world level, soil pollution represents 12% of all degradation and it affects a surface of about 235 millions of hectares (Adriano and others 1997). In particular, soil pollution due to heavy metals is very harmful for living organisms. This type of pollution is mainly caused by anthropic activities, such as vehicular traffic, burning of fossil fuels, soil fertilisation, use of pesticides, mining and metallurgical activities, and the disposal of sludge. The risks of toxicity due to pollution from heavy metals do not depend only on their concentration but also on their physical and chemical state (Kelly 1988, Marion and others 1997), in addition to their solubility and uptake by the vegetation in a complex system that involves soil, water, and plants (Adriano 1986, Kabata and others 1992, McBride 1994, McSweeney and others 1994, Merien 1991, Alloway 1990, Alloway 1995, Alloway 1997).

The reclamation of heavy metal–polluted soils can be achieved with different techniques and technologies depending on the purposes, the soil properties, the extent of the polluted area, and the economical cost. For example, it is possible to excavate the polluted soil and dump it into landfills, or to stabilise/solidify chemically the soluble pollutants to prevent their leaching and their absorption by plants (Pierzynski and Schwab 1991). Nevertheless, such techniques do not resolve the prob-

KEY WORDS: Heavy metals; Nickel polluted soil; Soil phytoremediation; Crop storing efficiency; Plant tissue storage

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*Author to whom correspondence should be addressed; *email:* camillo.zanchi@unifi.it lem, as in the first case pollutants are moved to another area, while in the second case, the soil remains polluted. Another technique used for reclamation of polluted areas consists of washing the soil with appropriate extractants (water and surface-active agents, diluted bases and acids, complexants, and chelating agents) and procuring their recovery. Such a technique is more or less applicable depending on the pollutant, its solubility and concentration. However, this technique cannot be used on wide areas because of its cost and the uncontrolled reliability.

Recognising that the above-mentioned methods are expensive and not always applicable, a possible alternative is to use a biological method that consists of the utilisation of plants that uptake and accumulate heavy metals in their tissues even if they are not needed for their growth (Lasat 2000, Lasat 2002). Obviously, different species of plants can store different amounts of these metals, while each of them presents symptoms of toxicity when the heavy metal exceeds certain limits. Consequently, the aim of the search in this field is to single out plants, suitable to specific pedoclimatic environments, able to uptake and store a considerable quantity of such polluting metals (iperaccumulation plants) without showing toxic effects.

The process of metal absorption and accumulation in the vegetation tissues is called phytoextraction. Such a process assumes that polluting heavy metals are present in the soil layer explored by the roots and that their concentration does not exceed certain levels. Some families of plants seem promising in the absorption of one or more heavy metals, but the problem is very complex as the accumulation capacity of each species does not depend solely on its genetic properties but also on the pedoclimatic and microbiological conditions of the site where they live. Another important aspect concerns the location in the plant where the accumulation of heavy metals takes place. This differs among species. Recognising the fact that some species have a different specific absorption capacity for different heavy metals, it is also very important to know if the metals are stored in roots, in stems and leaves, or in fruits. The knowledge of storage characteristics of each species would allow to know if the metals are stored in no edible parts of the plants. Aware of this fact, the objectives of the present research focus on the evaluation of the heavy metals uptake efficiency for different plants and in what part of plants the metals accumulate. Specifically, we report on experimental results related to the absorption of nickel by some agricultural crops. This research is part of a broader study that includes also other three heavy metals: lead, copper and zinc.

Table 1. Selected physical and chemical properties of the soil used in the experiments

Sand	%	35.2
Silt	%	50.5
Clay	%	14.3
C	%	0.52
N	%	0.04
P	ppm	3.0
Total $CaCO_3$	%	5.5
pH	—	8.0
Cation exchange capacity	$cmol_c kg^{-1}$	14.2
Cu	ppm	55.8
Ni	ppm	63.5
Pb	ppm	39.8
Zn	ppm	111.1

On our planet, nickel is distributed in ultramaphic, basaltic and sedimentary clayey rocks with an average concentration of about 2000, 150 and 68 ppm respectively (Krauskopf, Rose and others 1967; Cannon, 1978). Usually, nickel concentration in the soil ranges between 17 and 50 ppm, but the concentration can reach 5000 ppm (Alloway, 1995) depending on parent material and degree of pollution.

