ACTA UNIVERSITATIS AGRICULTURAE ET SILVICULTURAE MENDELIANAE BRUNENSIS

Volume LX 28 Number 5, 2012

INFLUENCE OF HEAVY METALS OCCURRENCE ON RESPIRATORY ACTIVITY OF MICROORGANISMS IN THE COMPOST

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Received: April 23, 2012

Abstract

VÍTĚZOVÁ, M., VÍTĚZ, T.: *Influence of heavy metals occurrence on respiratory activity of microorganisms in the compost.* Acta univ. agric. et silvic. Mendel. Brun., 2012, LX, No. 5, pp. 247–254

This work deals with the influence of selected heavy metals, copper, nickel, cobalt, zinc and cadmium on respiratory activity of microorganisms during the composting process. We focused on comparing the respiratory activity of microorganisms in the compost after composting process in the presence of different concentrations of above mentioned metals and the measured results were confronted with the valid Czech standard for industrial composts. The results show that the high inhibitory effect at low concentrations, about 1 mg/dm^3, has cobalt, for which the limit concentration is no set by Czech standard. Other heavy metals exhibit an inhibitory effect at concentrations higher than maximum allowable concentration, which is set in Czech standard for compost class I and II, with regard to their application to agricultural land.

municipal green solid waste, compost, heavy metals, microbial respiration

Composting represents the most common manner of aerobic processing of organic waste. It is a biotechnological process based on the ability of microorganisms and invertebrates to transform organic substances featuring the nature of waste material into utilisable products. Only few scientific papers dealing with the composition of microbial communities in compost. Conventional cultivation methods allow us to quantitatively assess the presence of selected groups of microorganisms (Lott Fischer *et al.*, 2001). Information about changes in the composition of micro flora in the composting process we can get by experiments based on the determination of PLFA (Phospholipid Fatty Acids) in cell walls of microorganisms that serve as markers of different microbial species (Szostková *et al.*, 2005) or modern molecular biological methods (Lott Fischer *et al.*, 2001). The growth of microorganisms in the compost is accompanied by their active metabolism which allows decomposition processes. Compost is, historically, one of the common natural fertilizers used for growing of plants as well as improvement of the quality of soil. One of the most hazardous groups polluting all components of the environment is due to the high toxicity, respectively genotoxicity, heavy metals (Holoubek, 2000). Microorganisms are usually the first organisms that come into contact with heavy metals, which may contaminate the environment. Influence of heavy metals is usually studied in pure microbial cultures. More important for practice has study of natural ecosystems, such as compost micro flora.

In the soil contaminated by heavy metals the ratio between eukaryotes and prokaryotes increases, soil mineralization potential declines and an advance in the bacterial community in behalf of resistant species can be found (Steritt and Lester, 1980). Bioaccumulation of heavy metals in soil fertilized with compost which includes concentrations of heavy metals exceeding the limit concentration (CSN 465735:1991) causes the entry of heavy metals into the food chain and risk exposure on human health.

MATERIAL AND METHODS

Samples of analyzed material were collected in the composting plant located near the city of Slavkov u Brna, which is in the South Moravian Region of the Czech Republic. The composting plant was erected and equipped in 2009, mainly on the basis of subsidies granted by the EU (Cohesion Fund), State Environmental Fund of the Czech Republic and Environmental Fund of the South Moravian Region. The composting plant processes, on average, 10.000–15.000 kilograms of waste per week. The waste originates mainly from maintenance of municipal vegetation and comprises also bio waste transported from residents of Slavkov u Brna. Sample No. 1 was collected from fresh bio waste, sample No. 2 was from waste composted in banded piles for the time period of 14 days, sample No. 3 was composted for 63 days and the last sample No. 4 represented fresh compost (75 days), more specifically its sub-sieve fraction prepared for distribution as a top quality fertilizer suitable for gardening or soil reclamation purposes. Total solid content in compost samples were determined by use of electric muffle furnace LMH 07/12 which is designed to measure incineration processes, drying, degradation, re-heating, thermal treatments etc. Analytical laboratory balances Radwag AS 220/X has been used for precise weighing, readability to 0.0001 g.

