



## ORIGINAL PAPERS

## PRESENCE OF PLANT HORMONES IN COMPOSTS MADE FROM ORGANIC FRACTION OF MUNICIPAL SOLID WASTE\*

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## ABSTRACT

Composting is a process of the biological decomposition of organic matter under aerobic conditions. Composts made from waste may still contain other substances that influence plant growth and development, such as vitamins or plant growth substances (plant hormones). Application of products containing phytohormones has an effect on numerous physiological processes in plants. Among observable results are: improved condition of the root system, increased absorption of nutrients, improved stress and disease resistance and delayed aging. The purpose of the research was to analyse the occurrence of plant hormones in mature composts made from selectively collected organic fraction of household municipal solid waste. Solid waste processing was carried out in a composting plant located at the Municipal Waste Management Plant in Żywiec (Silesian Province, Żywiecki District, Poland). The composting plant uses a Herhor bioreactor. Organic waste constituted 70% of the mixture, with the remaining 30% composed of structural material. Regarding the varying amounts of green waste (mainly grass clippings), the composts were divided into two groups: early spring composts and late spring composts. Four out of the five analysed substances (from the auxin and cytokinin groups) were found in composts made from waste collected in early spring. Composts made from waste collected from May to June contained mostly cytokinins and gibberellic acid. Total concentration of plant hormones in the composts was related to the content of plant waste in processed material.

**Keywords:** waste composting, plant growth substances, indole-3-acetic acid, phenylacetic acid, gibberellic acid, kinetin, 6-benzylaminopurine.

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## INTRODUCTION

Composting is a process of the biological decomposition of organic matter under aerobic conditions. Compost made from the organic fraction of municipal solid waste is increasingly used in agriculture for improving soil quality. It is estimated that 32% of compost produced in Europe is obtained from organic waste, 9% from mixed waste, and the remaining part originates mainly from green waste and sewage sludge. Nearly 50% of compost produced in Europe is used in agriculture (CESARO et al. 2015).

Proper composting and the quality of compost depend on the material which is used and the conditions in which the process takes place. Beside the organic fraction of municipal solid waste, good composting materials include: waste from green areas, sludge from municipal wastewater treatment plants, waste from animal husbandry, sugar, tobacco, fats and oils industry, as well as waste containing cellulose, straw and even biomass ashes (BERNAL et al. 1998, RAJ, ANTIL 2011, ESTEVEZ-SCHWARZ et al. 2012, GONDEK et al. 2014).

It is generally held that waste compost can be used to improve soil's structure and macro- and microelement content. A good supply of macroelements can be found in selectively collected kitchen waste or sewage sludge, the latter providing also a number of microelements (IŻEWSKA 2009, HIMANEN, HÄNNINEN 2011, IŻEWSKA et al. 2013, BOWSZYS et al. 2015). Compost mass can be enriched with substances stimulating the activity of microorganisms, thereby affecting the pace of the process, e.g. an addition of urea results in the decreasing of microbiological activity, while starch and oil may increase it (GONDEK et al. 2014).

Applying compost produced from municipal solid waste may reduce the harmful effect of soil salinity on plant growth and development, improve production of biomass and restrain the intake of heavy metals from soil (LAKHDAR et al. 2008). Composts made from waste constitute an alternative to traditional substrates, e.g. peat moss (DOBROWOLSKA, JANICKA 2014). However, fertilizing by means of compost may lead to soil contamination with heavy metals or polycyclic aromatic hydrocarbons, to name a few. Therefore, it is essential to keep regular control of the quality of used waste (IŻEWSKA 2009, SADEJ, NAMIOTKO 2010, DOBROWOLSKA, JANICKA 2014).

Composts made from waste may still contain other substances that influence plant growth and development, such as vitamins or plant growth substances (plant hormones). The content of these compounds is rather seldom studied, nevertheless their occurrence deserves attention, since composts containing such substances may replace plant growth stimulators used in agriculture.

Plant hormones (phytohormones) are small molecules which occur in low concentrations and influence plant growth and development. Application of products containing phytohormones has an effect on numerous physiological processes in plants. Among observable results are: improved condition of the

root system, increased absorption of nutrients, improved stress and disease resistance and delayed aging. The main groups of plant hormones regulating plant growth include: auxins (indole-3-acetic acid, indole-3-butyric acid, phenylacetic acid), cytokinins (kinetin, *trans*-zeatin, isopentenyladenine, 6-benzylaminopurine) and gibberellins (e.g. gibberellic acid) (DE BRUYNE et al. 2014, MIRANSARI, SMITH 2014).