Materials and Methods

The experimental plan includes seven agricultural crops: barley (*Hordeum vulgaris*), cabbage (*Brassica juncea*), spinach (*Spinacea oleracea*), sorghum (*Sorgum vulgare*), bean (*Phaseolus vulgaris*), tomato (*Solanum lycopersicum*), ricinus (*Ricinus communis*); three levels of treatment (150, 300 and 600 ppm of nickel) and one control, replicated three times. The experiments were repeated for two subsequent years (2000–2001). The above mentioned crops were sown or transplanted in 84 pots of 30 cm of diameter and 30 cm depth, each was filled with 18 kg of soil. The main physical and chemical properties of the used loamy soil are reported in Table 1. The analyses were executed using the official methodology indicated by Italian Ministry of Agriculture and Forestry (MiPAF, 1994).

The soil of each pot, following the experimental plan, was treated and mixed homogeneously with a solution containing 12.1 or 24.2 or 48.4 g of nickel sulphate corresponding to 150, 300, 600 ppm of nickel respectively. To avoid water stress the crops were irrigated with a dripping system.

At the end of the biological cycle of each crop, roots, stems and leaves, and fruits or seeds were separately collected, oven dried at 100°C, weighted, milled and analysed. The different components of the plants for each crop were ignited at a temperature of 550°C, the ashes were treated with nitric acid, and spettro-

Table 2. Bean: average nickel concentration (ppm) and average dry matter (g) produced in different parts of the plant in the two years of trials using different amounts of nickel in the soil

	Ni measured in different parts of the plant (ppm)						Dry matter measured in different parts of the plant (g)					
	2000			2001			2000			2001		
Ni added to the soil (ppm)	Leaves and stems	Legumes	Roots	Leaves and stems	Legumes	Roots	Leaves and stems	Legumes	Roots	Leaves and stems	Legumes	Roots
600	29.0	21.9	61.9	21.1	19.8	44.3	7.4	10.3	0.9	3.9	3.7	0.5
300	21.0	16.8	87.1	18.3	12.7	473	7.0	13.1	0.5	2.3	3.4	0.5
150	9.7	10.7	16.2	10.0	9.0	—	6.2	11.2	0.7	1.7	3.1	0.4
0	2.8	0.6	6.2	0.9	1.2	2.4	7.3	11.4	0.8	3.0	4.8	0.3
Lsd 0.05							3.33	6.80	0.71	2.29	4.56	0.30
Lsd 0.01							4.79	9.78	1.02	3.29	6.56	0.43

— NO data reported because of insufficient amount of material.

Lsd: Least significant difference.

photometrically determined at 231.604 nm using an ICP (Perkin Elmer).

For reducing the number of analyses and the relative costs, the nickel concentration was determined on a composite sample obtained by mixing three replications for each species and for each part of the plant (stems and leaves, fruits or seeds, and roots). Consequently, the statistical analyses of the results, performed with ANOVA together with a simple regression analyses, was performed only for the dry matter production data.

Results and Discussion

The data collected during the two years of experiments have been analysed for all crops and separately for each part of the plants: roots, stems and leaves, fruits or seeds for evaluating their nickel storage capacity and for assessing the effects of nickel soil treatments on plant dry matter.

Response of the Different Plants

Bean (Phaseolus Vulgaris). The data show a remarkable reduction of nickel concentration in leaves and stems of bean proceeding from the higher soil concentration (600 ppm) to the lower (150 ppm). In fact, at the maximum nickel soil concentration, its content in leaves and stems acquired values of 21 and 29 ppm respectively during the two years. At the lower concentration of nickel in the soil, the content of nickel in leaves and stems diminished at about 10 ppm. However this latter level appeared notably higher than that measured on leaves and stems of plants of bean grown on control. The same trend was observed in legumes and in the roots, even if in these latter the nickel concentration was remarkably higher, in particular at the middle and maximum concentration of nickel in the soil. During the length of the experiment were not noticed statistically significant differences among the various treatments as far as the amount of dry matter produced (Table 2).

