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# Soybean Phytotoxicity from Land-Applied Biosolids

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**ABSTRACT**: In 1999, marginal and entire leaf necrosis, interveinal chlorosis, leaf puckering and crinkling, and stunted overall growth were observed in field-grown soybeans (*Glycine max L.*) in Essex County, Virginia. The soybeans had been fertilized with biosolids treated by the Zimpro™ wet air oxidation process at the Passaic Valley Sewerage Commission (PVSC) Newark, NJ wastewater treatment plant. Our objectives were (1) to replicate the symptoms in the greenhouse using soils collected from fields where phytotoxicity symptoms were observed and (2) to determine whether the causative agent was a constituent in the primary sludge or was produced by the Zimpro™ treatment process. We replicated the field phytotoxicity symptoms in the greenhouse in soybeans grown in Zimpro™ biosolids-amended field soils from Essex County and in soils amended with Zimpro™-processed biosolids obtained from PVSC. Soybeans grown in soil amended with untreated primary sludge from PVSC had phytotoxic symptoms that were generally atypical of those observed in the field but may have masked symptoms more similar to those of the field-grown soybeans. We were thus unable to determine whether the toxicant was present in the primary sludge or was a product of the Zimpro™ process. We were also unable to identify any common inorganic element as the cause of the reduction in the growth and development of the field-grown soybeans. We hypothesize that the toxicant may have been a persistent organic compound whose slow degradation in the soil was able to cause phytotoxicity several years after biosolids land application.

## INTRODUCTION

**D**URING the summer of 1999, phytotoxicity symptoms in soybeans (*Glycine max L.*) that had been fertilized with biosolids from the Passaic Valley Sewerage Commission (PVSC) wastewater treatment plant in Newark, NJ were reported in numerous fields in Essex County, VA by a Virginia Cooperative Extension agent (Keith Balderson, personal communication). These symptoms included marginal necrosis, interveinal chlorosis, leaf puckering and crinkling, stunted plant growth and, in full expression, entire leaf necrosis. Corn and wheat were not affected, but soybean grown in the rotation with corn and wheat exhibited symptoms as late as the second or third year following the biosolids application, from which we inferred that the suspected toxicant was not easily degraded or transported from the root zone and/or was active at very low concentrations. The phytotoxicity symptoms appeared to be more severe under drought-stressed conditions. Analyses of the biosolids and routine soil and plant tissue testing failed to identify a common deficiency/toxicity factor. Paired sampling of adjacent affected and non-affected buffer areas showed higher nutrient status but no difference in other potential causes (trace element concentration, soluble salts/electrical conductance, pH) in soils under the symptomatic soybeans. Soybean tissue analysis did not identify any potential nutrient deficiency or trace element toxicity.

The PVSC wastewater treatment plant receives influent from approximately 260 km$^2$ of northern New Jersey (PVSC, 2006). The wastewater influent is 15% industrial by volume. Industries served by the plant include electroplaters, metal finishers, pharmaceutical and organic chemical manufacturers, textile dyers, hospitals, electronic products manufacturers, and newsprint recycling mills. Most of the wastewater from industries in the plant's service area requires pre-treatment because of high concentrations of heavy metals.

After settling and gravity thickening, primary sludge at the PVSC treatment plant undergoes secondary treatment using the Zimpro™ wet air oxidation process

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(PVSC, 2006; USFilter Corporation, 2005). The process involves mixing liquid primary sludge with air in a reaction vessel at 220°C (425°F) and 4.485 MPa (650 psi) which decreases BOD, COD, and insoluble volatile solids during this low pressure oxidation (PVSC, 1996; PVSC, 2006). The material is subsequently dewatered via a belt filter press, and the resulting Class A product meets the alternative pollutant limits of Title 40 of the Code of Federal Regulations (CFR) Part 503 (Standards for the Use or Disposal of Sewage Sludge, or the Part 503 Rule) (PVSC, 1996).