Auxins influence *inter alia* the creation of root primordia, cellular elongation through the loosening of cell wall structure, as well as cell expansion in diameter (annual rings). Gibberellins usually act synergically. Together with other hormones, they promote the elongation of stolons (e.g. of potatoes), whose proper growth requires the involvement of auxins. Cytokinins participate in metabolic processes. Their effect is much stronger than that of auxins and gibberellins; in some processes they act antagonistically to auxins. They influence both cell regeneration and volume through accumulation of some metabolites and regulation of hydration reactions. Many publications address the role of various plant hormones, mostly by analysing their involvement in plant growth and development processes (CASSINA et al. 2012, YANG et al. 2013, OUNI et al. 2014). A body of research has also been carried out into the application of plant hormones in restoration of contaminated soils. Foliar fertilization of plants grown in such soils with a preparation containing cytokinins brought about an increase in mercury absorption and in biomass production, thus improving the efficiency of phytoextraction (CASSINA et al. 2012).

Research into plant hormone levels has showed variations related to the impact of fertilizing with compost produced from organic waste. Given the fact that hormone levels may either increase or decrease, it is recommended to apply moderate compost doses (OUNI et al. 2014). Also vermicomposting, which entails interactions between microorganisms and earthworms, encourages the occurrence of phytohormones, e.g. indole-3-acetic acid (ARANCON et al. 2006). Analyses of biochemical properties of compost extracts (compost tea) revealed the occurrence of phytohormones from the gibberellin group, which contributed significantly to plant growth (PANT et al. 2012).

Composting appears to be a good method of utilizing plants which are undesired in a given area. Examples include giant salvinia (*Salvinia molesta*), an invasive plant growing in many tropical countries, which, once removed from a waterbody, can be composted. Research showed the presence of 16 compounds from the cytokinin group and indole-3-acetic acid (IAA), from the auxin group (ARTHUR et al. 2007).

Analysis of plant hormone presence is frequently carried out with high pressure liquid chromatography (HPLC). This technique allows simultaneous identification of a large number of components, as well as a relatively quick and highly accurate analysis, thereby proving useful in identifying phytohormones in plant matter and composts (ARTHUR et al. 2007, CUI et al. 2015, PANT et al. 2012).

## MATERIAL AND METHODS

The paper presents preliminary results of research into the occurrence of plant hormones in composts made from the organic fraction of municipal solid waste. The analyses were performed on samples of mature compost. The composted waste selectively collected from household waste (Figure 1).



Fig. 1. Waste used in composting (KLIMAS 2014)

The structural material, which is essential for the process of composting, consisted of clippings of tree and shrub branches (green waste). The research involved the preparation of experimental compost mixture, a two-stage composting process, as well as quality and quantity analyses of selected plant hormones (PIECYK, ANDERS 2012).

Solid waste processing was carried out in a composting plant located at the Municipal Waste Management Plant in Żywiec (Silesian Province, Żywiecki District, Poland). The composting plant uses a Herhor bioreactor fitted with a feedstock aeration and humidification system. It processes such waste as food scraps (of animal and plant origin), shredded tree and shrub branches, grass clippings, fallen tree and shrub leaves and other organic wastes (e.g. weeds and waste from wholesale fruit and vegetable outlets).

Preparation of the composting mixtures involved shredding the organic waste (Figure 2), adding the structural material and maintaining the humidity level at 60%. Organic waste constituted 70% of the mixture, while the structural material made up the remaining 30%. The research included 10 cycles of two-stage composting. Regarding the varying amounts of green waste (mainly grass clippings), the composts were divided into two groups:

- early spring composts, made from waste collected from March to April, whose major part was household organic waste (60%), and waste from urban greenery constituted 10%;
- late spring composts, made from waste collected from May to June,



Fig. 2. Shredding of the organic waste (KLIMAS 2014)

with the dominance of waste from urban greenery (50%) and a 20% addition of household organic waste.

The first stage of composting involved intensive decomposition of organic substances in the computer controlled Herhof reactor (Figure 3). The process



Fig. 3. Interior of the Herhof bioreactor (KLIMAS 2014)

of intensive composting included four phases: initial (temp. rise up to 40°C, duration 2 days), decomposition (temp. 40-45°C, duration *ca* 3 days), hygienization (temp. up to 60°C, duration *ca*. 4 days) and cooling (temp. drop below 40°C) (ANDERS, NOWAK 2008). The bioreactor was monitored with regard to the volume of air supplied to the composting mixture, the temperature and



carbon dioxide content in the air removed from the bioreactor. The basic parameters of the composting process in the bioreactor are presented in Table 1.

