J. Mater. Environ. Sci. 3 (3) (2012) 469-476
ISSN: 2028-2508
CODEN: JMESCN

Bio-accumulation of the metal elements traces by the radish (Raphanus sativus) cultivated on grounds amended by muds of a sewage treatment plant

OUIHMAN EL Mahdi*, HBAIZ EL Mahdi, LEBKIRI Mustapha, LEBKIRI Ahmed, RIFI El Housseine, OUZAIR Abdelhamid

Laboratory of Organic Synthesis and Extraction Processes, Faculty of Science, University Ibn TOFAIL, BP 133 Kenitra 14000, Morocco

Received 26 aug 2011, revised Feb 2012, accepted Feb 2012.
* Corresponding author**:** E-mail: ouihman@yahoo.fr , Tél: (+212) 662 38 78 66 or (+212) 661 32 56 03.

Abstract
This work consists under investigation of the effect of the spreading of the mud of the sewage treatment plant of the city of Beni Mellal in Morocco on the accumulation of certain elements metal traces (Pb, Cu, Ni, Zn and Cd) in a market gardening: the radish (Raphanus sativus). The results show that the mud contribution involves an increase in the accumulation of the metal elements traces in radish. This increase informs about the risks caused in the long run by the spreading of this waste. In addition, the accumulation of the metal elements traces by radish is influenced by the mud concentration (0, 10, 20, 30, 40 and 50%). This effect is variable according to metal, of the mud amount brought and the plant species.

Keywords: metal sewage treatment plant, mud, radish, elements traces, accumulation.

1. Introduction:

The spreading of muds of sewage treatment plants of waste waters in agriculture is in net increase these last decades. These muds can be used to improve the fertility of the grounds and the production of the cultures **[1]**. This practice is one of the ways of organic waste recycling. However, it must be conditioned by the composition of muds. Indeed, besides the nauseous odors, muds can contain pathogenic micropolluants and toxic elements, in particular of the metal elements traces **[2]**. These elements, no biodegradable, can accumulate in the grounds and, under certain conditions, migrate in the major horizons **[3, 4]** and be thus at the base of a risk of contamination of the water tables. These elements metal traces can be absorptive by the plants cultivated on the grounds amended by muds and be transferred along the trophic chains, thus constituting a risk of contamination of the man **[5, 6]**. In order to minimize these risks, of the standards relating to the spreading of muds the limiting contents of metal elements traces in this waste and the grounds likely fix to receive these spreading **[7]**.

The behavior of the metal elements traces in the system ground-plant is controlled by the nature and the physicochemical characteristics of the grounds and the muds used as well as species and variety of the plants. These characteristics are responsible for the chemical shape of the elements traces, thus being able either to increase or to decrease their availability with the plants **[8, 9]**.

We followed accumulation, by radish, of the metal elements traces (Pb, Cu, Zn and Cd) contained in mud coming from the system of treatment of waste waters of this city.

ISSN: 2028-2508
CODEN: JMESCN

2. Experimental

2.1 Station of study

It is the sewage treatment plant of waste waters Béni Mellal (Morocco). It is dimensioned in order to provide an effluent than 40 mg/l of DBO and 30 mg/l of suspended matter.

It understands works of pretreatment, intended for the biological treatments of water and muds.

2.2 Material used

2.2.1 Ground

In the experimentation one used as substrate two soil types of different nature, the table below represents the physico-chemical characteristics of the two grounds:

Table 1: Physico-chemical characteristics of the two grounds.

Ground of Maâmora	Ground of Béni Mellal
Sandy ground, containing fine sand 66.6%, to slightly basic pH (7.53). It contains sufficient organic matter (0.7%), of nitrogenized and cogitated compound and trace elements for the culture **[10]**.	Ground isohumic with fine clay limono-sand spreader texture containing 38.8% from clay and 41,6% of silts and with alkaline pH (7.81). Its content of organic matter is average (2.22%).

In the two grounds the content of heavy metals is lower than the standards of the EEC **[11]**.