Few researchers have specifically addressed the effects of Zimpro™-processed biosolids on plant growth. Neel et al. (1978) observed no adverse effects in a warm-season turfgrass sod grown in 10 cm of composted Zimpro™-processed biosolids from Fort Lauderdale/Hollywood, FL. The composted biosolids produced better turf sod than a local soil and other composted organic waste products. Wei et al. (1985) found that Zimpro™-processed biosolids from Oshkosh, WI applied at rates up to 112 Mg/ha for a single application, or at 134 Mg/ha in six annual applications, lowered soil bulk density; increased hydraulic conductivity, aggregate stability, and large pore volume; and did not adversely affect the growth of sudangrass (*Sorghum bicolor* sudanensis) and corn (*Zea mays* L.). Vail and Dewey (1985) applied Zimpro™-processed biosolids from Port Elizabeth, South Africa to turf and pastureland in a study designed to develop metal application rate guidelines. The biosolids contained relatively high levels of Zn (4554 mg/kg) by today's standards and Pb (1193 mg/kg) that exceeds the Part 503 Ceiling Concentration Limit, but a one-time application at rates up to 224 Mg/ha produced high-quality forage and turf whose plant tissue metal concentrations were well below reportedly phytotoxic levels.

Our objectives for this study were (1) to determine whether residual phytotoxicity existed in soils that exhibited yield suppression and/or various toxicity symptoms in the field, and (2) to determine whether the phytotoxicity exhibited by the Zimpro™-processed PVSC biosolids was due to a constituent in the primary sludge or was a result of the Zimpro™ wet air oxidation treatment process. We subjected the soybean plants used in the bioassay to both adequate moisture and drought-stressed conditions in order to determine whether the toxicity was exacerbated by moisture stress.

## MATERIALS AND METHODS

### Experiment 1

Two coastal plain soil series, Kempsville sandy loam (Fine-loamy, siliceous, subactive, thermic Typic Hapludults; Field A) and Suffolk sandy loam (Fine-loamy, siliceous, semiactive, thermic Typic Hapludults; Field B) were collected in spring 2000 from fields in Essex County, VA that had been amended with Zimpro™ biosolids in March 1997 (Table 1) and where phytotoxicity symptoms were observed in 1999. According to the biosolids applicator's records, Field A sustained "total plant loss," and the soybeans in Field B were "severely affected." A Suffolk sandy loam collected from an unamended buffer strip adjacent to the treated area and which had the same surface texture as the Zimpro™-amended Kempsville

***Table 1. Treatments for Greenhouse Experiments 1 and 2.***

| Experiment 1 | | |
|---|---|---|
| **Treatments** | **Field-applied Zimpro™-processed Biosolids Rate (Mg/ha)** | **Additional Amendments** |
| Control | 0 | Lime, P, and K applied to greenhouse pots |
| Field A | 23.1 | K applied to greenhouse pots |
| Field B | 21.3 | Lime P, and K applied to greenhouse pots |
| Reclamation site | 78.4 | 33.6 Mg/ha Blue Plains wastewater treatment plant (Washington, DC) lime-stabilized biosolids applied in field |
| **Experiment 2** | | |
| **Treatment** | **Description** | |
| Control | P and K were applied according to routine soil test recommendations. Lime was applied to adjust soil pH to 6.2. | |
| Pre-Zimpro™ | 22.4 Mg/ha PVSC primary sludge before Zimpro™ processing | |
| Post-Zimpro™ | 22.4 Mg/ha PVSC biosolids after Zimpro™ processing | |

and Suffolk soils was used as a control. We also included a treatment consisting of soil from a reclaimed sand and gravel mine in Aylett, VA (Reclamation Site) that had been amended with a reclamation (higher than agronomic) rate of Zimpro™ biosolids and lime-stabilized biosolids (Table 1) in fall 1998 (Daniels et al., 2002). No phytotoxic symptoms had been reported in the plants used to revegetate the mined land, but soybeans were not grown at this site.

