

**MEDICINAL PLANTS GROWN IN SOIL AMENDED WITH STRUVITE RECOVERED FROM ANAEROBICALLY PRETREATED POULTRY MANURE WASTEWATER**

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Corresponding author E-mail: [yetilmez@yildiz.edu.tr](mailto:yetilmez@yildiz.edu.tr)**ABSTRACT**

Struvite (magnesium ammonium phosphate hexahydrate,  $\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$ , MAP) recovered from up-flow anaerobic sludge blanket (UASB) pretreated poultry manure wastewater ( $\text{MgCl}_2 \cdot 6\text{H}_2\text{O} + \text{KH}_2\text{PO}_4$ ,  $\text{Mg}^{2+}:\text{NH}_4^+:\text{N}:\text{PO}_4^{3-}:\text{P} = 1:1:1$ ,  $\text{pH} = 9.0$ ) was tested as a slow release fertilizer on the growth of four medicinal plants including garden rocket (*Eruca sativa*), dill (*Anethum graveolens*), fennel (*Foeniculum vulgare*) and parsley (*Petroselinum crispum*) in a series of lab-scale greenhouse experiment. Pot trial tests indicated that rates of increase in fresh weights, dry weights and fresh heights of plants grown in soil fertilized with the recovered struvite were determined as 405%, 488%, and 51% for garden rocket; 154%, 191%, and 44% for dill; 152%, 379%, and 27% for fennel; 141%, 208%, and 22% for parsley, respectively, compared to the control pot. Results of a static bioassay test proved that the use of plants cultivated in MAP pots as the feeding material did not cause any acute toxicity symptoms or mortality in guppy fish (*Lebistes reticulatus*), and all survived and exhibited normal visual responses at the end of 170-h exposure. Findings of this study confirmed that the recovered struvite from UASB effluent provided a valuable slow release fertilizer for the agricultural use, resulting an edible multi-nutrient animal feed.

**Key words:** Poultry manure wastewater; up-flow anaerobic sludge blanket; magnesium ammonium phosphate; medicinal plants; fertilizer; acute toxicity.

**INTRODUCTION**

Poultry sector demonstrates a rapid growth to meet the needs for animal proteins of the population day by day. Considering the need for white meat, this development may be expected to rise gradually in both developing and developed countries. However, this brings about several issues of environmental pollution, particularly in plants located in the large settlements. For instance, dumping of untreated wastes from poultry and farm animals (i.e., chicken, duck, bovine animals, etc.) to agricultural lands leads to deterioration of product diversity and quality, disruption of soil stability and decrease in favorable properties of the soil. Besides, when the storage of animal wastes does not comply with the standards, problems of flies and pests arise, and health of living things is adversely affected. Furthermore, as well as nitrogen and phosphorus pollution in receiving waters and soil, certain gases (i.e.,  $\text{NH}_3$ ,  $\text{CH}_4$ ,  $\text{CO}_2$ , etc.) from accumulation of improperly managed animal wastes pollute the air in the region, and cause generation of pathogenic microorganisms and severe diseases (Yetilmezsoy, 2008; Yetilmezsoy and Sakar, 2008a; Yetilmezsoy *et al.*, 2009a).

Magnesium ammonium phosphate (MAP, struvite) precipitation is suggested as an alternative and

promising physicochemical method due to its high efficiency in recovery of residual nitrogen and in industrial wastewaters, and production of a beneficial by-product in the end of the process (Yetilmezsoy and Sapci-Zengin, 2009). Although struvite precipitation has been widely studied by many researchers as an established and promising method for  $\text{NH}_4^+$  or  $\text{PO}_4^{3-}$  removal from various types of wastewaters (Kim *et al.*, 2007; Suzuki *et al.*, 2007; Ronteltap *et al.*, 2007; Battistoni *et al.*, 1997; Kalyuzhnyi *et al.*, 2002), so far, only a few of studies (Goto, 1998; Li and Zhao, 2003; Diwani *et al.*, 2007; Yetilmezsoy and Sapci-Zengin, 2009; Yetilmezsoy *et al.*, 2009b) dealing with this problem have earlier addressed the fertilizing value of the MAP precipitate using plant studies. However, as a further step, the ameliorative effect of the struvite recovered from anaerobically pretreated poultry manure wastewater on the various medicinal plants growth has not been fully investigated in the literature. For this reason, the present work aims at fulfilling the gap in this field by focusing upon studies on the growth of four medicinal plants including garden rocket (*Eruca sativa*), dill (*Anethum graveolens*), fennel (*Foeniculum vulgare*) and parsley (*Petroselinum crispum*) in a series of lab-scale agricultural tests.

The specific objectives of this study were (1) to appraise the fertilizing potential of the MAP precipitate by greenhouse experiments using four quick-growth

medicinal plants; (2) to examine characteristics of the recovered product by means of X-ray diffraction analysis; (3) to investigate the possible acute toxicity of the selected medicinal plants grown up in soil fertilized with the struvite recovered from anaerobically pretreated poultry manure wastewater by using guppy fish (*Lebistes reticulatus*) as the test organism; and (4) to make a statistical evaluation of the present experimental data by means of several appropriate parametric and non-parametric tests.

## MATERIALS AND METHODS

**Previous findings and present experimental conditions:** Anaerobic treatability studies on high strength poultry manure wastewater were fully investigated in our previous studies (Yetilmezsoy, 2008; Yetilmezsoy and Sakar, 2008a; Yetilmezsoy and Sakar, 2008b; Yetilmezsoy and Sapci-Zengin, 2009; Yetilmezsoy *et al.*, 2009a). The chemical combinations and the corresponding  $\text{NH}_4^+\text{-N}$ , total chemical oxygen demand and color removals obtained in the previous study (Yetilmezsoy and Sapci-Zengin, 2009). Previous findings (Yetilmezsoy and Sapci-Zengin, 2009) showed that  $\text{MgCl}_2\cdot 6\text{H}_2\text{O} + \text{KH}_2\text{PO}_4$  was found to be most efficient combination in terms of the MAP precipitate obtaining.