2.2.2 Muds

The mud used in the experimentation comes from the sewage treatment plant of waste waters of the city from Beni Mellal (Morocco).

In addition, a taking away of 250 kg mud was carried out in the month of April 2010, then dried with the free air, crushed and filtered using a sieve of diameter of 2 mm. Table 2 represents the physicochemical characteristics of mud.

Table2: Physico-chemical characteristics of mud.

Parameter	Result	* EEC normalizes
pH-water	6,57	-
Conductivity (µs/cm)	6,01	-
Organic carbon (%)	22,56	-
Organic matter (%)	22,4	-
Nitrogenize total (%)	2,11	-
C/N	10,69	-
Cogitates total (‰)	1,16	-
Assimilable phosphorus (Olsen) (‰)	1,66	-
EC (ns/kg)	5,8	-
Cadmium (ppm)	□ limit of detection	20
Zinc (ppm)	775	3000
Copper (ppm)	134	1000
Lead (ppm)	155	800
Nickel (ppm)	35	200

* Standards of the European Economic Community (86/278/CEE of June 12th, 1986).

The pH of mud is slightly acid, of 6.57. It corresponds to the new recommendations of the Superior council of Public health of France (SCPHF), which requires pH > 6 to be used as amendment (Table 2). These muds are rich in organic matter (22.4 % of the matter weight dry).

ISSN: 2028-2508
CODEN: JMESCN

The organic matter of an amendment is an agronomic parameter which is of a great importance in agriculture, since it improves porosity and the power of retention of water of the grounds **[12]**. It thus supports the rooting of the crop plants, their water provision and in nutritive elements while bringing a source of food to the earthworms and the micro-organisms useful **[13]**. This mud contains high percentages of compounds nitrogenized (2.11 %) and cogitated (1.16 %). Report C/N, ranging between 9 and 11, indicates a good mineralization of the organic matter. The contents zinc, copper, lead, cadmium and nickel are below standards of the EEC. However, raised electric conductivity, about 6 µs.cm^{-1}, could harm the cultures sensitive to salts.

2.2.3 Vegetable material

We chose like vegetable material the plant of the radish (Raphanus sativus), it is a bi-annual vegetable, of the family of Brassicacées, cultivated for its fleshy hypocotyle and soup believed like vegetable.

2.3 Experimental protocol

2.3.1 Conditions of culture

On samples of two kilograms matter dry of ground, filtered to 2 mm and put in plastic pots (20 cm diameter and 25 cm height), irrigated three times per week with concentrations correspond to 0 (witness), 25,50,75 and 100% out of waste water. For each amount of concentration tested, three repetitions were carried out.

After watering, the seeds of the radish (Raphanus sativus) were sown at a rate of 3 seeds per pot.

The experimentation was carried out under greenhouse for the period being spread out from May to July 2010. The irrigation is made in order to maintain the ground to 90 % of the capacity to the field.

During this work, the two bodies of radishes (sheet and root) collected each pot are washed abundantly with distilled and dried water with 75 °C during 48 hours.

Fig. 1: Glasshouse cultivation of radish.

2.3.2 Measured agronomic parameters

Harvest took place after two months of culture. The given parameters are six:

- Length of the sheets;
- Many sheets per plant;
- Number of the sheets deteriorated by plant;
- Diameter of the root of radish;
- Fresh weight of the root;
- Dry weight of the root.

ISSN: 2028-2508
CODEN: JMESCN

2.4 Method of analysis
The proportioning of the metal elements traces in the collected dry matter was carried out by ICP-AES (Inductively coupled plasma atomic emission spectroscopy).

The apparatus used is a spectrometer of the type: ULTIMA 2 JY at the national center of the scientific research and technique (C.N.R.S.T) in Morocco after mineralization of the samples according to the method of **[14]**. Indeed, 2g of dry matter of the plant were calcined in an oven with 450 °C during 4 hours. Ashes were mineralized by water levels: 25 % HNO_3 and 75 % HCl, then brought back dry until discoloration of the minéralisat on a sand bath. The dissolution of the residue was made with 10 ml of HCl to 5 % hot during 15 minutes then filtered on Wattman paper 0.45 µm, the solution obtained was adjusted to 20 ml by distilled water.