Each soil was air dried, and sieved to < 2 mm. Subsamples of each soil were analyzed for pH, soluble salts, and Mehlich-1 extractable P, K, Ca, Mg, Zn, and Mn by the methods of Mullins and Heckendorn (2005) (Table 2). Total N was determined by EPA method 351.3 (U.S. EPA, 1979); $NO_3$-N by method SM 4500-$NO_3$ F (AWWA, 1998); $NH_4$-N by EPA method 350.2 (U.S. EPA, 1979), and total organic C by EPA method 415.1 (U.S. EPA, 1979). Particle size analysis was performed by the pipette method using oven-dry samples (Method 3A1: UDSA-NRCS, 1996). Moisture content at field capacity was determined by pressure-plate extraction at 33 kpa (1/3 bar) (Method 4B1a, USDA-NRCS, 1996).

Phosphorus and K as solutions of reagent grade $KH_2PO_4$ and/or KCl were added to each soil at rates recommended by Virginia Tech Soil Testing Laboratory for soybean production (Donohue and Heckendorn, 1994). Soils having a pH of less than 5.9 were limed to 6.2 with $Ca(OH)_2$ according to Virginia Tech Soil Testing recommendations prior to potting the soils (Donohue and Heckendorn, 1994). Plastic-lined pots were used to prevent leaching of growth-inhibiting constituent(s) and to accurately control soil moisture during irrigation. Six kg of each soil was added to each per pot, except for the Reclamation Site soil, whose higher organic matter content allowed only 5 kg per pot. Each soil treatment consisted of eight pots.

Water was applied to each pot to achieve 80% field capacity to permit equilibration of inorganic soil amendments in preparation for seeding. Five soybean seeds (*Glycine max L.* var. FFR-493) treated with Captan 50™ fungicide were planted to a depth of 1.5 cm in the surface soil of each pot. Soil moisture was maintained using mist irrigation until one week after germinated seeds emerged from the soil. Emergence and plant development data were collected until the first true leaves appeared. A week after emergence, the soybeans were thinned to 3 plants/pot, at which time we began watering the pots to 80% field capacity. Treatment observations were made several times per week throughout the course of the experiment. We thinned the soybean plants to 2 per pot and 1 per pot at two and three, respectively, weeks after emergence.

Treatments included two moisture regimes: 80% field capacity (WET) and 40% field capacity (DRY) × three soils (Suffolk without biosolids, Suffolk with Zimpro™-processed biosolids, and Kempsville with Zimpro™-processed biosolids). The Reclamation Site soil was only used in the WET (80% field capacity moisture) regime. Each of the 7 treatment combinations was replicated four times. The DRY moisture regime treatment was established at 30 days after emergence by gradually decreasing moisture content to 50% field capacity for one month before reducing it to 40%.

***Table 2. Properties of Soils Used in Experiments 1 and 2 before Liming and Fertilization.***

| | Control (Suffolk sl) | Field A (Kempsville sl) | Field B (Suffolk sl) | Reclamation Site |
|---|---|---|---|---|
| pH | 4.80 | 6.20 | 5.40 | 7.60 |
| Total Organic C (%) | 1.40 | 1.68 | 1.34 | 2.38 |
| Total N (%) | 0.08 | 0.09 | 0.08 | 0.29 |
| $NH_4$-N (mg/kg) | 11.0 | 12 | 14 | 4 |
| $NO_3$-N (mg/kg) | 23 | 20 | 16 | 19 |
| P* (mg/kg) | 17 | 112 | 42 | 89 |
| K (mg/kg) | 97 | 66 | 71 | 30 |
| Ca (mg/kg) | 228 | 774 | 330 | 4,988 |
| Mg (mg/kg) | 40 | 42 | 50 | 99 |
| Mn (mg/kg) | 19 | 9 | 12 | 12 |
| Zn (mg/kg) | 2 | 9 | 7 | 21 |
| Fe (mg/kg) | 19 | 44 | 12 | 15 |
| Cu (mg/kg) | 0.43 | 0.74 | 0.36 | 0.47 |
| B (mg/kg) | 0.11 | 0.12 | 0.09 | 0.21 |
| Sand (%) | 70 | 76 | 74 | 87 |
| Silt (%) | 26 | 20 | 24 | 9 |
| Clay (%) | 4 | 4 | 2 | 4 |