1.1 The compost sample preparation for microbial respiration determination

The suspension has been prepared by adding of compost sample corresponding to 100 g of its dry mass into 1 000 ml of sterile distilled water for 24 hours in a shaker (Heidolph Promax 1020, 130 rpm). After filtration, through filter paper of porosity 10 μm, the suspension has been used for following microbiological tests.

1.2 The determination of heavy metals concentration

The concentration of cobalt, copper, cadmium, nickel and zinc in compost samples No. 1, 2, 3 and 4 have been determined by flame atomic absorption spectrometry technique (according to CSN 465735:1991). The concentration of the determined elements has been determined from a calibration curve. The values have been recalculated per kilogram of dry weight of each sample of compost (Tab. I).

1.3 The determination of microbial respiration

Microbial respiration, expressed by biochemical oxygen demand (BOD) has been measured on the OxiTop Control device (WTW, Germany). During measuring of microbial respiration, selected chlorides of heavy metals ($NiCl_2{\cdot}6H_2O$ p.a., $CoCl_2{\cdot}6H_2O$ p.a., $ZnCl_2$ p.a., $CuCl_2$ p.a. and $CdCl_2$ p.a.) have been added to the solution to the required total concentration. Additions have been converted to an exact total concentration of the ions of heavy metals, including concentrations of these heavy metals found in the original sample of compost No. 1. Solid part of filtered aqueous extract of this sample have been determined. Into glass bottles, intended for measuring at the device OxiTop, 20.7 ml of an aqueous extract of compost have been trasfered (sample No. 1), 1 ml of 10% sucrose and 1 ml of the heavy metal solution of appropriate concentration. Each bottle contained a different concentration of heavy metal. One bottle has been used as a control, without further addition of heavy metal. It contained 20.7 ml of an aqueous extract of sample No. 1, 1 ml of 10% sucrose and 1 ml of sterile distilled water. To each bottle sterile teflon stirrer with a metal core and rubber cap with pips of NaOH have been added, subsequently the bottles have been closed by manometrical measuring heads. Bottles have been placed on a magnetic stirrer (stirring speed of 390 rpm) to thermo box Q-cell 140/40 (Poland), where they have been tempered for 60 minutes at 26 °C. After tempering the heads have been restarted and the measuring has been started for at least 140 hours. Data from display of OxiTop device have been multiplied by multiplier factor of 100, which corresponds to 22.7 ml volume used (according to the manufacturer's instructions).

RESULTS AND DISCUSSION

Samples of compost contained different amounts of water. The determined content of dry solids ranged, as regards individual samples, between 45.8 % and 64 % (Tab. I). It is well known that compost can be polluted with heavy metals. Many scientifical works clarify the leaching or residue of heavy metals when compost was added to soil (Chen *et al.*, 2010). Concentration of cobalt, copper, cadmium, nickel and zinc in samples after mineralization have been determined (Tab. I). The results show that in neither case has not been exceeded the limit concentrations of heavy metals set in the standard CSN 465735:1991 for the input material as well as for the compost before application to agricultural land according to class I or II (Tab. II).

I: *Total solids and heavy metal content in samples*

sample label	total solids [%]	Ni mg/kg of solids	Co mg/kg of solids	Zn mg/kg of solids	Cu mg/kg of solids	Cd mg/kg of solids
input material	45.8	54.9	1,5	11.3	35.0	< 0.2
14 days	51.2	64.2	< 1	21.4	33.2	< 0.2
63 days	56.3	42.3	< 1	18.6	30.6	< 0.2
75 days	64.0	37.8	< 1	7.0	35.0	< 0.2

II: *The limit concentrations of heavy metals in the compost CSN 465735:1991*

	class I mg/kg of solids	class II mg/kg of solids
Zn	300	600
Co	no limit	no limit
Ni	50	70
Cu	100	400
Cd	2	4

The accurate determination of heavy metals availability is becoming more important, as risk assessments and remediation efforts confirm that total heavy metals concentrations are not the best predictors of metals availability. Various extractants are used for estimating the fraction of heavy metals in composts that is available to plants (Chen *et al.*, 2010), but little work has been done to determine the availability of these elements to microbes in compost processes. The oxygen consumption of sucrose oxidation of microbial cells from composted green waste in the presence of different concentrations of heavy metals has been monitored (Fig. 1–5). Oxygen consumption indicates the respiratory activity of present microorganisms, and thus of their viability. The values of oxygen consumption recorded in different time intervals have been related per gram of dry matter in the measured sample.