3. Results and discussions

3.1 Effects of the incorporation of mud on the ground
The study of the effect of the rate of the mud incorporated in the ground on the evolution of the pH and electric conductivity led to the following results:

3.1.1 pH
Figures 2 and 3 represent the effect of the incorporation of mud on the pH of the two grounds Maâmora and Béni Mellal.

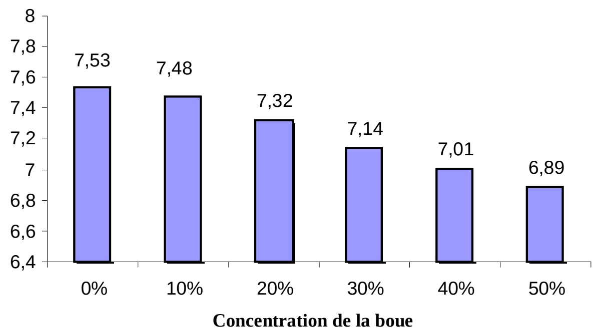

Fig. 2: Effect of mud on the pH of the ground of Maâmora.

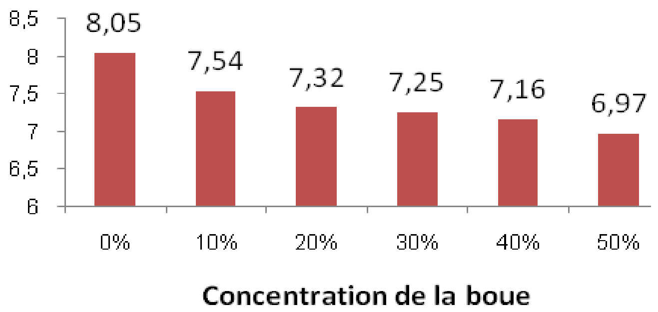

Fig. 3: Effect of mud on the pH of the ground of Beni Mellal.

The results show on the level of the two grounds which the pH of the substrate is alkaline (pH > 7) and decreases gradually with the increase in the mud amount compared to the witness (for the ground of Maâmora pH = 7.53 and for the ground of Béni Mellal pH = 8.05).

Indeed, with a concentration of 10% the recorded pH is about 7.66 for the ground from Maâmora and 7.54 for the ground of Béni Mellal. It is also noted that the plant can more or less well be nourished and push according to the pH of the substrate.

3.1.2 Electric conductivity

The effect of mud on the conductivity of the ground was also studied. Figures 4 and 5 illustrate the data obtained.

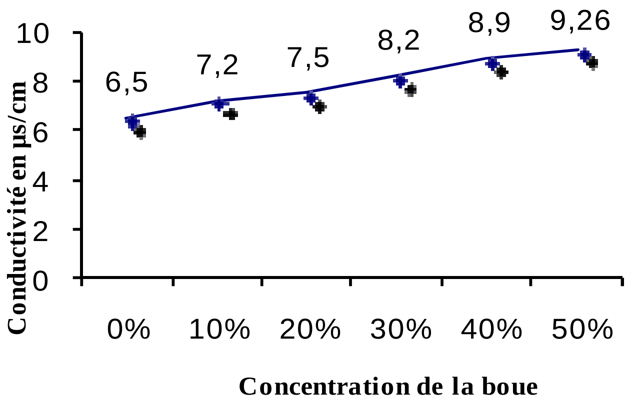

Fig. 4: Influence mud contribution on the electric Conductivity of the ground of Maâmora with 25°c.