Following to MAP precipitation tests, in the present study, four quick-growth medicinal plants including garden rocket (*Eruca sativa*), dill (*Anethum graveolens*), fennel (*Foeniculum vulgare*) and parsley (*Petroselinum crispum*) were tested to explore the ameliorative effect of the MAP precipitate obtained under the optimum operating conditions (Yetilmezsoy and Sapci-Zengin, 2009). The present study, being the continuation of those analyses, is devoted to the investigation of the second stage experiments using the MAP precipitate obtained at the previous optimum experimental conditions ( $\text{MgCl}_2\cdot 6\text{H}_2\text{O} + \text{KH}_2\text{PO}_4$ ,  $\text{Mg}^{2+}:\text{NH}_4^+\text{-N}:\text{PO}_4^{3-}\text{-P} = 1:1:1$ ,  $\text{pH} = 9.0$ , a stirring time of 15 min, and a stable temperature of  $25^\circ\text{C}$ ). Therefore, a series of lab-scale greenhouse experiments were further conducted to investigate the ameliorative effect of the MAP precipitate (as obtained in the first stage) on the growth of four medicinal plants selected as the model plants in the scope of this study.

**Characteristics of MAP precipitate:** In this study, an XRD analysis was first conducted to characterize the MAP precipitate obtained in the previous study (Yetilmezsoy and Sapci-Zengin, 2009). It was determined that the XRD of the obtained precipitate coincides with the XRD result of the MAP standard by positions and densities of peaks. This confirms that the obtained solid phase is the crystal MAP (Fig. 1). Besides XRD analysis, the precipitate was also morphologically studied in

laboratory conditions with stereo and digital microscopes (Fig. 2).

**Plant growth tests:** The ameliorative effect of different doses of the MAP precipitate was investigated in a series of greenhouse experiment using garden rocket (*Eruca sativa*), dill (*Anethum graveolens*), fennel (*Foeniculum vulgare*) and parsley (*Petroselinum crispum*). Details on the morphologies of medicinal plants used in greenhouse experiments can be found in the work of Yilmaz and Kaleli (2010). Garden soil was used as raw solid media for germination and also growth of the plants for the present study. The characterization of the used garden soil was  $\text{pH} 5.5\text{--}6.0$ , electrical conductivity (EC)  $1.0\text{--}1.2\text{ mS/cm}$ , potassium (K)  $70\text{--}100\text{ ppm}$  and calcium (Ca)  $40\text{--}60\text{ ppm}$ . Based on the dimensions of conical plastic pots, about 450 g of garden soil sample were filled in each of the identical pots used for the present greenhouse experiment.

Required dosages of macro elements (N, P, K) for fertilizer applications for Turkish vegetable growth has been recommended as 150-1000 mg N/L (of fertilizer solution), 100-400 mg K/L, and 50-100 mg P/L (Sevgican, 1999). When the lower limits of the suggested ranges for K and N were considered, the required dosage of the MAP precipitate in 0.45 kg of solid media were determined to be about 1.2 g. In the cultivation of plants, following steps were implemented as described by others (Li and Zhao, 2003; Yetilmezsoy and Sapci-Zengin, 2009): (1) each plastic pot was filled with the garden soil; (2) about 2 cm of top layer was removed from the pots; (3) pre-weighted MAP precipitate were mixed into the packed media prior to planting; (4) 100 mL of tap water was added evenly over the surface of each pot; (5) pre-weighted plant seeds (each of 1 g) were laid evenly on the excavated surface; (6) plant seeds were covered with the removed solid media sample, and surfaces of the backfilled media were then gently compressed by the reverse side of a tablespoon; (7) a second 100 mL of tap water was added evenly over the planted and covered surface.

Pots were randomly placed in an artificially illuminated growth chamber at about  $25^\circ\text{C}$  under suitably sterile conditions. In the chamber, ultraviolet radiation was continuously provided by a modulated fluorescent lamp. Based on the humidity of the ambient air, pots were irrigated by spraying the required volume of tap water collected in a graduated glass vessel to ensure equal watering to all plants during the agricultural process. During the rest of the study, a model chemical equivalent to MAP was applied to the plant (herein rocket) that grew the most rapidly, and it was compared to the MAP. This model fertilizer was prepared with  $\text{NH}_4\text{NO}_3 + \text{KH}_2\text{PO}_4$  chemicals. The dosage calculations of the model fertilizer was made as in the MAP application, and 0.2 g  $\text{NH}_4\text{NO}_3$

and 0.1 g  $\text{KH}_2\text{PO}_4$  were separately weighed and added to the soil (Fig. 3).