2.1 The influence of copper on the respiration of microbial communities in compost

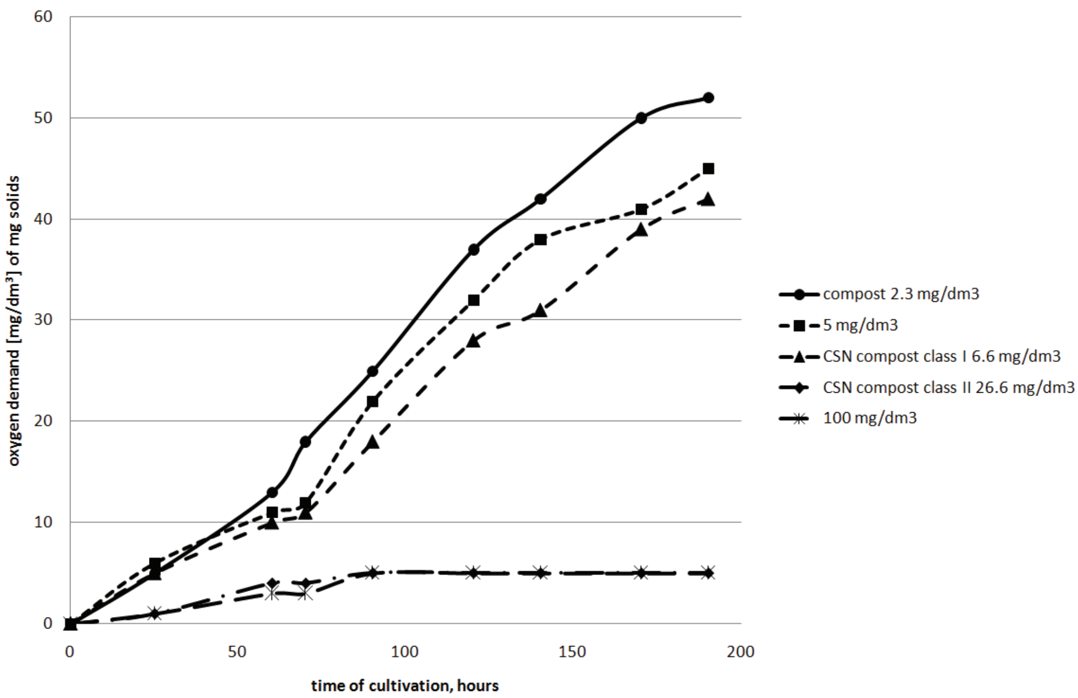
Effect of copper on the respiration of cells has been observed in the concentration ranged from 2.3 mg/dm^3 to 100 mg/dm^3. Pattern of oxygen consumption in the samples and the control is shown in Fig. 1.

The course of respiratory curves showed that the respiration of microorganisms at the lowest concentration of copper used (2.3 mg/dm^3, control sample No. 1) is not significantly toxic to microorganisms (Fig. 1). Authors Zhenrong and Tan (1999) stress the importance of copper in small concentrations as essential trace element in the nutrition of microorganisms. Copper at concentrations of 26.6 mg/dm^3 or higher strongly inhibits respiration of microbial cells. BOD decreased in these samples after 190 hours at 9.6% of the control sample.