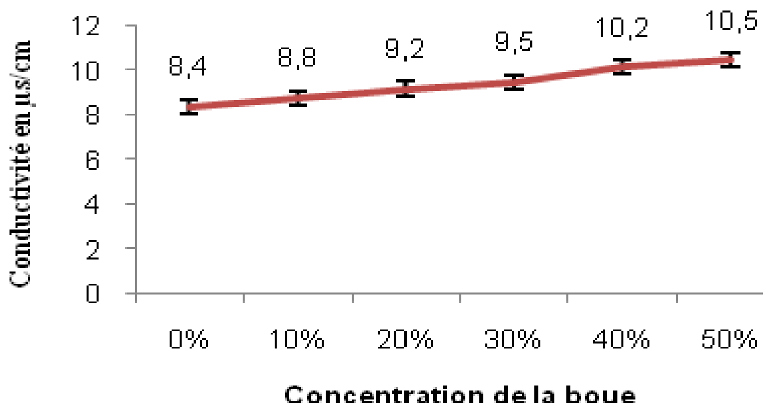

Fig. 5: Influence mud contribution on the electric Conductivity of the ground of Béni Mellal with 25°c.

It should be noted that the got results show that all the concentrations tested generate a progressive increase in the salinity of the ground compared to the witness (0% of mud). The values measured on the level of the ground of Maâmora are of 7.02; 7.5; 8.2; 8.9 and 9.26µs.cm^{-1} respectively for 10,20,30,40 and 50% out of mud and on the level of the ground of Béni Mellal are of 8.8; 9.2; 9.5; 10.2 and 10.5 µs.cm^{-1} respectively for 10,20,30,40 and 50%.

The increase in the electric conductivity of the grounds can be explained by the accumulation of the biogenic salts in the parts amended by the grounds.

3.2 Effect of mud on the agronomic parameters of Radish

Table 3 represents the effect of mud on the various agronomic parameters studied after the culture in the two grounds. The comparison of the various got results reveals a significant growth of the diameter of the root of the plant according to the concentration of mud. This increase can be due to the important assimilation of the plant of the biogenic salts. Best results of the contribution of mud on the diameter of the root of the plant one obtained for the amount of 50% with a diameter of 16 mm on the level of the ground of Maâmora and 14 mm on the level of the ground of Béni Mellal. As we note as the number of sheets is influenced by the contribution of mud: at the plants treated with 10,20,30,40 and 50% of mud. This number of sheets varies between 4 and 5.33 on the level of the two grounds.

For the number of sheets deteriorated by plant, the results show that the contribution of mud influences slightly on this number which varies between 2.33 and 3.12 while passing from 10 to 40% of mud/ground of Maâmora and 2.45 to 3.10 while passing from 10 to 40% of mud/ground of Béni Mellal.

Nevertheless on the level of the ground of Maâmora, this influence is marked by a high number of faded sheets which is about 4.64 sheets for the treatment with mud 50%, on the other hand on the level of the ground of Beni Mellal it reaches 4.44 of faded sheets.

Table 3: Effects of mud on the agronomic parameters of radish in the two grounds.

	GROUND OF MAAMORA						GROUND OF BENI MELLAL					
% of mud	**0**	**10**	**20**	**30**	**40**	**50**	**0**	**10**	**20**	**30**	**40**	**50**
Diameter of the root of the radish (mm)	11,7	12,5	13	14,2	15,3	16	12	13,2	13,5	13,5	13,8	14
Many sheets per plant	4	4,66	4,66	5	5	5	4	4,66	5	5	5,33	5
Many sheets deteriorated by plant	1,33	2,33	2,33	2,66	3,12	4,64	1	2,45	3	3	3,10	4,44
Fresh weight of the root (g)	1,2	1,5	1,5	1,6	1,66	3	1,1	1,33	1,35	2,1	2,2	2,7
Dry weight of the root (g)	1,1	1,25	1,36	1,4	1,4	2,8	1,6	1,9	2,1	2,2	2,3	3,1

N.B: We point out that the values mentioned are the means of three repetitions.

The other studied agronomic parameter which is the fresh weight of the root passes respectively on the level of the ground of Maâmora of 1.5 to 3g and 1.33 to 2,7g on the level of the ground of Béni Mellal while going from 10% to 50%.

For the dry weight it reaches the maximum with 50% with a value of 2,8g for the ground of Maâmora and 3,1g for the ground of Béni Mellal.