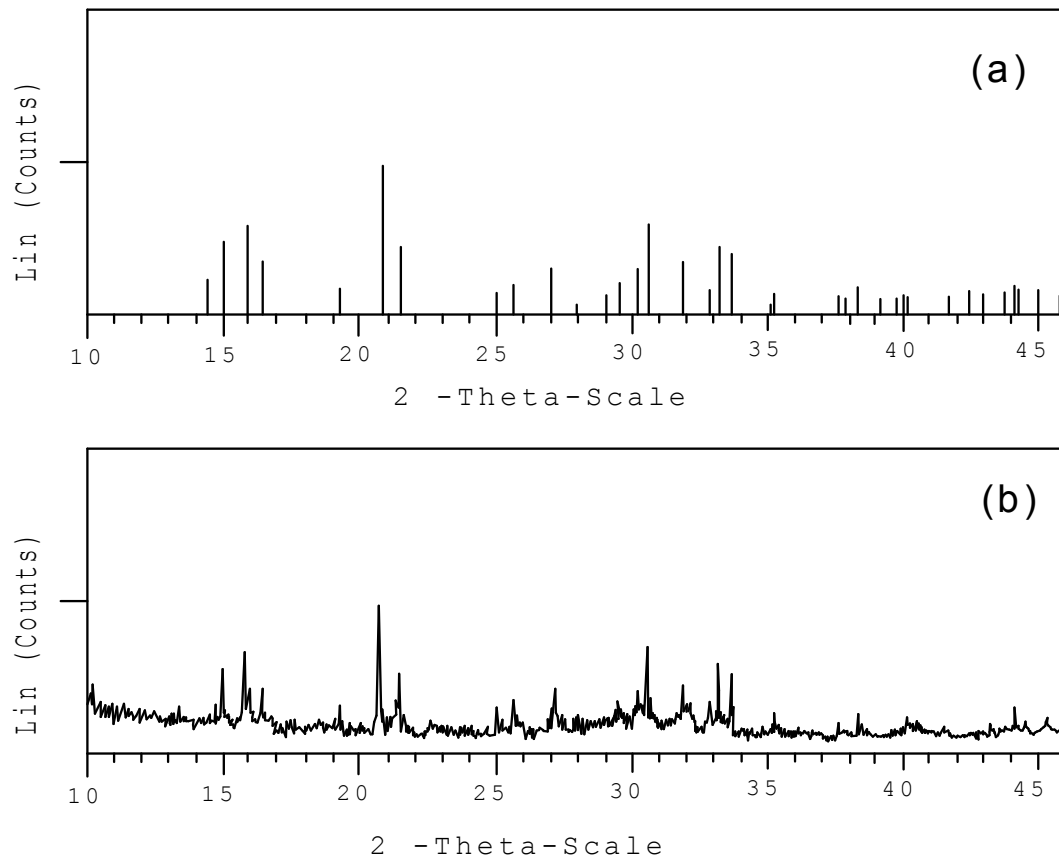
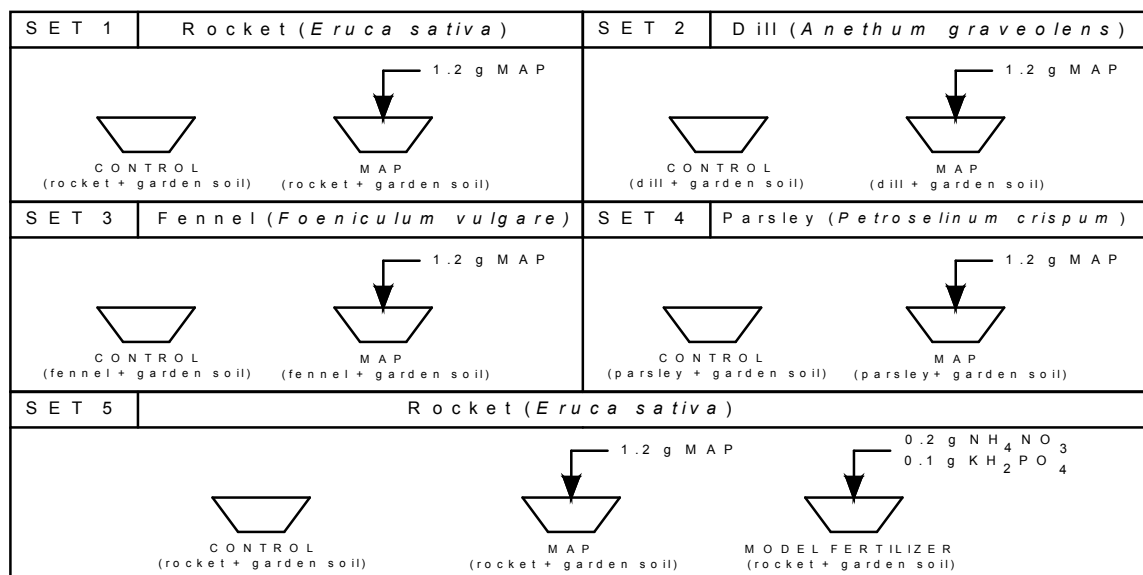


Fig. 1 XRD result of the MAP precipitate by positions and densities of peaks: (a) MAP standard; (b) obtained precipitate.



Fig. 2 Physical morphology of the original MAP product and 40 times magnification with stereo microscope.



**Fig. 3 A schematic of the experimental set-up.**

**Fish toxicity test (static bioassay experiment):** Toxicity trials are conducted to identify the level of effects of products that are natural or synthetically produced and contain dangerous or non-dangerous, organic or inorganic chemical substances on the environment and human health. Identification of toxicity requires bio-analyses. During bio-analyses, the effects on the test organism used as a bio-sensor are monitored. Among them, fish bio-test is a standard trial that helps to state toxicity in relation to dilution ratios, by identifying the toxic effects of waste waters on fish species used as indicator organisms, and determining the survival percentages of fish during certain time such as 48 h, 72 h, 96 h at varying dilution ratios of wastewaters, as defined in Turkish Water Pollution Control Regulation (2004).

Fish toxicity test was applied using the plant (herein rocket) that grew most rapidly among the plants cultivated with the obtained MAP precipitate to determine whether it had any potential toxic effect. Another objective of the toxicity test conducted under this study was to determine feasibility of the plant cultivated with the MAP precipitate as feed in a macro living thing such as experiment fish. Fish bio-experiments are usually used to determine toxic effects of waste waters, whereas it was used in this study to identify the toxicity of a solid substance.