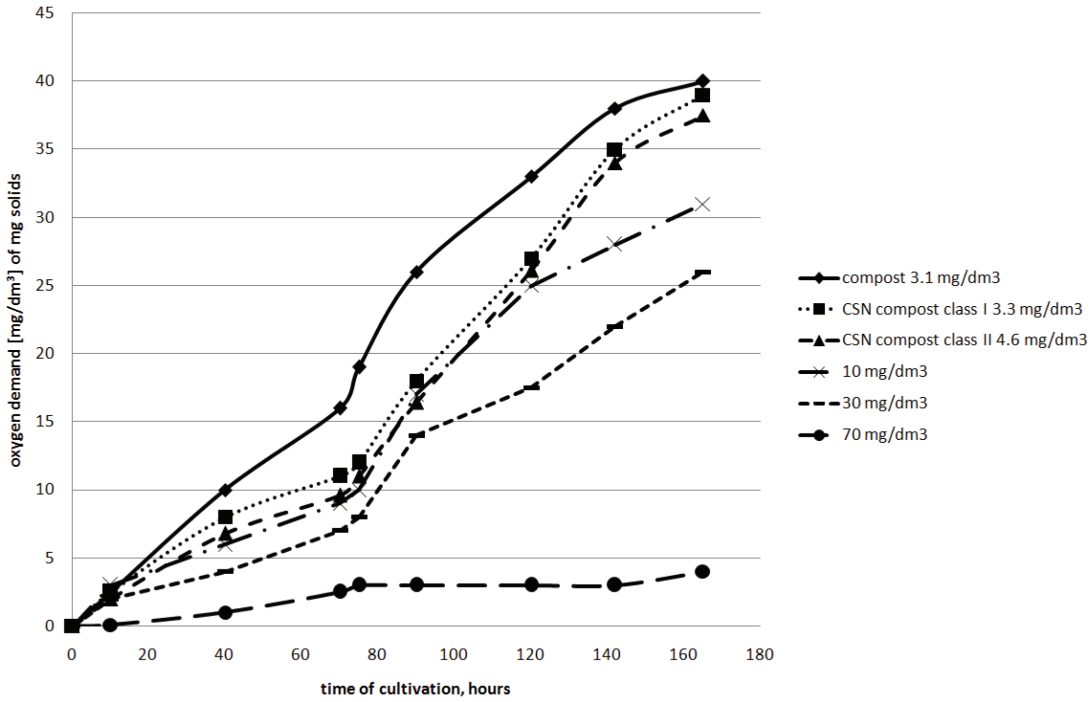
2.2 The influence of nickel on the respiration of microbial communities in compost

Effect of nickel on the respiration of cells has been observed in the concentration ranged from 3.1 mg/dm^3 to 70 mg/dm^3. Pattern of oxygen consumption in the samples and the control is shown in Fig. 2.

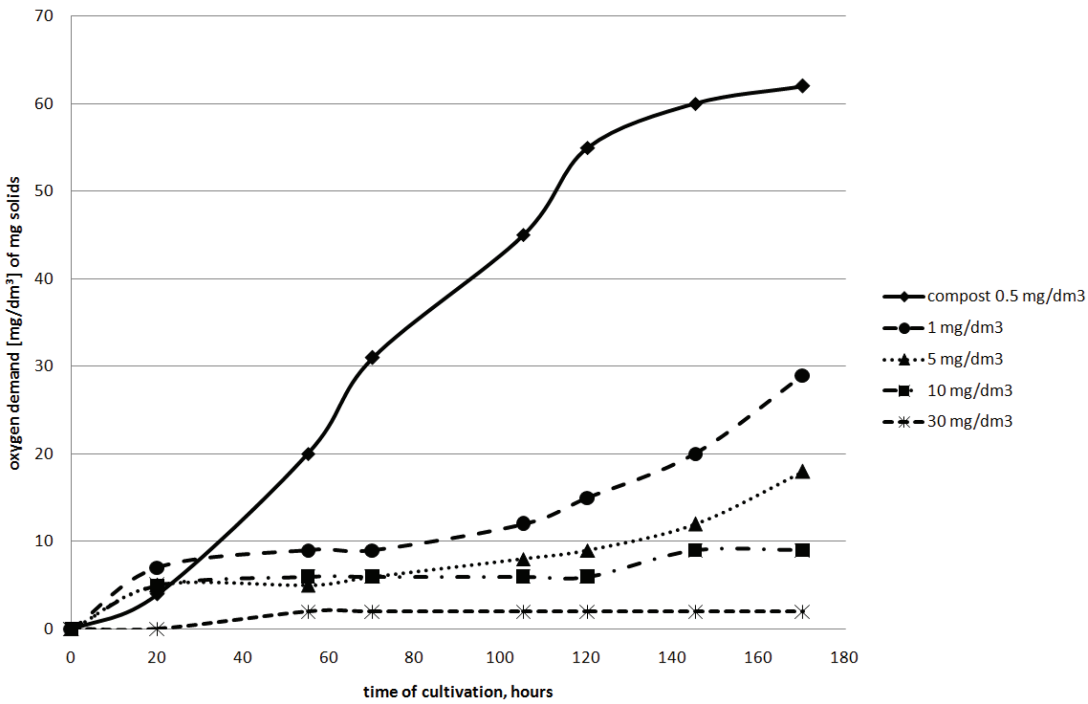
The figure clearly shows that the Ni^{2+} inhibits cell respiration in samples with concentration of 10 mg/dm^3. In these samples, cell respiration reached BOD values approximately 77% of the control sample. Nickel at a concentration of 70 mg/dm^3 strongly inhibits respiration of microbial cells. BOD in these samples decreased after 165 hours at 10% of the control sample.