After seven days, the bioreactor was emptied. Maturation of pre-composted mixtures proceeded in compost piles (Figure 4). During the process,

Table 1

Basic parameters of composting process in a bioreactor

Sample	Air flow (dm <sup>3</sup> min <sup>-1</sup> kg <sup>-1</sup> )	Maximum CO <sub>2</sub> content (%)	Duration of (h)			Maximum temperature of the process (°C)
			temperature increase phase	hygienization phase	cooling phase	
Early spring composts						
Compost 1	1.40	4.7	53	75	40	66
Compost 2	1.36	4.9	74	75	19	70
Compost 3	1.37	4.7	74	80	14	70
Compost 4	1.42	4.7	45	90	33	68
Range	1.36 - 1.42	4.7 - 4.9	45 - 74	75 - 90	13 - 40	66 - 70
Late spring composts						
Compost 5	1.33	4.9	57	69	42	68
Compost 6	1.48	4.7	33	107	28	68
Compost 7	1.45	4.7	48	76	44	69
Compost 8	1.44	4.7	25	110	33	69
Compost 9	1.37	4.9	38	95	35	68
Compost 10	1.39	4.7	34	91	43	68
Range	1.33 - 1.48	4.7 - 4.9	25 - 57	69 - 110	28 - 44	68 - 69

the mass was controlled for its temperature and humidity, and it was also turned and watered. The operations were carried out until the temperatures were stabilized, which signals the completion of the composting process. The basic parameters characterizing maturation in piles are presented in Table 2. Each compost batch was turned during maturation 8 to 12 times.

As a result of the two-stage composting process, 10 batches of mature compost were obtained. Samples were taken from every batch with the coning technique for further study.

The samples were prepared for chemical analyses in two stages. During the first stage, they were dried at 50°C in 1.5 hour and then the water content percentage was determined by the weight technique (within 1-99% range). In the second stage, compost samples (50 g of weight each) were extracted with 200 cm<sup>3</sup> methanol by ultrasonic extraction in 3-hour spans. When the percolation ended, the solvent was removed by rotary evaporation, then a test portion (0.1 g) was dissolved in 10 cm<sup>3</sup> of methanol pure for anal-



Fig. 4. Compost pile made after emptying the bioreactor (KLIMAS 2014)

Table 2

Basic parameters of maturation process in compost heaps

Sample	Total maturation process (days)	Maximum temperature of the process (°C)	Duration of temperatures between 50°C and 70°C (days)	Humidity (%)	Number of turning cycles
Early spring composts					
Compost 1	207	68	42	61	12
Compost 2	210	68	86	59	10
Compost 3	197	63	61	46	10
Compost 4	190	69	76	50	8
Range	190 - 210	63 - 69	42 - 86	46 - 61	8 - 12
Late spring composts					
Compost 5	194	69	51	51	8
Compost 6	203	65	68	49	8
Compost 7	214	67	64	47	8
Compost 8	197	67	72	47	10
Compost 9	187	70	69	49	8
Compost 10	204	64	48	48	12
Range	187 - 214	64 - 70	48 - 72	47 - 51	8 - 12

ysis. The mixture was filtered through a nylon syringe filter, pore diameter 0.45  $\mu\text{m}$ , and analysed by reverse-phased chromatography. The flow velocity was 1  $\text{cm}^3 \text{min}^{-1}$ , the volume of a single injected sample was 20  $\text{mm}^3$ . An analysis lasted for 10 min for standard substances, and 20 min for real samples. Measurements were taken at the wavelengths of 208, 254 and 280 nm.

The substances were identified by comparison to standards of plant hormones from the groups of auxins (indole-3-acetic acid (IAA) and phenylacetic acid (PAA)), gibberellins (gibberellic acid (GA)) and cytokinins (kinetin (KA) and 6-benzylaminopurine (6-BA)).

The equipment and reagents used for chemical analyses:

- methanol and acetonitrile pure for analysis (POCH, Gliwice/Poland, Merck, Darmstadt/Germany); double distilled water by MilliQ-system (Millipore, MA/USA); standards: IAA, PAA, KA, GA and 6-BA pure for analysis (Sigma Aldrich);
- syringe filters with pore diameter 0.45  $\mu\text{m}$ ;
- HPLC column Luna C-18 100 A, 150x4.6 mm, 5  $\mu\text{m}$ , Phenomenex;
- chromatograph HPLC-PDA Ultimate 3000 with an autosampler Dionex.