*P, K, Ca, Mg, Mn, Zn, Fe, Cu, B are expressed as extractable by Mehlich #1 method.

We sampled the lower symptomatic trifoliolate leaves from each plant of the Control and Zimpro™-amended treatment soils at anthesis (R2, 64 days after emergence). At 66 days after emergence, we sampled the three upper (younger) completely developed trifoliolate leaves from the Field A, Field B, and Control treatments to test for nutrient deficiency/toxicity in soybean (Sabbe et al., 2000). There were too few leaves remaining on the Reclamation Site treatment plants to allow sampling at this time. All plant tissue samples were dried for 4 to 6 days at 55°C, ground in a stainless steel Wiley mill, weighed into Pyrex beakers, and ashed in a muffle furnace at 450°C for 16 h. Ash was dissolved in 2 mL of concentrated $HNO_3$ on a hot plate and then refluxed for 2 h with 10 mL of 3 *M* HCl. After digestion, solutions were filtered and diluted to 25 mL with 0.1 *M* HCl. Samples were analyzed for Zn, Cu, B, Mn, Fe, P, Mg, Ca and K by inductively coupled plasma (ICP) atomic emission spectrometry using 40 mg $L^{-1}$ Y as an internal standard.

We harvested whole plants 145 days after emergence by cutting plants at the base. At this point, all of the seeds in the Reclamation Site plants had attained maturity, but some of those in the Field A and Field B treatments had not. Pods were separated from plants, and both pods and whole plants were oven dried at 55°C until constant weight was attained. We hand-removed as much of the root mass as possible from each pot and visually compared root mass, nodulation, and other rooting characteristics. The soil from each pot was then mixed and subsampled. Soil samples were analyzed for pH, soluble salts, and Mehlich 1 extractable P, Ca, Mg, K, Mn, Zn, Fe, Cu, and B by the methods of Mullins and Heckendorn, 2005.

## Experiment 2

For experiment 2, we used both primary sludge from the PVSC plant (Pre-Zimpro™) and the product of this same primary sludge after undergoing the Zimpro™ process at the PVSC plant (Post-Zimpro™) (Table 3). The primary sludge from the PVSC wastewater treatment plant was collected after screening and settling, but before digestion. The sludge was then dried to approximately 50% moisture at the wastewater treatment plant without Zimpro™ method processing. Chemical and physical analyses of both the primary sludge and Zimpro™-processed biosolids was performed by the following methods: percent solids (SM 25408; AWWA, 1998), total N (Method 351.3; U. S. EPA, 1979); $NO_3$ (SM 4500-$NO_3$ F; AWWA, 1998); NH4-N (Method 350.2; U.S. EPA, 1979); total organic C (Method 415.1; U.S. EPA, 1979); chloride (SM 4500-CL D; AWWA, 1998); and P, K, S, Ca, Mg, Na, Fe, Al, Mn, Cu, Zn, and B (SW 846-6010B; U.S. EPA, 1995). Calcium carbonate equivalence was determined by ASTM method C602-95a (ASTM, 2001).