1: *Effect of different concentrations of copper on respiratory activity of microorganisms in the compost*



2: *Effect of different concentrations of nickel on respiratory activity of microorganisms in the compost*



3: *Effect of different concentrations of cobalt on respiratory activity of microorganisms in the compost*

2.3 The influence of cobalt on the respiration of microbial communities in compost

In this experiment, cell respiration has been monitored in the presence of Co^{2+} at concentrations ranged from 0.5 mg/dm³ to 30 mg/dm³ (Fig. 3).

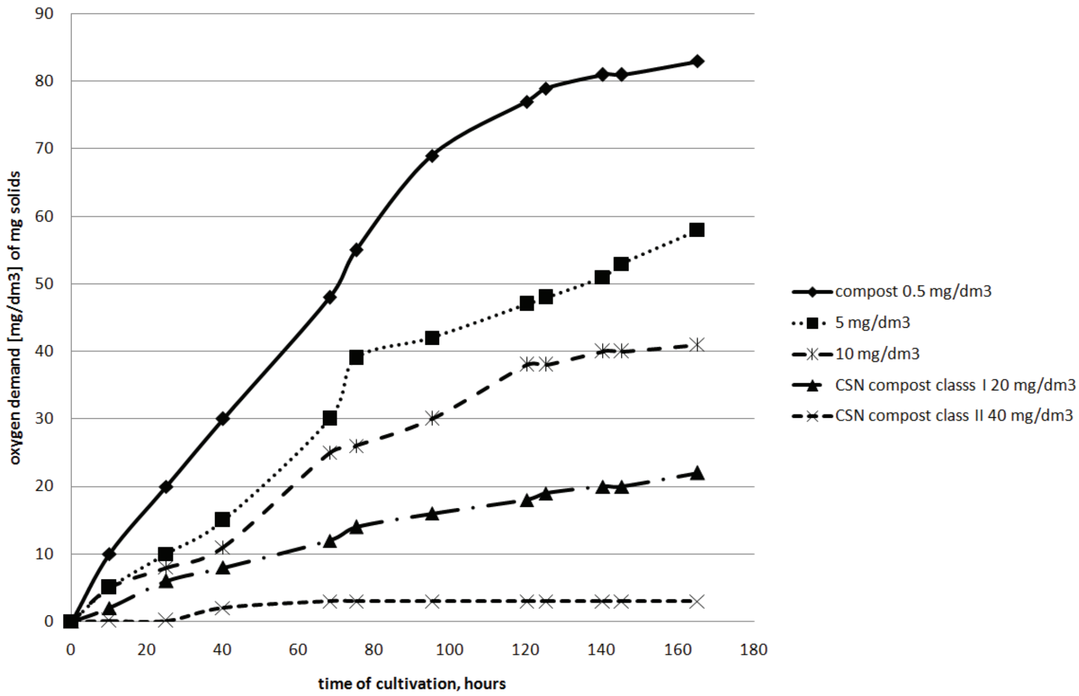
Reduction of oxygen consumption for the oxidation of sucrose expressed by BOD value was reflected not only after the application of higher concentrations of heavy metal, but it has been already observed in the sample, which contained 1 mg/dm³ of cobalt. The value of BOD reached here, compared to control, only 46.7 % (Fig. 3). The strong inhibition of the respiratory activity of cells, however, occurs in samples containing 30 mg/dm³