3.3 Effect of mud on the bio-accumulation of heavy metals in the bodies of radish

The contents of heavy metals in the bodies of radish cultivated on the two grounds and fertilized by muds are given in tables 4 and 5.

Table 4: Effect of the fertilization by muds on the accumulation of heavy metals (mg/kg) in the bodies of radish cultivated on the ground of Maâmora.

		copper	Lead	Zinc	Nickel	Cadmium
Break into leaf of the plant	**0%**	3,05	0,25	2,26	0,50	<LD
	10%	4,86	0,44	8,12	3,25	<LD
	20%	5,15	1,08	12,84	5,95	<LD
	30%	12,11	1,36	15,55	7,66	<LD
	40%	14,30	1,65	15,87	9,33	<LD
	50%	16,6	1,83	17,05	10,16	<LD
Root of the plant	**0%**	3,56	0,46	3,41	1,46	<LD
	10%	5,02	0,62	9,21	4,02	<LD
	20%	6,12	1,76	13,41	6,51	<LD
	30%	13,97	1,89	16,20	7,20	<LD
	40%	14,55	1,90	16,84	9,50	<LD
	50%	17,85	2,01	17,74	10,52	<LD

ISSN: 2028-2508
CODEN: JMESCN

Table 5: Effect of the fertilization by muds on the accumulation of heavy metals (mg/kg) in the bodies of radish cultivated on the ground of Béni Mellal.

		copper	Lead	Zinc	Nickel	Cadmium
Break into leaf of the plant	**0%**	5,5	1,23	2,55	1,54	<LD
	10%	5,66	2,20	2,69	2,08	<LD
	20%	6,55	2,22	5,50	3,11	<LD
	30%	7,88	2,56	6,21	7,62	<LD
	40%	7,96	3,10	7,62	9,13	<LD
	50%	8,23	3,45	9,45	10,24	<LD
Root of the plant	**0%**	6,13	2,66	3,13	2,60	<LD
	10%	7,06	3,13	3,66	3,21	<LD
	20%	7,65	3,64	6,45	3,54	<LD
	30%	8,02	4,05	7,03	8,55	<LD
	40%	8,66	3,56	8,60	10,11	<LD
	50%	8,9	3,75	11,55	11,41	<LD

The results of the spectroscopic analysis by ICP-AES of the two bodies of radish show that Cadmium does not accumulate in the bodies of radish, this is explained by the complete lack of this element in the two grounds.

On the other hand the bio-accumulation of copper in the root and the sheets of radish increases with the mud amount brought. On the level of the ground of Maâmora and to 50% it reaches values higher than the allowed standard which is 15mg/kg **[15]**. However it is noted that copper accumulates more in radish cultivated on ground of Maâmora than on ground of Béni Mellal. Indeed the use of a going mud amendment from 10 to 50%, the copper concentration in the root passes from 5.02 to 17.85 ppm for a culture carried out on ground of Maâmora and 7.06 to 8.9 on ground of Béni Mellal. This difference can be interpreted by the argillaceous structure of the ground which allows an important adsorption of the metal elements traces. Moreover in the two grounds, the bio-accumulation of copper is more important in the roots of the plant.

With regard to lead, in the two soil types, the contribution of mud intensifies its accumulation in the two bodies of radish, with a higher content in the root compared to the sheets for the two grounds. The maximum values of accumulated organic lead are obtained with waste water 50%, they are, respectively for the root and the sheet, about 2.01 and 1.83 ppm on ground from Maâmora and 3.75 and 3.45 ppm on ground of Béni Mellal. These values are higher than the allowed standard which is of 1 ppm **[16]**.

Also the accumulation of nickel in the two bodies of the crop plant on the two grounds is all the more important as the contribution of mud is high. During the amendment with increasing percentages of muds of about 10, 20, 30, 40 and 50%, the contents of nickel in the root are respectively 4.02, 6.51, 7.20, 9.50 and 10.52 ppm on ground of Maâmora and 3.21, 3.54, 8.55, 10.11 and 11.41 ppm on ground of Béni Mellal. It is thus noted that when the mud contents become higher than 30%, the concentration of nickel exceeds the threshold of toxicity which is of 8 mg/kg **[17]**. The results show that Nickel accumulates preferably in the root for the two grounds.