In the toxicity analysis conducted in this study, guppy fish (*Lebistes reticulatus*) was used as a bio-sensor organism. An experimental setting was prepared with 3 fish groups to study feasibility of plant species cultivated in control, MAP and non-MAP conditions as feed. In the study, test fish were kept in sterile aquarium water supplied by the commercial aquarium seller from whom the fish were purchased. The plant growing the most rapidly among the plants dosed with MAP and the non-

MAP derivative of the same plant were dried in a drying oven after harvesting. Thus, they were easily rendered into a form that can be consumed by fish in water. The fish in the control group was fed with its own feed (Sanyu Tropical fish feed), the fish in the non-MAP group was fed with the plant cultivated in an environment where MAP precipitate was not used as feeding material, and the fish in the MAP group was fed with the plant that was cultivated in the environment where MAP was used feeding material. The amount of the feed was determined according to the information provided by the aquarium from which the fish were taken. The dried and rendered plants cultivated in MAP and non-MAP environment were weighed at the same amount for feeding the guppy fish.

In the experimental setting, the oxygen supply for the fish was provided through an air pump with a multi-air outlet. The air pipes connected to the pump were placed in the aquarium with the fish. The air flow was adjusted so as to avoid shaking the fish and to ensure even distribution of air. The air flow was disconnected when feeding the fish, and reconnected after feeding. Under these circumstances, the behaviour of the fish was observed during the monitoring period of at least 48 h. This monitoring period was later extended to 170 hours.

**Analytical procedure:** During the agricultural study conducted under this work, a modulated fluorescent lamp (Panlight daylight lamps, 3011 T8 36W) was used as artificial light in the cultivation experiment setting. The plants were dosed with MAP and model chemical substance ( $\text{NH}_4\text{NO}_3 + \text{KH}_2\text{PO}_4$ ) and their harvest weights were identified with an electronic sensitive scientific balance (Avery Berkel). The harvested plants were dried in a drying oven (Electro-mag, M6040p) at  $105^\circ\text{C}$ . The

plant seeds and the MAP precipitate were imaged under laboratory conditions with a stereo microscope (Prior-James Swift, 240V.AC, F80 mA) combined with a Sony Cyber-shot DSC-N1 model digital camera. MAP powders were also imaged by using a digital microscope (Keyence VHX-1000) consisted of a high resolution CCD camera-based system with a high intensity halogen lamp. During the fish bio-experiment, oxygen supply for the fish was provided through an air pump with a multi-air outlet (Resun, Air Pump AC-9906). The chemical composition of MAP was identified by using Philips Panalytical X'Pert Pro X-ray diffractometer (45 kV, 40 mA, CuK $\alpha$ ) at the Chemical Engineering Laboratory of Yildiz Technical University. The pH, conductivity, potassium and calcium data for the garden soil used in the study was supplied by the manufacturer of the soil.

**Statistical analysis:** Each experiment was performed in triplicate and repeated at least three times to observe the reproducibility. StatsDirect (version 2.7.2, Copyright© 1990–2008 StatsDirect Ltd.) software package was used for the statistical analysis of the experimental data. In all calculations, spreadsheets of Microsoft Excel® or DataFit® scientific software (version 8.1.69, Copyright© 1995-2005 Oakdale Engineering) used as an open database connectivity data source running under

Windows. An alpha ( $\alpha$ ) level of 0.05 (or 95% confidence) was used to determine the statistical significance in all analyses. Various descriptive statistics such as ranges of growth yields, minimum and maximum values, medians, means, standart deviations, variances, variance coefficients, standard errors of means, skewness and Kurtosis values for each subset were analysed in order to characterize the selected groups and identify the extreme values. Sprout and growth rates used as quantitative measures of the fertility level of the MAP precipitate were statistically evaluated by means of appropriate statistical tests. Prior to parametric tests, the Shapiro-Wilk  $W$  and the Levene's tests were consecutively conducted as preconditions to ensure that the experimental subsets had a normal or non-normal distribution, and variances (or standard deviations) of the paired groups were homogeneous or unequal. Test results were assessed with various descriptive statistics such as two-tailed  $p$  values,  $t$  statistics, combined (or pooled) standard errors ( $se_p$ ), and power values (PW or probability of detecting a true effect for 5% significance) to reflect the statistical significance between paired groups. Flowchart of the statistical analysis conducted to evaluate the experimental results is depicted in Fig. 4.

