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CORRELATION BETWEEN BIOLOGICAL AGENTS AND LEVELS OF HEAVY METALS IN MUNICIPAL SEWAGE SLUDGE

Urszula Bożek, Teresa Kłapeć

Department of Environmental Hygiene, Institute of Agricultural Medicine, Lublin, Poland

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Abstract: During the period 2003-2004, 110 samples of sewage sludge were examined from 18 treatment plants located in the territory of the whole of Poland, the majority in the Lublin Province. Samples of fermented and dehydrated sewage sludge were taken from sludge drying beds. The bacteriological and parasitological determinations covered bacteria of the *Salmonella* genus and eggs of the helminths *Ascaris* spp., *Trichuris* spp., and *Toxocara* spp. Chemical examinations for the presence of the heavy metals: chromium, zinc, cadmium, copper, nickel and lead were carried out by the method of atomic absorption flame spectrophotometry. The results were subjected to statistical analysis with the use of ANOVA and Student's t-tests. In the sewage sludge samples examined, *Salmonella* bacteria were isolated from 9 samples (8.2%), and the eggs of helminths from 31 samples (28.1%). The eggs of *Ascaris* spp. were most frequently detected – in 24 samples of sewage sludge (21.8%), followed by *Trichuris* spp. eggs – in 11 samples (10.0%), and *Toxocara* spp. eggs - in 6 samples (5.4%). Several samples of sewage sludge were infected by eggs of all the parasites examined (ATT). A very high variation was observed with respect to *Salmonella* bacteria and ATT eggs according to the origin, type of sanitation and duration of deposition of sludge. Levels of chromium, zinc, copper and lead in the sewage sludge samples, in the majority of samples examined, remained below allowable values. Very high values exceeding the allowable values for the levels of heavy metals were observed only in the samples of sewage sludge from one treatment plant. This concerned a cadmium level (201.1 mg/kg d.m.) which exceeded by 20 times the most rigorous allowable contents of this metal for sewage sludge designed for agricultural use, and nickel (288.7 mg/kg d.m.) – the standard being exceeded 3 times. A significant variation of the levels of heavy metals was observed depending on treatment type. A longer period of deposition of sludge on plots (over 6 months) causes a significant decrease of cadmium level, a decrease in the amount of *Salmonella* bacteria, and total elimination of *Toxocara* eggs. In the majority of cases no significant correlation was observed between the level of heavy metals and biological contaminants. A weak correlation was found between the concentrations of lead and chromium and the presence of the eggs of helminths (ATT), as well as between the concentration of zinc and the presence of *Salmonella* bacteria.

Address for correspondence: Dr Urszula Bożek, Department of Environmental Hygiene, Institute of Agricultural Medicine, Jaczewskiego 2, 20-090 Lublin, Poland.
E-mail: urszula.bozek@op.pl

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INTRODUCTION

The exploitation of sewage treatment plant, is associated with the necessity for proper sludge economy. Sludge produced in the process of treatment must be subject to processing, and then properly utilized, managed or neutralized. One of the forms of sewage sludge management, in Poland and abroad, is its use in agriculture, mainly for the production of fertilizers. With respect to the production of food, the sanitary requirements concerning sewage sludge

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are high and there is a need for constant monitoring [8]. Sanitary evaluation is conducted based on the results of chemical, bacteriological and parasitological studies [12].

Chemical studies consist in the determination of the level of contamination with heavy metals.

The most dangerous heavy metals present in sewage sludge, which are characterised by a high bio-accumulation coefficient, are: chromium, zinc, cadmium, copper, nickel and lead [1]. Heavy metals – without any harm to the environment, cultivated plants and their consumers – may be introduced into soil in precisely defined amounts [2]. The conditions which must be satisfied while using communal sewage sludge are defined by the Polish Regulations of the Ministry of Environment of 1 August 2002 [12].