## RESULTS OF INVESTIGATIONS

The analytical examination of the composting process parameters carried out in a bioreactor. Whose results are presented in table 1, revealed no significant differences in the air flow or maximum  $\text{CO}_2$  content. A more rapid increase in temperature occurred in waste composted in late spring, in which the hygienization and cooling phases lasted longer, which may have been induced by higher ambient temperature. However, this made no difference with respect to the temperatures obtained in the bioreactor, which were comparable for both early and late spring composts.

Table 2 presents parameters of the maturation process in compost piles. No significant differences in the duration of the process or the maximum temperatures obtained in the composts were observed. However, in early spring composts, temperatures between 50 and 70°C extended over a longer period and humidity was higher, which may have been caused by a higher content of household organic waste.

Available literature neglects the question of the content of plant hormones in composts derived from organic wastes. Studies usually deal with compost extracts or plants fertilized with composts (PANT et al. 2012, OUNI et al. 2014). Our results of the determination of the presence of phytohormones in mature composts can be found in Table 3. Four out of the five analysed substances were found in composts made from waste collected in early spring (March-April), namely: indole-3-acetic acid (IAA), phenylacetic acid (PAA), kinetin (KA) and 6-benzylaminopurine (6-BA), which belong to the auxin and cytokinin groups. None of the early spring composts contained gibberellic acid (GA). The total content of the analysed phytohormones in early spring composts ranged from 3.692  $\text{mg kg}^{-1}$  to 14.56  $\text{mg kg}^{-1}$ , and the mean concentration was 9.489  $\text{mg kg}^{-1}$ .

The content of plant hormones in composts made from waste collected



Table 3

Results of analyses of plant hormone content in mature composts

Sample	Active substances in composts					Total content (mg kg <sup>-1</sup> )
	IAA	PAA	GA	KA	6-BA	
Early spring composts						
Compost 1	X	-	-	X	-	13.18
Compost 2	X	X	-	X	-	14.56
Compost 3	X	X	-	X	X	3.692
Compost 4	-	X	-	X	X	6.524
Average						9.489
Late spring composts						
Compost 5	-	-	X	X	-	23.20
Compost 6	-	-	-	X	X	21.85
Compost 7	-	-	-	X	X	5.105
Compost 8	-	-	-	X	X	4.752
Compost 9	-	-	-	X	X	22.03
Compost 10	-	-	-	X	X	9.422
Average						14.39

(X) – detected, (-) – not detected

from May to June was different. None of the samples contained substances from the auxin group (indole-3-acetic acid (IAA), phenylacetic acid (PAA)), only one contained gibberellic acid (GA). Similarly to early spring composts, kinetin (KA) was found in all late spring composts, and 6-benzylaminopurine (6-BA) occurred in five out of six samples. The total content of the phytohormones in late spring composts ranged from 4.752 mg kg<sup>-1</sup> to 23.20 mg kg<sup>-1</sup>. The mean concentration in this group of composts reached 14.39 mg kg<sup>-1</sup>, which is higher than in the early spring group. Since no substantial differences were observed regarding the conditions in which the composting took place (both of the March-April and the May-June waste), it is justifiable to suppose that a higher concentration of phytohormones in late spring composts was related to a higher content of green waste (from urban greenery) in the processed material.

## CONCLUSIONS

Composting is a recycling process whereby organic waste is converted into a valuable product (fertilizer). Compost can be a source of numerous soil conditioning substances. Apart from nutritional components, organic waste

(plant material waste in particular) can supply the environment with substances promoting plant growth and development, such as plant hormones. The study on the composting of the municipal waste organic fraction has led to the following conclusions:

1. The application of a two-stage composting technology facilitates the monitoring of the process.

2. The application of high-performance liquid chromatography with diode array detection allowed simultaneous identification of several plant hormones in compost samples.

3. Total concentrations of plant hormones in composts were highly varied: from 3.692 mg kg<sup>-1</sup> to 14.56 mg kg<sup>-1</sup> in early spring composts and from 4.752 mg kg<sup>-1</sup> to 23.20 mg kg<sup>-1</sup> in late spring composts. The average content of the analysed substances was higher in late spring composts, which were made from mixtures containing larger amounts of plant waste (from urban greenery).

4. The phytohormones that were found most frequently in the analysed composts belonged to cytokinins, e.g. kinetin occurred in all the samples (ten), and 6-benzylaminopurine was determined in seven samples. Hormones from the auxin group were found only in early spring composts – both indole-3-acetic acid and phenylacetic acid were detected in three samples each. A hormone from the gibberellin group was identified only in one sample (of late spring compost).

5. Apart from differences in the average content of plant hormones, the number of substances occurring in composts was also observed to vary: early spring composts comprised from two to four phytohormones, whereas late spring composts had only two.

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