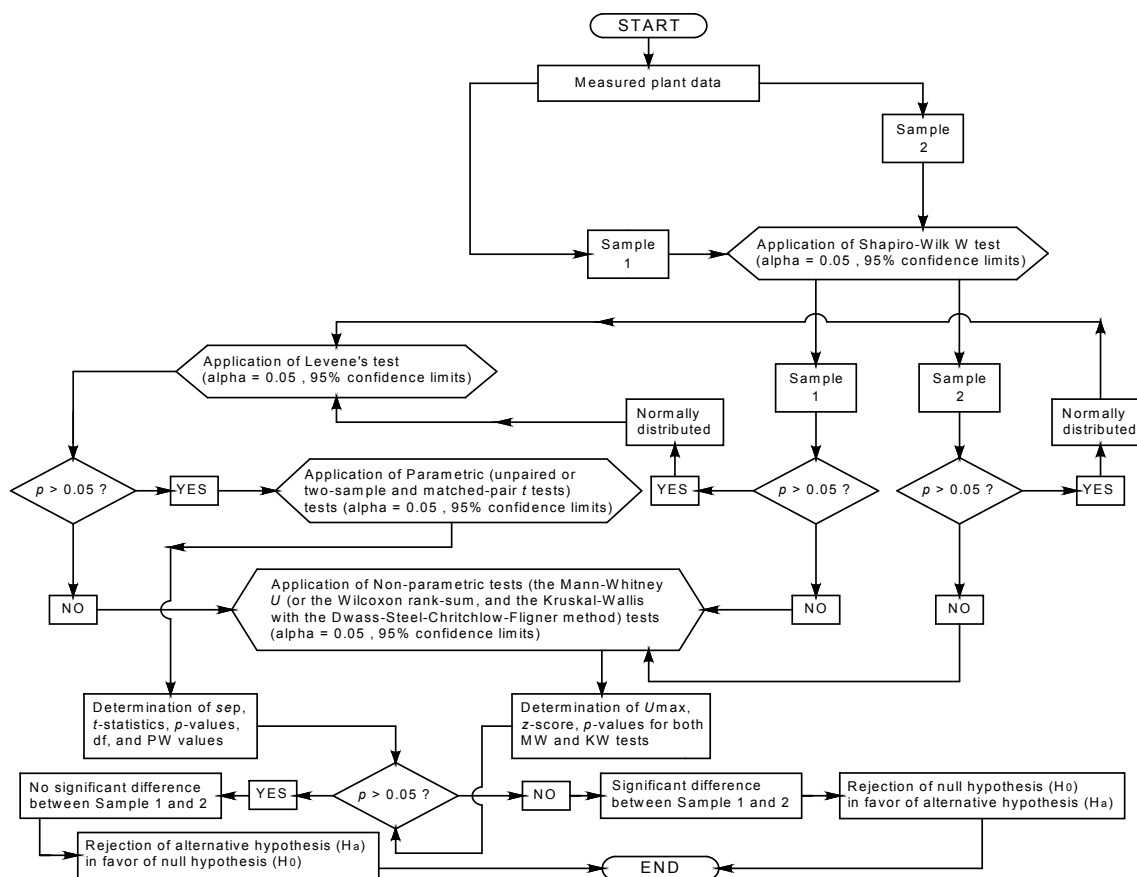


Fig. 4 Flowchart of the statistical analysis conducted to evaluate the experimental results.



## RESULTS AND DISCUSSION

**Greenhouse experiment:** The present greenhouse study showed that test plants sprouted for about 3–5 days. To explore the ameliorative effect of the collected MAP sludge ( $\text{MgCl}_2 \cdot 6\text{H}_2\text{O} + \text{KH}_2\text{PO}_4$ , pH 9.0,  $\text{Mg}^{2+}:\text{NH}_4^+:\text{N}:\text{PO}_4^{3-}:\text{P} = 1:1:1$ ) on the plant growth, all plants were harvested on the 15th day after seeding. Prior to harvesting, the plants in the pots were sprayed with deionized water to wash the dust off, as conducted by others (Li and Zhao, 2003; Yetilmezsoy and Sapci-Zengin, 2009). After drying in ambient conditions, the three tallest plant individuals in each pot were selected to compare their fresh heights. It was observed that the plants in the MAP pots grew much faster than those in the control pots, but all plants grew at different rates depending on their species (Fig. 5). Based on the comparative appearance of MAP, model chemical ( $\text{NH}_4\text{NO}_3 + \text{KH}_2\text{PO}_4$ ) and control sets of the most rapid growth plant (rocket), the MAP group grew better than both the chemical and control groups.

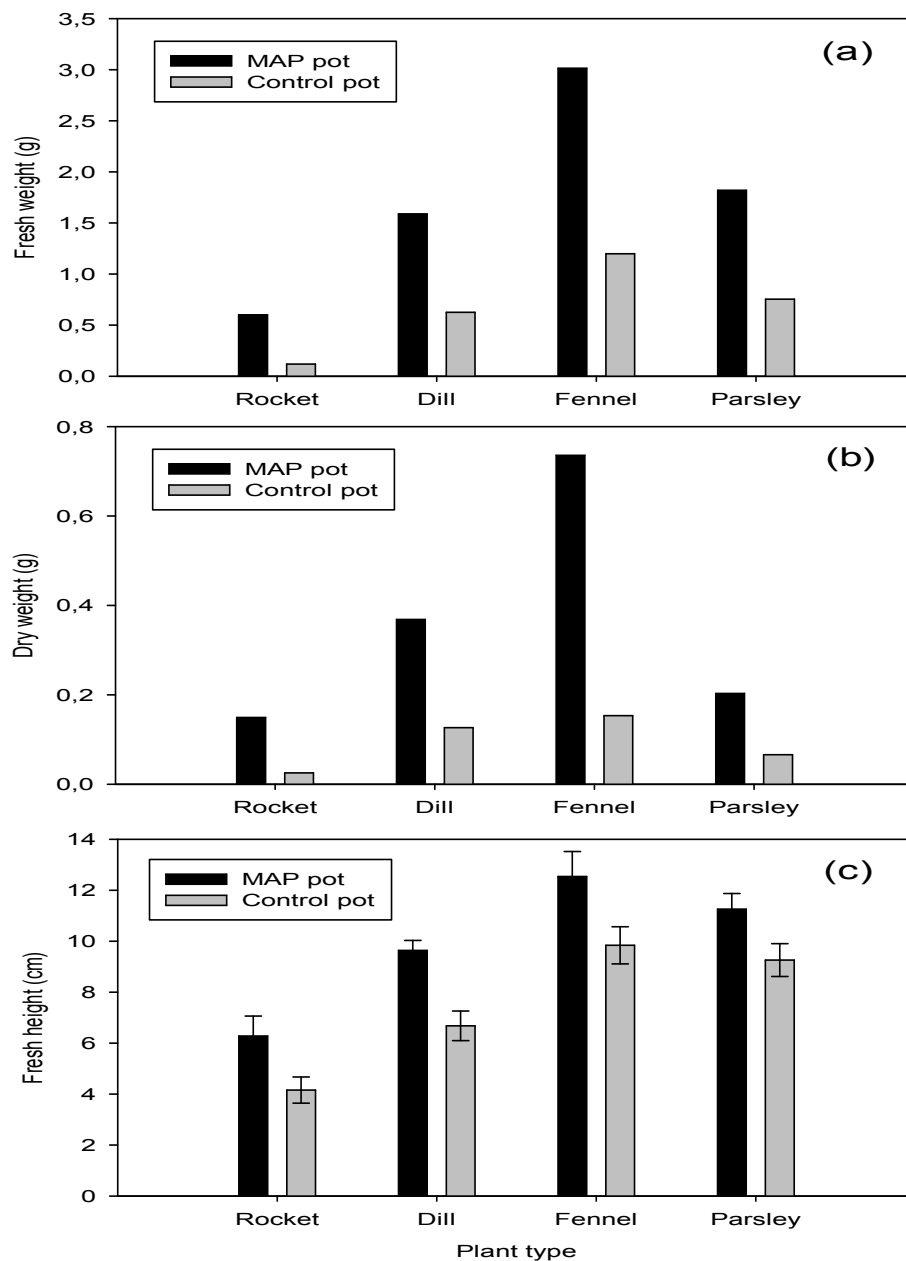
Based on the wet-dry weights and fresh height measurements of plants after the harvest, the results obviously indicated that the plants in the MAP groups grew better than the control groups (Fig. 6). According to the obtained results, the plants in the MAP group have also higher growth rates than the model chemical set. The dry-wet weights and fresh heights of the plants which were dosed with MAP were increased by 488%, 405% and 51% for rocket, 191%, 154% and 44% for dill, 379%,