The biological composition of sludge reflects the state of health of the inhabitants, as well as epidemiological risk in the area from which sewage flows to the treatment plant. Sludge produced in treatment plants are subject to biological or chemical stabilization procedures which considerably reduce the abundance, but which, however, do not totally eliminate pathogenic organisms. The bacterial sanitary indicator for sludge and fertilizers produced from sludge are *Salmonella* bacteria [4, 6]. Eggs of helminths including: *Ascaris lumbricoides*, *Trichuris trichiura*, *Toxocara canis*, and *Toxocara cati*, penetrate into sludge already during the preliminary treatment. They create a special risk because they are capable of surviving in various forms of sludge, and already their small amount may cause pathological symptoms in humans [3, 7, 15]. Soil-transmitted helminths infections constitute a major public health problem in poor and developing countries [14]. The introduction of sludge into the soil environment may mean an inclusion into the biochemical processes of a large chemical and biological load. An important factor are pathogenic organisms which, together with the sludge, may be transferred beyond the area of their direct application and create a hazard for humans and animals [5, 7, 13].

In the literature available, no reports were found concerning the correlation between bacteriological and parasitological agents and levels of heavy metals in sewage sludge sediments. Therefore, studies were undertaken in order to find out whether such a correlation exists.

MATERIAL AND METHODS

Sewage treatment plants examined. The study covered 18 municipal sewage treatment plants from which sludge is agriculturally used. The plants were located in the area of the whole of Poland; however, the majority of samples (80 out of 110) were taken from treatment plants in the Lublin province (Eastern Poland). The numbers of examined samples of sewage sludge from individual plants are specified below in brackets. These were treatment plants located in: Biała Podlaska (4), Białystok (5), Czarnków (10), Krasnystaw (5), Kraśnik (3), Lubartów (10), Lublin (4), Łęczna (5), Łowicz (10), Milejów (10), Parczew (10), Piaski (5), Radzyń (4), Rejowiec (5), Ryki (5), Swarzewo (5), Tomaszów Lubelski (5), and Zamość (5).

Sampling of sewage sludge. A total number of 550 samples of fermented and dehydrated sewage sludge were taken from drying beds. 110 samples of sludge were designated for laboratory studies after the preparation of mean samples. Sludge samples of ca. 0.5 kg were taken from drying beds by the envelope method (4 samples from each corner and 1 in the middle of the bed). Sludge was sampled into sterile containers or clean plastic bags, with the use of disinfected shovels. Each sample included information concerning date and place of sampling, type of sample, and date of sewage discharge. The quality of the sludge produced and duration of its deposition in treatment beds varied from 0.5–10 years, according to the type of sewage treatment plant and technological processes applied.

Due to their volume, composition and characteristics, municipal sewage sludge is subject to processing in the area of the plant by concentration and dehydration, which leads to the reduction of the volume of the sludge through the disposal of water contained within it (thus increasing the contents of dry mass) and stabilisation of its chemical composition, i.e. elimination of its capability for putrefaction and reduction or total elimination of pathogenic organisms. The bacteriological-parasitological studies of sewage sludge covered *Salmonella* bacteria and eggs of helminths.

Bacteriological examination. Identification of *Salmonella* bacteria was conducted based on the Polish Standard [9]. This standard is developed for soil, but is also used for of *Salmonella* bacteria in sewage sludge. *Salmonella* bacteria were identified with the use of bacteriological and serologic methods. The following media were used for primary isolation and differentiation of *Salmonella*: agar SS, Rambach Agar, and bismuth sulfite agar according to Wilson-Blair. Sodium selenite medium was used for propagation of isolates. *Salmonella* isolates were identified with API 20E microtests (bioMérieux, Marcy I'Etoile, France). Serologic identification was carried out with latex test (BIOMEX, Kraków, Poland). Helminthological examination. Eggs of intestinal parasites were detected based on the Polish Standard [10]. The eggs of *Ascaris* spp. and *Trichuris* spp. were detected by the flotation method according to Wasilkowa, while eggs of *Toxocara* spp. – by the floatation method according to Quinn *et al.* [11].

Determination of heavy metals. Chemical studies were aimed at the determination of the level of contamination of sewage sludge with heavy metals: chromium, zinc, cadmium, copper, nickel and lead. The preliminary preparation of samples for chemical examinations consisted in air drying of the sample of sewage sludge, and then homogenisation in an agate mortar. For chemical examinations 1 g of air-dried averaged sample of sludge was weighed. The mineralization of sewage sludge samples was performed with the use of a mixture of concentrated acids (nitric and hydrofluoric) – by microwave method, in the microwave type Milestone MLS 1200 Mega. The level of heavy metals was determined by the method of atomic absorption flame spectrophotometry, with use of Philips Scientific Pye Unicam PU 9280. In addition, the concentration of hydrogen ions was measured by the potentiometric method in a mean sample of sewage sludge suspended in freshly re-distilled water at the ratio of 1:10.