Co^{2+}, where the value of BOD is 2 mg/dm³, which represent 3.2 % of the control sample.

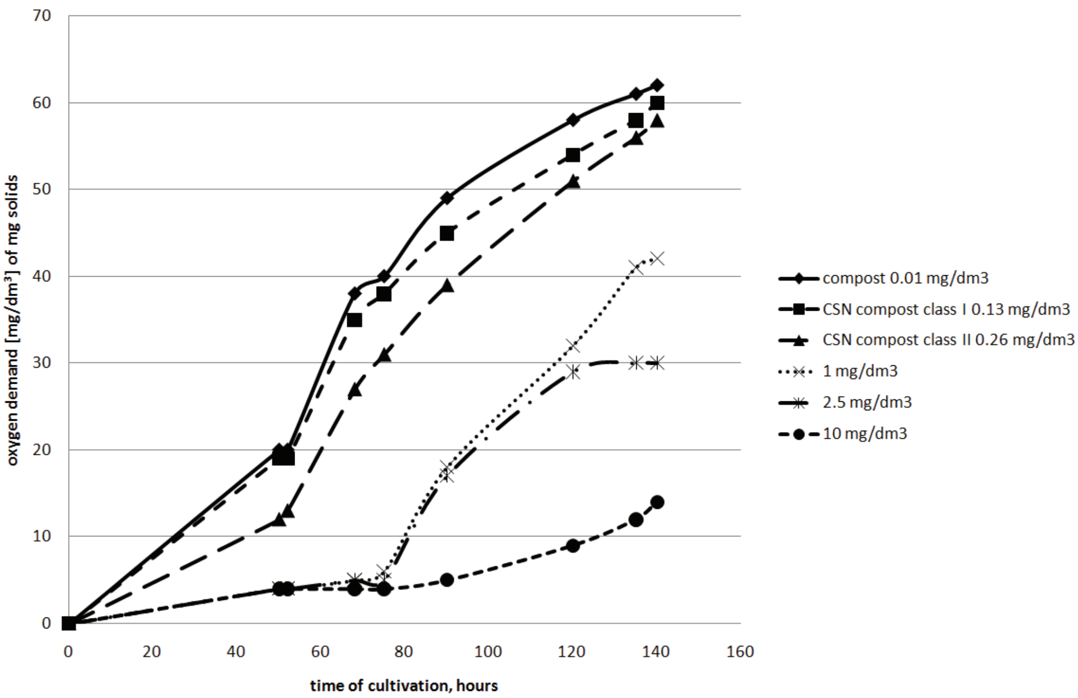
2.4 The influence of zinc on the respiration of microbial communities in compost

Effects of zinc on respiratory activity of cells have been observed in the concentration ranged of 0.5 mg/dm³ to 40 mg/dm³. Respiration of cells has been recorded for 165 hours (Fig. 4).

From the measured values is evident that when the highest concentration of 40 mg/dm³ of Zn^{2+} have been applied a significant reduction in BOD to 3.6 % of the value of the control sample of composted material have been reached (Fig. 4). The sample, which contained Zn^{2+} at a concentration of 20 mg/



4: *Effect of different concentrations of zinc on respiratory activity of microorganisms in the compost*



5: *Effect of different concentrations of cadmium on respiratory activity of microorganisms in the compost*

dm^3 BOD reached 26.5% consumption of oxygen compared to control cells. Zhenrong and Tan (1999) marked zinc as the essential metal for a variety of microorganisms. Tolerant organisms are apparently able to resist lower concentrations of zinc by sorption process on the cell surface (Hassen *et al.*, 1998).