Sorghum (Sorgum Vulgare). In the leaves and stems of sorghum some storage of nickel, particularly in the year 2001, was revealed even if the quantity remains low (9 ppm) also at the maximum concentration of nickel in the soil. The same trend, with decreasing storage with the decrease of nickel concentration in the soil, was noticed also in the grains. On the contrary, a considerable storage of nickel was noticed in roots even if the reduction of nickel concentration in the soil tended to decrease. No statistically significant differences were evidenced as far as dry matter production among the various treatments (Table 3).

Barley (Hordeum Vulgaris). The nickel concentration in the leaves and stems of barley appeared to be remarkably greater during the 2000 than in 2001. Also in the grains the concentration of nickel resulted moderate with a decreasing trend following the decrease of concentration of nickel in the soil. The statistical analysis of dry matter of grains showed a difference highly significant ($P < 0.01$) between the various levels of soil treatments (low, middle and high), and between the maximum level versus the control. The difference between this latter and the low level of treatment resulted significant at $P < 0.05$. It seems interesting to remark that at the low level a significant increase of dry matter ($P < 0.05$) was noticed in comparison with control; moreover the dry matter production decreased rapidly with the increase of nickel concentration in the soil, particularly at the maximum level. No significant differences were noticed for the amount of dry matter either of leaves and stems, or of roots (Table 4).

Table 3. Sorghum: average nickel concentration (ppm) and average dry matter (g) produced in different parts of the plant in the two years of trials using different amounts of nickel in the soil

	Ni measured in different parts of the plant (ppm)						Dry matter measured in different parts of the plant (g)					
	2000			2001			2000			2001		
Ni added to the soil (ppm)	Leaves and stems	Grains	Roots	Leaves and stems	Grains	Roots	Leaves and stems	Grains	Roots	Leaves and stems	Grains	Roots
600	2.6	6.3	62.6	9.0	3.2	58.8	19.6	34.2	4.3	15.6	12.2	10.0
300	2.9	4.3	57.6	5.5	2.6	42.3	19.3	34.1	5.8	14.7	10.6	8.4
150	0.5	3.0	19.6	4.5	1.6	26.0	18.6	32.3	4.2	163	11.4	9.0
0	1.8	0.2	4.4	0.9	0.8	4.6	20.5	25.3	4.0	23.0	14.7	5.0
Lsd 0.05							3.98	19.14	6.30	8.52	7.79	5.42
Lsd 0.01							5.72	27.53	9.07	12.25	11.21	7.80

Lsd: Least significant difference.

Table 4. Barley: average nickel concentration (ppm) and average dry matter (g) produced in different parts of the plant in the two years of trials using different amounts of nickel in the soil

	Ni measured in different parts of the plant (ppm)						Dry matter measured in different parts of the plant (g)					
	2000			2001			2000			2001		
Ni added to the soil (ppm)	Leaves and stems	Grains	Roots	Leaves and stems	Grains	Roots	Leaves and stems	Grains	Roots	Leaves and stems	Grains	Roots
600	30.2	—	—	9.0	4.0	—	18.7	—	1.6	0.7	0.6	0.2
300	17.8	—	—	5.9	2.6	—	21.2	—	2.2	1.8	2.0	0.3
150	27.8	—	—	3.6	1.6	—	16.2	—	2.7	1.6	3.1	0.4
0	1.9	—	—	0.7	0.1	4.3	10.2	—	1.8	3.1	2.3	0.6
Lsd 0.05							13.70	—	1.56	1.74	0.67	0.39
Lsd 0.01							19.70	—	2.24	2.50	0.96	0.56

—: No data reported because of insufficient amount of material.
Lsd: Least significant difference.

Spinach (Spinacea Oleracea). The spinach was particularly efficient in uptaking nickel. In the leaves it has been evidenced considerable storage of the metal that progressively increase with increasing nickel concentration in the soil. From the storage point of view this plant seems very promising but a further confirm is necessary as the data collected regard only the leaves for one year. No significant differences in the dry matter production were found between the different treatments (Table 5).

Tomato (Solanum Lycopersicum). A notable storage of nickel was found in the roots of tomato. The storage of nickel progressively decreased in leaves and stems, and in fruits with decreasing of nickel concentration in soil. As far as the leaves and stems, the dry matter production of the first year showed a significant statistical difference among the three levels of nickel versus the control. The second year showed a significant difference between the maximum level of nickel in the soil and the other treatments. In fact, the regression analyses (n = 12) related to year 2000 and 2001 showed a determination coefficients (R^2) of 0.60 and 0.48 respectively. Moreover, it was found a significant difference ($P < 0.05$) in the dry matter production of fruits between the maximum level of nickel in the soil and the control and the soil treated at the lower level (Table 6).