In addition the analysis of the table shows that the bio-accumulation of zinc varies according to the body, of the mud contents and the type of substrate of culture. Indeed for the two grounds, the content of the bodies of radish zinc is all the more important as the mud contribution is high. The maximum values are reached with mud 50%, they are respectively for the root and sheet 17.74 and 17.05 ppm on ground from Maâmora and 11.41 and 9.45 on ground of Béni Mellal. Thus the bio-accumulation of the metal element is more important in the root and as higher on sandy substrate as on argillaceous substrate. Studies carried out on the accumulation of the elements traces at the colza cultivated on ground amended by waste muds showed a strong accumulation of zinc, inducing disturbances of synthesis of photosynthetic pigments causing of foliar deteriorations, also recorded in radish **[18]**.

ISSN: 2028-2508
CODEN: JMESCN

Conclusion

The spreading of muds of the sewage treatment plant of waste waters on the agricultural grounds seems to be a way of interesting improvement of the outputs of the cultures. However, this improvement can be accompanied by an increase in the accumulation of the metal elements traces by the cultures.

The mud contribution appears to have an effect on the transfer of the metal elements traces towards radish. This effect is variable according to metal and would be in keeping with its concentration in mud and its chemical form (exchangeable).

Moreover, the contents recorded in the cultures of radish can exceed the standards recommended. So a treatment of muds before their spreading on the agricultural grounds would be essential. But these results were got in controlled conditions, the cultures were carried out in phase of vegetation.

Under these conditions, the metal elements traces are biodisponibles. It is thus very possible that a culture of radish realized in full field would have led to concentrations out of Cd, Cu, Ni, Zn and weaker Pb in the plant.

Acknowledgements

This research was supported by the grant of Laboratory Team Organic Synthesis and Extraction Processes, Faculty of Science, University Ibn Tofail, BP 133 Kenitra 14000, Morocco

References

1. Schauer, PS., Wright, WR., Pelchat, J. *J Envir. Qual.* 9 (1980) 69-73.
2. Nejmeddine, A., Fars, S., Echab, A. *Environnemental technology.* 21 (2000) 225-230.
3. Legret, M., Divet, L., Juste, C. *Water research.* 22 (1988) 953-959.
4. Baveye, P., Mc Bride, MB., Bouldin, D., Hinesly, TD., Dahdoh, MS., Abdel sabour, MF. *Sci total environ*. 227 (1999) 13-28.
5. Dudk, S., Miller, wp. *J Environ. Sci Health* B. 34 (1999) 681-708.
6. Manz, M., Weissfloy, L., Kuhne, R., Schurmann, G. *Ecotoxicol environ safety*. 42 (1999) 191-201.
7. Afnor. Permanent code environment and nuisance. 245 (1998) 6605-6612.
8. Morel, JL., Pierrat, JC., Guckert A., J Agronomy. 8 (1988) 107-113.
9. Hooda, PS., Alloway, BJ. *J Soil Sci*. 44 (1993) 97-110.
10. Kassaoui, H. *Bull soc pharm Bordeaux*. 148 (2009) 77-90.
11. CEE. Standards of the European economic community. 278 (1986) 86.
12. Dayegamiye, A., Drapeau, S., Huard, Y. *Agrosol.* 15 (2004) 83-90.
13. Estevez, B., Coderre, D. *Agrosol.* 5 (1992) 26-31.
14. Tauzin, A. *Agric France.* 56 (1986) 31-34.
15. Heller, St., Lance, C. *Vegetable physiology*. 6 (1998) 306.
16. Hooda, M. Wat., *Air Soil Poll.* 74 (1994) 235-250.
17. Godin, P. *Sources of contamination and challenges.* 6 (1982) 306.
18. Rifi, E.H. *Reports chemistry* 8 (2004) 215-218.