2.5 The influence of cadmium on the respiration of microbial communities in compost

Effects of cadmium on respiratory activity of cells have been observed in the concentrations ranged of 0.01 mg/dm^3 to 10 mg/dm^3. The control sample of composted material contained less than 0.2 mg of Cd^{2+} per kg of dry matter. Respiration of cells has been recorded for 140 hours (Fig. 5).

Cd^{2+} inhibited microbial respiration already at a concentration of 1 mg/dm^3, when reduced the BOD on 67.7% of control. The highest tested concentration of this metal, 10 mg/dm^3, decreased BOD value by 77.4% compared with the control. Cadmium is physiologically widely regarded as a foreign element to the cells, because it is no serve any function in the cell metabolism and according to the work of Knight *et al.* (1997) do not shows positive effect of any concentration on soil microorganisms. Its influence is always negative, which also confirmed Smith and Giller (1992). Hassen *et al.* (1998) tested the effect of cadmium on the cells of bacterial strain *Pseudomonas aeruginosa* and concluded that the value of the minimum inhibitory concentration for this heavy metal as compared with values for other heavy metals is very low and corresponds to a concentration of 1.5 mM Cd^{2+}. They also demonstrated that cadmium inhibits the growth of *P. aeruginosa* cells and prolongs their lag-phase already at concentration of 0.5 mg/dm^3 Cd^{2+}. Some microbial cells are able to adsorb heavy metal to its surface, or eliminate the toxic effect with amplification of the cell wall. The role of cell wall structure in the process of protecting bacterial cells against the effects of cadmium, as well as other heavy metals also confirmed Baudo and Block (1990). In certain concentrations, however, the heavy metal can penetrate through these defence mechanisms in the cell and cause damage that can result loss of cell viability or the disruption of metabolic activity. According to Renella *et al.* (2002) have separately applied heavy metal lesser inhibitory effects on respiration of soil microorganisms than mixtures of metals in various combinations.

CONCLUSION

In our work, we analyzed the contents of selected heavy metals in samples of compost originating from composting facility Slavkov u Brna. In these samples, from different stages of the composting process, has been no exceeding the limits concentrations of selected heavy metals set in standard CSN 465735:1991. The influence of zinc, copper, nickel, cobalt and cadmium to microorganisms involved in composting has been studied in terms of volume of their respiratory activity. In the presence of excess concentrations of heavy metals there was a strong inhibition of microbial respiration. From this perspective seems to be above the limit concentration of selected heavy metals such as purposefully selected. Effects of cobalt could not be compared with the standard, since its maximum permissible concentration of this standard is not established. Even its very low concentration (1 mg/dm^3) acts on toxic microorganisms in the compost, where oxygen consumption was compared with the control decreased to 46.7%. For other metals a similar reduction occurs when cell respiration at higher concentrations.

SUMMARY

One of the major environmental pollutants are heavy metals. Their impact on man and its health is essential. There are many sources from which heavy metals can get into the food chain. One of it is agriculture, where the compost, which comes from the processing of biodegradable waste from cities and villages, is very often used. In our work we focused on qualitative analysis of the compost in which we monitored the content of heavy metals. The measured values were then confronted with the values specified in CSN 465735:1991 for industrial composts. The performed measurements show that the analyzed compost met the requirements of CSN 465735:1991 for heavy metals content. In addition we evaluated if the limit concentrations of heavy metals in the standard is well chosen and so we're adding to compost samples heavy metals at concentrations specified in the standard, but in higher, eventually in lower concentrations and the respiratory activity of microorganisms have been monitored. We concluded that the limit concentrations of heavy metals in the standard are chosen optimally. Only the cobalt would be appropriate to consider introducing a limit concentration are not set yet, because cobalt at low concentrations about 1 mg/dm^3 inhibits the composting process.

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