152% and 27% for fennel, and 208%, 141% and 22% for parsley, as compared to the plants in the control group.

According to the highest growth rates, garden rocket was selected for the further step to compare the ameliorative effects of the recovered struvite with those provided by a model fertilizer of  $\text{NH}_4\text{NO}_3 + \text{KH}_2\text{PO}_4$  chemicals. Dry-wet weights and fresh heights of rocket, which was dosed with model chemical, was increased by 246%, 384% and 28%, respectively in comparison to the control. Results of the present agricultural experiments clearly confirmed that growth rates of garden rocket cultivated in MAP pots were found to be superior than others in terms of both measured weights and heights (Table 1). According to the experimental results obtained under the present study, the fresh height values were statistically evaluated, and the differences between control, MAP and model sets were appraised. Statistical analysis of the experimental results proved that the plants in the MAP groups grew better than the control and model groups after the harvest (Tables 2 – 4). The obtained results demonstrated that MAP precipitate is comparatively more effective in growth of plants than the synthetic model fertilizer. As seen in Table 3, the difference between fresh heights of rocket plants cultivated with model fertilizer and MAP was found to be statistically significant ( $t = 2.5228$ ,  $p = 0.0357$ ). The statistical tests demonstrated that there are statistically significant differences between the control and MAP sets for the values obtained for rocket ( $t = 5.0677$ ,  $p = 0.001$ ), dill ( $t = 4.2588$ ,  $p = 0.0028$ ), fennel ( $t = 4.9443$ ,  $p = 0.0011$ ) and parsley ( $t = 5.0443$ ,  $p = 0.001$ ) in terms of fresh heights (Table 2).



**Fig. 5** Photos of three plants at different cultivation conditions after 15 days growth (control pots at left-hand side and MAP pots at right-hand side): (a) rocket (*Eruca sativa*); (b) dill (*Anethum graveolens*); (c) fennel (*Foeniculum vulgare*); (d) parsley (*Petroselinum crispum*).



**Fig. 6** Average growth rates (g) of test plants for the control and MAP groups after 15 days growth based on wet and dry mass and fresh height: (a) fresh weights, (b) dry weights, (c) fresh heights.

**Table 1:** Average growth rates of test plant cultivated with MAP and model fertilizer (only the rocket) after 15 days growth based on wet and dry mass and fresh heights compared to the control groups

Plant type	Rate of increases compared to the control (%)		
	Fresh weight	Dry weight	Fresh height
Rocket ( <i>Eruca sativa</i> ) – MAP	405	488	51
Dill ( <i>Anethum graveolens</i> ) – MAP	154	191	44
Fennel ( <i>Foeniculum vulgare</i> ) – MAP	141	208	22
Parsley ( <i>Petroselinum crispum</i> ) – MAP	152	379	27
Rocket ( <i>Eruca sativa</i> ) – Model chemical (NH <sub>4</sub> NO <sub>3</sub> + KH <sub>2</sub> PO <sub>4</sub> )	384	246	28