Statistical analysis. The results of studies were subject to statistical analysis with the use of SPSS v. 4 package, by ANOVA, and Student's t-test.

RESULTS AND DISCUSSION

The results of the studies showed that sewage from municipal sewage treatment plants are biologically contaminated, which may be determined in figures by isolating *Salmonella* bacteria, and a conventional ATT index. The ATT index is the sum of 3 types of helminths eggs: *Ascaris* spp., *Toxocara* spp., and *Trichuris* spp., calculated per 1 kg dry mass of sludge. This method of calculation has been imposed by the Polish Regulation by the Minister of the Environment of 1 August 2002 in the matter of municipal sewage sludge [12]. According to this regulation, sludge designed for use in agriculture must not contain *Salmonella* bacteria, nor live eggs of helminths: *Ascaris* spp., *Toxocara* spp., and *Trichuris* spp.

The above-mentioned regulation covers also the allowable amounts of heavy metals (in mg/kg dry mass) in sewage sludge used in agriculture: lead – 500, cadmium – 10, nickel – 100, zinc – 2500, copper – 800, and chromium – 500 [12].

Tables 1–4 present the results of bacteriological, parasitological, and chemical studies of sewage sludge from the 18 treatment plants. *Salmonella* bacteria were isolated from 9 samples of sewage sludge (8.2%) from 7 plants. Helminth eggs were observed in 31 samples of sludge (28.1%) from 13 plants. The most contaminated sludge came from 5 treatment plants (6 samples) – this sludge was contaminated with *Salmonella* bacteria and helminth eggs (Tab. 1). Only sewage sludge from 3 treatment plants may be – without any reservations – designed for agricultural use, because it contained neither *Salmonella* bacteria nor helminth eggs.

Table 1. *Salmonella* bacteria, helminths eggs and heavy metals in sewage sludge samples.

Locality	SE[1]	Biological factors		Heavy metals [ppm]											
		SP[2]		Cr		Zn		Cd		Cu		Ni		Pb	
		Sal[3]	ATT[4]	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Krasnystaw	5	0	2	44.9	23.9	384.5	10.5	4.6	1.3	108.3	10.7	24.6	5.2	83.1	18.5
Biała Podlaska	4	0	0	47.6	26.0	360.3	54.3	3.6↓	0.7	106.9	40.9	34.9	2.8	90.1	6.7
Piaski	5	0	1	29.0	10.0	377.6	18.1	2.5↓	0.9	88.7↑	38.2	19.4↓	3.4	100.7	21.1
Zamość	5	0	3	67.7	11.5	378.2	2.5	4.0↓	0.5	110.6	6.1	43.6↑	6.0	66.3	12.4
Tomaszów Lubelski	5	2	4	42.8↓	3.2	381.9	5.8	2.9↓	0.5	95.4	10.1	26.6	2.2	125.9↑	22.9
Parczew	10	1	0	32.6↑	10.0	396.5↑	13.6	7.2↑	1.7	130.4↑	20.2	26.9↓	4.4	51.2↓	18.4
Lubartów	10	1	1	48.6	17.2	284.9↓	78.5	4.9	1.4	57.4↓	13.6	26.7↓	7.6	77.4	23.7
Ryki	5	0	3	61.6	19.3	358.7	25.9	2.5↓	1.1	87.6↓	30.1	35.6↑	5.2	128.7↑	37.3
Radzyń Podlaski	4	1	1	24.1↓	1.4	354.0	12.9	1.7↓	0.4	113.0	15.7	25.7	0.9	44.2↓	11.0
Kraśnik	3	0	2	51.5	2.3	379.5	11.8	1.5↓	0.8	135.8↑	25.9	36.3	2.2	108.0	6.5
Łęczna	5	0	0	47.8	23.8	329.1	49.1	6.1	1.3	89.3↓	28.4	26.2	8.7	70.3	16.2
Milejów	10	0	5	29.3	5.7	356.7	15.0	6.5↑	1.5	68.0	6.8	20.5↓	2.2	63.4↓	8.7
Łowicz	10	1	1	53.4	6.0	402.3↑	7.2	5.3	0.8	139.9↑	7.6	33.8↑	7.6	51.6↓	10.8
Czarnków	10	0	3	58.5	11.7	361.7	12.8	5.0	0.8	93.1↓	8.3	36.3↑	4.0	64.8↓	14.6
Rejowiec	5	0	0	17.1↓	4.1	375.4	33.1	6.2	0.5	77.8↓	22.2	16.1↓	2.8	57.7↓	11.0
Swarzewo	5	2	0	25.8	2.0	375.9	2.5	8.4↑	0.7	104.2	19.9	23.6↓	2.5	57.6↓	18.0
Białystok	5	0	4	71.9↑	24.1	395.6↑	7.5	4.1	1.2	129.1↑	12.6	26.5	3.7	67.2	7.8
Lublin	4	1	1	80.3↑	3.7	391.8↑	1.1	201.1↑	2.4	165.8↑	3.4	288.7↑	6.5	81.9	6.2
TOTAL	110	9	31	45.7	21.0	367.0	44.1	12.1	36.8	102.7	33.7	37.8	49.4	73.3	28.3
Limit[5]				500		2500		10		800		100		500	