Ricinus (Ricinus Communis). The amount of nickel stored in the roots and in the leaves and stems of ricinus increased progressively in accordance with the increasing metal concentration in the soil. The values were notably greater in comparison with the control. Also in the seeds, only at intermediate level of treatment, the concentration of metal was notably higher in comparison with the control. The first year the plants treated with 600 and 150 ppm did not produce fruits. In the second year there was not production of fruits at all. The production of leaves and stems dry matter of all treatments levels resulted significantly different from the control, even if the regression analysis (n = 12) shows a determination coefficient rather low ($R^2 = 0.20$). As far as the roots dry matter production, the statistical analysis evidenced highly significant differences ($P < 0.01$) between the maximum and intermediate level of treatment and control (Table 7).

Table 5. Spinach: average nickel concentration (ppm) and average dry matter (g) produced in different parts of the plant in the two years of trials using different amounts of nickel in the soil

	Ni measured in different parts of the plant (ppm)						Dry matter measured in different parts of the plant (g)					
	2000			2001			2000			2001		
Ni added to the soil (ppm)	Leaves and stems	Grains	Roots	Leaves and stems	Grains	Roots	Leaves and stems	Grains	Roots	Leaves and stems	Grains	Roots
600	65.5	—	—	—	—	—	7.8	—	—	—	—	—
300	42.3	—	—	—	—	—	4.3	—	—	—	—	—
150	21.7	—	—	—	—	—	8.4	—	—	—	—	—
0	4.6	—	—	—	—	—	2.3	—	—	—	—	—
Lsd 0.05							9.38	—	—	—	—	—
Lsd 0.01							13.49	—	—	—	—	—

—: No data reported because of insufficient amount of material.

Lsd: Least significant difference.

Table 6. Tomato: average nickel concentration (ppm) and average dry matter (g) produced in different parts of the plant in the two years of trials using different amounts of nickel in the soil

	Ni measured in different parts of the plant (ppm)						Dry matter measured in different parts of the plant (g)					
	2000			2001			2000			2001		
Ni added to the soil (ppm)	Leaves and stems	Fruits	Roots	Leaves and stems	Fruits	Roots	Leaves and stems	Fruits	Roots	Leaves and stems	Fruits	Roots
600	24.2	8.1	53.9	3.3	1.5	47.6	55.0	21.8	3.3	27.9	15.5	4.4
300	18.9	6.2	35.5	14.9	3.8	28.9	49.2	9.6	3.3	17.4	3.6	1.5
150	10.3	5.2	16.3	11.5	2.0	16.0	44.3	5.7	3.0	13.0	3.2	2.1
0	1.0	0.4	2.3	2.7	0.6	4.3	27.8	3.9	4.6	18.5	5.4	2.4
Lsd 0.05							12.89	13.02	4.32	6.63	16.28	3.50
Lsd 0.01							18.53	18.73	6.21	9.53	23.41	5.04

Lsd: Least significant difference.

Cabbage (Brassica Juncea). Also the cabbage proved to be a good nickel storer, particularly in the leaves and, to a lesser extent, in the roots. The statistical analysis showed a significant difference ($P < 0.05$), in the dry matter production, between the higher level of treatment and the control (Table 8). The regression analysis ($n = 12$) showed determination coefficients (R^2) of 0.50 and 0.18 for 2000 and 2001 respectively.

Conclusions

This study points out the effectiveness of some agricultural crops, not examined in previous researches, for the capacity of up taking and storing nickel in parts of the plants. Spinach and ricinus appeared to be very effective in removal of nickel from the soil. The cabbage, the only species utilised in previous research on phytoremediation of nickel polluted soils (Panwar and others 2002), seems effective in nickel uptake and storage, but the results obtained are not comparable with those obtained by other investigators in as much as the latter used chelating agents.