**Table 2: Statistical comparison of fresh heights of test plants obtained for control and MAP pots**

Pairs of groups	Normality or non-normality test (Shapiro – Wilk W test) <sup>a</sup>	Homogeneity of variances (Levene's test) <sup>b</sup>	Test type	Statistical outputs <sup>c</sup>
Rocket (Control) – Rocket (MAP)	Rocket (Control) <i>p</i> = 0.2964 (Normal) Rocket (MAP) <i>p</i> = 0.1402 (Normal)	<i>F</i> = 0.2154 <i>df</i> = 1.8 <i>p</i> = 0.6549 (Homogeneous)	Unpaired <i>t</i> -test	<i>se<sub>p</sub></i> <sup>d</sup> = 0.41833 <i>df</i> = 8 <i>t</i> = 5.067769 <i>p</i> = 0.001 PW <sup>e</sup> > %99.99
Dill (Control) – Dill (MAP)	Dill (Control) <i>p</i> = 0.8591 (Normal) Dill (MAP) <i>p</i> = 0.4618 (Normal)	<i>F</i> = 0.2154 <i>df</i> = 1.8 <i>p</i> = 0.6696 (Homogeneous)	Unpaired <i>t</i> -test	<i>se<sub>p</sub></i> <sup>d</sup> = 0.314643 <i>df</i> = 8 <i>t</i> = 4.2588 <i>p</i> = 0.0028 PW > %99.91
Fennel (Control) – Fennel (MAP)	Fennel (Control) <i>p</i> = 0.0903 (Normal) Fennel (MAP) <i>p</i> = 0.1013 (Normal)	<i>F</i> = 0.2154 <i>df</i> = 1.8 <i>p</i> = 0.8925 (Homogeneous)	Unpaired <i>t</i> -test	<i>se<sub>p</sub></i> <sup>d</sup> = 0.546077 <i>df</i> = 8 <i>t</i> = 4.944358 <i>p</i> = 0.0011 PW = %99.99
Parsley (Control) – Parsley (MAP)	Parsley (Control) <i>p</i> = 0.525 (Normal) Parsley (MAP) <i>p</i> = 0.8678 (Normal)	<i>F</i> = 0.2154 <i>df</i> = 1.8 <i>p</i> = 0.8207 (Homogeneous)	Unpaired <i>t</i> -test	<i>se<sub>p</sub></i> <sup>d</sup> = 0.396485 <i>df</i> = 8 <i>t</i> = 5.044333 <i>p</i> = 0.001 PW = %99.99

<sup>a</sup> *p* values > 0.05 shows that normal distribution.

<sup>c</sup> *p* values < 0.05 were considered to be significant.

<sup>e</sup> Power value (for 5% significance).

<sup>b</sup> *p* values > 0.05 shows that variances are homogeneous.

<sup>d</sup> Combined (or pooled) standard error.

**Table 3: Statistical comparison of fresh heights of rockets obtained for control, MAP and model fertilizer pots by appropriate statistical tests**

Pairs of groups	Normality or non-normality test (Shapiro – Wilk W test) <sup>a</sup>	Homogeneity of variances (Levene's test) <sup>b</sup>	Test type	Statistical outputs <sup>c</sup>
Rocket (Control) – Rocket (Model)	Rocket (Control) <i>p</i> = 0.2964 (Normal) Rocket (Model) <i>p</i> = 0.7374 (Normal)	<i>F</i> = 0.2119 <i>df</i> = 1.8 <i>p</i> = 0.6575 (Homogeneous)	Unpaired <i>t</i> -test	<i>se<sub>p</sub></i> <sup>d</sup> = 0.2739 <i>df</i> = 8 <i>t</i> = 4.2357 <i>p</i> = 0.0029 PW <sup>e</sup> = %99.9
Rocket (Model) – Rocket (MAP)	Rocket (Modell) <i>p</i> = 0.7374 (Normal) Rocket (MAP) <i>p</i> = 0.1402 (Normal)	<i>F</i> = 0.6722 <i>df</i> = 1.8 <i>p</i> = 0.4360 (Homogeneous)	Unpaired <i>t</i> -test	<i>se<sub>p</sub></i> <sup>d</sup> = 0.3805 <i>df</i> = 8 <i>t</i> = 2.5228 <i>p</i> = 0.0357 PW = %88.2

<sup>a</sup> *p* values > 0.05 shows that normal distribution.

<sup>c</sup> *p* values < 0.05 were considered to be significant.

<sup>e</sup> Power value (for 5% significance).

<sup>b</sup> *p* values > 0.05 shows that variances are homogeneous.

<sup>d</sup> Combined (or pooled) standard error.

**Fish toxicity test:** The toxicity test conducted for approximately 48 h demonstrated that the plants did not have any acute toxic effect on guppy fish (*Lebistes reticulatus*). The fish exhibited usual behaviour during the test process, and no behavioural abnormality and neurotoxic effect were observed after feeding. After 48 h of test period, guppies were not netted from test jars and behavioural changes of individuals were monitored for an additional 120 h of incubation. Results indicated that all

guppies were still alive and showed normal behaviour even at the end of about 170 h of exposure. Thus, it is concluded that the struvite from anaerobically pretreated poultry manure wastewater can be used as a valuable slow release fertilizer for the growth of medicinal plants. Furthermore, it is demonstrated that dried and rendered form of the plants grown up in soil fertilized with the recovered struvite can be evaluated as an edible multi-nutrient feed without any acute toxic effects.



**Table 4: Detailed descriptive statistics of fresh heights for the present greenhouse experiment**

Plant type	Mean	Variance	Standard deviation	Variance coefficient	Standard error of mean	Skewness	Kurtosis value	Maximum	Median	Minimum
Rocket (Control)	4.16	0.260	0.513	0.123	0.229	0.929	2.528	5.0	4.1	3.7
Rocket (MAP)	6.28	0.612	0.782	0.125	0.350	1.094	2.756	7.6	6.2	5.6
Dill (Control)	8.82	0.177	0.421	0.048	0.188	-0.404	2.067	9.3	8.8	8.2
Dill (MAP)	10.16	0.318	0.564	0.056	0.252	-0.691	2.019	10.7	10.4	9.3
Fennel (Control)	9.84	0.533	0.730	0.074	0.326	-0.351	1.213	10.5	10.2	9.0
Fennel (MAP)	12.54	0.958	0.978	0.078	0.438	1.153	2.749	14.2	12.2	11.8
Parsley (Control)	9.26	0.413	0.643	0.069	0.287	-0.494	2.020	9.9	9.2	8.3
Parsley (MAP)	11.26	0.373	0.611	0.054	0.273	-0.055	2.349	12.1	11.3	10.4