[1] SE = samples examined; [2] SP = samples positive; [3] Sal = *Salmonella* spp.; [4] ATT = *Ascaris* spp. and/or *Trichuris* spp. and/or *Toxocara* spp.; [5] Limit = limit in [ppm] established for heavy metals in sewage sludge according to [12]; ↓, ↑ = means established by one-way ANOVA as stastistically lower (↓) or higher (↑) than the rest of localities ($p<0.05$); grey background for values exceeding limits.

Table 2. *Salmonella* bacteria, helminths eggs and heavy metals concentration in sewage sludge samples depending on treatment type.

Treatment	N	*Salmonella* positive samples	Helminths eggs positive samples				Heavy metals (mean ± SD) [ppm]					
			Ascaris	*Trichuris*	*Toxocara*	ATT	Cr*	Zn*	Cd*	Cu*	Ni*	Pb*
Fermentation	45	2 (4.4%)	11 (24.4%)	4 (8.9%)	4 (8.9%)	14 (31.1%)	52±22	381±22	22.5±56	114±33	53±74	67±18
Liming	7	1 (14.3%)	2 (28.7%)	0	0	2 (28.7%)	31±18	305±50	3.1±0.9	72±33	25±11	57±13
Other method	58	6 (10.3%)	11 (19.0%)	7 (12.1%)	2 (3.4%)	15 (25.9%)	43±19	364±49	5.0±2.3	98±31	27±7	80±34
Total	110	9 (8.2%)	24 (21.8%)	11 (10.0%)	6 (5.4%)	31 (28.1%)	46±21	367±44	12.1±37	103±34	38±49	73±28

* statistically significant differences between treatments as assessed by ANOVA ($p<0.05$)

Table 3. *Salmonella* bacteria, helminths eggs and heavy metals concentration in sewage sludge samples depending on treatment time.

Time	N	*Salmonella* positive samples	Helminths eggs positive samples				Heavy metals (mean ± SD) [ppm]					
			Ascaris	*Trichuris*	*Toxocara*	ATT	Cr	Zn	Cd*	Cu	Ni	Pb**
Below 6 mo.	88	8 (9.1%)	20 (22.7%)	7 (8.0%)	6 (6.8%)	25 (28.4%)	44±21	369±38	14±41	102±32	40±55	67±24
Above 6 mo.	22	1 (4.5%)	4 (18.2%)	4 (18.2%)	0	6 (27.3%)	51±21	359±63	4.5±1.5	104±40	29±8	97±33
Total	110	9 (8.2%)	24 (21.8%)	11 (10.0%)	6 (5.4%)	31 (28.1%)	46±21	367±44	12.1±37	103±34	38±49	73±28

*, ** statistically significant differences between treatment times as assessed by ANOVA (* $p<0.05$; ** $p<0.01$)

Tables 2 and 3 show that *Ascaris* spp. eggs were most frequently found (24 samples), followed by *Trichuris* spp. eggs (11 samples), and *Toxocara* spp. eggs (6 samples).