From a practical point of view, it is interesting to know the capacity of metal removal, per the unit dry matter produced, and if the removal is influenced by the nickel concentration in the soil. Consequently, the dry matter of different parts of plants were multiplied by the relative nickel concentration and summed together. The result was divided by total dry matter produced for obtaining the weighted concentration of nickel for each gram of dry matter produced.

The spinach was the most efficient in the removal of the nickel, such removal increased linearly with the increase of metal concentration in the soil. At the higher nickel concentration in the soil the uptake reached 65 g for each ton of dry matter produced. The other species showed a decreasing efficiency in the

Table 7. Ricinus: average nickel concentration (ppm) and average dry matter (g) produced in different parts of the plant in the two years of trials using different amounts of nickel in the soil

	Ni measured in different parts of the plant (ppm)						Dry matter measured in different parts of the plant (g)					
	2000			2001			2000			2001		
Ni added to the soil (ppm)	Leaves and stems	Fruits	Roots	Leaves and stems	Fruits	Roots	Leaves and stems	Fruits	Roots	Leaves and stems	Fruits	Roots
600	48.7	—	67.2	34.9	—	42.7	49.7	—	2.9	11.1	8.7	2.1
300	35.2	17.0	45.3	19.0	—	37.5	40.3	—	2.9	9.7	12.0	1.3
150	20.4	—	33.9	19.0	—	21.8	47.2	—	2.4	9.3	10.3	1.5
0	2.3	0.6	48.8	1.8	0.6	2.6	28.0	—	2.4	6.5	6.7	1.2
Lsd 0.05							24.73	—	1.13	3.03	1.67	0.50
Lsd 0.01							35.56	—	1.63	4.36	2.40	0.72

—: No data reported because of insufficient amount of material.
Lsd: Least significant difference.

Table 8. Cabbage: average nickel concentration (ppm) and average dry matter (g) produced in different parts of the plant in the two years of trials using different amounts of nickel in the soil

	Ni measured in different parts of the plant (ppm)						Dry matter measured in different parts of the plant (g)					
	2000			2001			2000			2001		
Ni added to the soil (ppm)	Leaves and stems	Grains	Roots	Leaves and stems	Grains	Roots	Leaves and stems	Grains	Roots	Leaves and stems	Grains	Roots
600	40.6	—	17.7	21.5	—	—	16.7	—	0.8	1.9	—	0.4
300	23.0	—	21.3	15.9	—	7.3	12.3	—	0.6	2.1	—	0.4
150	15.1	—	12.3	26.0	—	—	13.7	—	0.8	0.9	—	0.3
0	0.9	—	1.5	1.3	—	1.2	10.3	—	0.5	1.0	—	0.4
Lsd 0.05							4.96	—	0.35	1.23	—	0.45
Lsd 0.01							7.13	—	0.51	1.77	—	0.64

—: No data reported because of insufficient amount of material.
Lsd: Least significant difference.

Table 9. Average amounts of nickel (grams per ton of dry matter produced) absorbed by each species at different amounts of nickel in the soil

Ni added to the soil (ppm)	Bean	Sorghum	Barley	Spinach	Tomato	Ricinus	Cabbage
600	24.3	20.4	18.7	65.5	13.3	42.8	31.0
300	18.4	16.5	10.4	42.3	15.9	25.9	19.1
150	10.1	8.1	12.1	21.7	10.2	20.2	20.5
0	1.4	1.8	1.8	4.6	1.8	1.8	1.1

removal of metals. When the higher concentration of the metal in the soil is considered, the uptake of ricinus, cabbage, bean, sorghum, barley and tomato corresponds, respectively, to 42.8, 31.0, 24.3, 20.5, 18.7 and 13.3 grams per ton of dry biomass (Table 9). Knowing that the dry matter production for ricinus is 3.6 t ha^{-1}, 3.0 t ha^{-1} for spinach and 11.0 t ha^{-1} for cabbage, the amounts of Ni per ha removed is 154.5 g, 196.5 g and 341.0 g respectively.

In conclusion, the phytoremediation capability depends on the level of nickel concentration in soil. At very high nickel concentration the phytoremediation, even if appropriate, needs considerable time for lowering the nickel at acceptable levels. On the other hand, we must consider that the costs of phytoremediation are not excessive, as they are represented only by the normal cultivation costs. In terms of utilisation, the dry matter produced could be burned for energy production while the metal could be retrieved and eventually reutilized.

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