**Conclusion:** In this study, the struvite obtained from effluent of the UASB reactor treating poultry manure wastewater was explored as a slow release fertilizer on growth of four different medicinal plants, such as garden rocket (*Eruca sativa*), dill (*Anethum graveolens*), fennel (*Foeniculum vulgare*) and parsley (*Petroselinum crispum*) in a series of lab-scale greenhouse experiment. A static bioassay test was also performed to explore the possible acute toxicity of the plants grown up in soil fertilized with the recovered struvite using guppy fish (*Lebistes reticulatus*) as the test organism. The increase ratio of wet-dry weights and heights of plants which were fertilized with struvite were determined to be 405%, 488% and 51% for rocket, 154%, 191% and 44% for dill, 152%, 379% and 27% for fennel, and 141%, 208% and 22% for parsley, respectively, as compared to the plants in the control group. In the second set experiments conducted with the rocket plant, which demonstrated the most rapid growth among the other plants used in the study, the increase ratio of wet-dry weights and heights for the model fertilizer ( $\text{NH}_4\text{NO}_3 + \text{KH}_2\text{PO}_4$ ) were as high as 384%, 246% and 28%, as compared to the rocket control sets. Under the same conditions, the wet-dry weight and heights of rocket were 0.5769 g, 0.0878 g and 5.32 ( $\pm 0.33$ ) cm for the model fertilizer, and 0.6014 g, 0.1265 g and 6.28 ( $\pm 0.78$ ) cm for MAP, respectively. As a result of the bio-toxicity analysis, which was conducted with guppy fish (*Lebistes reticulatus*) for about 170 h (approximately 3.5 folds of the regular 48-h test procedure), dried and rendered plants given as the multi-nutrient feed did not have any acute toxic or neurotoxic effects on guppies. Thus, it has been determined that the struvite obtained from the UASB effluent is feasible in agriculture as a valuable fertilizer, and the final product be used as a healthy animal feed.

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## REFERENCES

- Battistoni, P., G. Fava, P. Pavan, A. Musacco and F. Cecchi (1997). Phosphate removal in anaerobic liquors by struvite crystallization without addition of chemicals: preliminary results. *Water Res.* 31: 2925–2929.
- Diwani, G. E., Sh. E. Rafie, N. N. E. Ibiari and H. I. El-Aila (2007). Recovery of ammonia nitrogen from industrial wastewater treatment as struvite slow releasing fertilizer. *Desalination* 214: 200–214.
- Goto, I. (1998). Application of phosphorus recovered from sewage plants. *Environ. Conserv. Eng.* 27: 418–422.
- Kalyuzhnyi, S., V. Sklyar, A. E. I. Arkhipchenko, I. Barboulina, O. Orlova, and A. Klapwijk (2002). Combined biological and physico-chemical treatment of filtered pig manure wastewater: Pilot investigations. *Water Sci. Technol.* 45: 79–87.
- Kim, D., H-D. Ryu, M-S. Kim, J. Kim, and S-III. Lee (2007). Enhancing struvite precipitation potential for ammonia nitrogen removal in municipal landfill leachate. *J. Hazard. Mater.* 146: 81–85.
- Li, X. Z. and Q. L. Zhao (2003). Recovery of ammonium-nitrogen from landfill leachate as a multi-nutrient fertilizer. *Ecolog. Eng.* 20: 171–181.
- Ronteltap, M., M. Maurer and W. Gujer (2007). Struvite precipitation thermodynamics in source-separated urine. *Water Res.* 41: 997–984.
- Sevgican, A. (1999). *Ortuali sebzeçiligi*, Topraksiz Tarim, 2nd ed., EUZF Public., No. 526, p.130, 1999 (in Turkish).
- Suzuki, K., Y. Tanaka, K. Kuroda, D. Hanajima, Y. Fukumoto, T. Yasuda and M. Waki (2007).

- Removal and recovery of phosphorous from swine wastewater by demonstration crystallization reactor and struvite accumulation device. *Bioresour. Technol.* 98: 1573–1578.
- TWPCR (Turkish Water Pollution Control Regulation), Official Gazette No. 25687 dated December 31, 2004.
- Yetilmezsoy, K. (2008). Treatability of poultry manure wastewater using anaerobic sludge bed reactor. Ph.D. Thesis, Institute of Science, Department of Environmental Engineering, Yildiz Technical University, Istanbul, Turkey.
- Yetilmezsoy, K. and S. Sakar (2008a). Improvement of COD and color removal from UASB treated poultry manure wastewater using Fenton's oxidation. *J. Hazard. Mater.* 151: 547–558.
- Yetilmezsoy, K. and Z. Sapci-Zengin (2009). Recovery of ammonium nitrogen from the effluent of UASB treating poultry manure wastewater by MAP precipitation as a slow release fertilizer. *J. Hazard. Mater.* 166: 260–269.
- Yetilmezsoy, K., F. Ilhan, Z. Sapci-Zengin, S. Sakar and M. T. Gonullu (2009a). Decolorization and COD reduction of UASB pretreated poultry manure wastewater by electrocoagulation process: a post-treatment study. *J. Hazard. Mater.* 162: 120–132.
- Yetilmezsoy, K., B. Sertyesilisik, E. Kocak, and Z. Sapci-Zengin (2009b). Ameliorative effect of different doses of  $MgNH_4PO_4 \cdot 6H_2O$  precipitate recovered from the effluent of UASB treating poultry manure wastewater: Growth of *Lolium perenne*. *Inter. J. Food Agric. Environ.* 7: 823–831.
- Yilmaz, T. and M. Kaleli (2010). Investigation of growth of medicinal plant cultivated with struvite recovered from poultry manure wastewater pretreated by anaerobic sludge bed reactor. B.Sc. Thesis, Faculty of Civil Engineering, Department of Environmental Engineering, Yildiz Technical University, Istanbul, Turkey.