Very high levels of heavy metals were observed in the samples of sewage sludge from the Treatment Plant in Lublin (Tab. 1). This concerns especially the level of cadmium (201.1 mg/kg d.m.) – exceeding by 20 times the most rigorous allowable contents of this metal established for sludge used in agriculture, and the level of nickel (288.7 mg/kg d.m.) – 3 times exceeding this standard. Individual places were compared by means of ANOVA test, with respect to the differences in the levels of heavy metals with the threshold of $p < 0.05$. The levels of Cr, Cd, Cu, and Ni in sewage sludge samples from the Treatment Plant in Lublin were significantly the highest (Tab. 1). Due to such considerable levels of the above-mentioned metals and the presence of *Salmonella* bacteria and ATT eggs – this sludge is not used in agriculture, but consigned to the municipal dumping ground.

Table 4. Comparison of heavy metals concentration in sewage sludge samples with the presence of *Salmonella* and ATT index.

Metal [ppm]	*Salmonella* spp.		ATT	
	negative	positive	negative	positive
	N=101	N=9	N=79	N=31
Cr	46.3±21.2	38.1±19.9	42.6±19.9	53.7±22.5*
Zn	365±46	384±16*	363±50	377±20
Cd	10.7±33.5	27.5±65.7	12.6±37.6	10.6±35.7
Cu	101±34	121±27	102±35	104±30
Ni	36.1±45.3	57.2±86.3	37.5±51.0	38.6±46.8
Pb	73.0±27.3	76.6±41.1	68.6±24.6	85.3±34.0*

*statistically significant difference assessed by Student's t-test ($p<0.05$)

Sludge from the remaining treatment plants showed low levels of heavy metals, which would qualify it for agricultural use. However, the presence of the eggs of intestinal parasites does not allow for agricultural use of the sludge.

Table 2 presents the levels of heavy metals and the presence of *Salmonella* and eggs of helminths depending on type of treatment (fermentation, liming and other methods). The highest values for Cr, Zn, Cd, Cu and Ni were noted at fermentation treatment, whereas the highest level of Pb was observed at ”other methods”. The lowest levels of heavy metals were noted at liming treatment. No method proved to be efficient for the elimination of bacteria and parasites eggs (ATT).

In “younger” sludge, deposited for a period up to 6 months, the levels of zinc, cadmium, and nickel were higher (Tab. 3). A statistically significant, higher level was observed with respect to cadmium (14 mg/kg d.m.). In “older” sludge, a higher significantly level of lead was found (97 mg/kg d.m.). A longer period of deposition of sludge on plots causes a decrease in the amount of *Salmonella* bacteria and total elimination of eggs *Toxocara*.

Table 4 shows that there is a weak correlation between the occurrence of *Salmonella* bacteria, the eggs of helminths, and the level of heavy metals. In sludge where *Salmonella* bacteria and helminths eggs were found, the levels of heavy metals were usually higher. The analysis by Student's t-test showed that in samples with a positive ATT index, the levels of chromium and lead were significantly higher ($p < 0.05$). The levels of 5 out of 6 examined metals were higher in the samples positive for *Salmonella*, and this relationship was significant in the case of zinc (Tab. 4). In the presence of *Salmonella* bacteria higher levels of zinc were noted only in a few samples.

CONCLUSIONS

Low levels of heavy metals not exceeding the allowable values were recorded in all but one examined sewage sludge deposition plants.

The prevalence of *Salmonella* spp. and the eggs of helminths were not high, 8.2% and 28.1%, respectively.

A significant variation of the levels of heavy metals was observed depending on treatment type.

A longer period of deposition of sludge on plots (over 6 months) causes a significant decrease of the cadmium level, a decrease in the amount of *Salmonella* bacteria, and total elimination of *Toxocara* eggs.

A weak correlation was found between the concentrations of lead and chromium and the presence of the eggs of helminths (ATT), as well as between the concentration of zinc and the presence of *Salmonella* bacteria.

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