***Table 3. Properties of Primary Sludge and Zimpro™-processed Biosolids from PVSC Wastewater Treatment Plant.***

| | PVSC primary Sludge (Pre-Zimpro™) | PVSC Zimprotm-processed Primary Biosolids (Post-Zimpro™) |
|---|---|---|
| Solids (g/kg) | 500.78 | 820.44 |
| CCE (g/kg) | <0.1 | <0.1 |
| Total Organic C (g/kg) | 434.2 | 347.5 |
| Total N (g/kg) | 53.4 | 29.8 |
| C/N ratio | 81.0 | 117.0 |
| $NH_4$-N (g/kg) | 8.9 | 10.1 |
| $NO_3$-N + $NO_2$-N (g/kg) | 0.02 | nd* |
| Organic N (g/kg) | 44.5 | 19.7 |
| P (g/kg) | 18.1 | 28.1 |
| K (g/kg) | 5.9 | 2.1 |
| S (g/kg) | 9.6 | 4.1 |
| Ca (g/kg) | 18.4 | 22.3 |
| Mg (g/kg) | 5.0 | 5.8 |
| Na (g/kg) | 9.4 | 2.4 |
| Fe (g/kg) | 8.7 | 16.0 |
| Al (g/kg) | 12.4 | 23.5 |
| Cl (g/kg) | 9.9 | 2.4 |
| Mn (mg/kg) | 355 | 544 |
| Cu (mg/kg) | 947 | 1,210 |
| Zn (mg/kg) | 82 | 1,580 |
| B (mg/kg) | 20 | 12 |
| Cd (mg/kg) | 6.0 | 11.0 |
| Ni (mg/kg) | 49 | 59 |
| Pb (mg/kg) | 91 | 125 |
| As (mg/kg) | 3.4 | 2.9 |
| Hg (mg/kg) | 1.6 | 2.7 |
| Se (mg/kg) | 2.2 | 1.9 |
| Mo (mg/kg) | 93 | 30 |

*nd = not detected

All treatments were applied to the limed control soil (Suffolk sandy loam) that was used in Experiment 1. Plastic-lined, 4.5 liter pots were filled with 5 kg soil and amended with the equivalent of 22.4 Mg/ha (dry weight basis) of the two PVSC materials. Both the primary sludge and the Zimpro™-processed biosolids were hand-mixed into the soil. A third treatment consisted of 5 kg of unamended control soil. As in Experiment 1, all three soil treatments received P and K as solutions of reagent grade $KH_2PO_4$ and/or KCl at rates recommended by Virginia Tech Soil Testing Laboratory for soybean production (Donohue and Heckendorn, 1994). Treat-

ments again included two moisture regimes: 80% field capacity (WET) and 40% field capacity (DRY) × three soil treatments (Suffolk without biosolids, Suffolk with PVSC primary biosolids, and Suffolk with PVSC Zimpro™-processed biosolids). Each of the 7 treatment combinations was replicated four times.

We used the same watering, seeding, germination, observation, and soybean thinning procedures as in Experiment 1. We initiated drought stress for the 40% field capacity treatments three weeks after emergence. The soybean plants were harvested 65 days after emergence, at which time approximately half of the plants were flowering. We harvested the whole plant because their small size required that the entire plant be used to provide enough material for analysis. Whole plant sampling and drying, root observations, and soil sampling and analysis were conducted as described for Experiment 1.

### Pest Control

Pest control for each greenhouse experiment was provided by spraying Avid 0.15 EC™ miticide (Abamectin) and Conserve SC™ insecticide (Spinosad, including Spinosyn A and Spinosyn D).

### Statistical Analyses

The WET (80% field capacity) and DRY (40% field capacity) treatments of each experiment were statistically analyzed as separate completely randomized experiments with four replications per treatment. Thus, we did not statistically compare data between the WET and DRY treatments of the experiments. Within each moisture regime of each experiment, treatment variations were analyzed by a least squares analysis of variance procedure (SAS Institute, 2002). Where the overall experiment-wide F-test was significant ($p < 0.05$), treatment means were separated by Fisher's LSD procedure.

## RESULTS AND DISCUSSION

### Soil Properties

Neither of the agricultural soils from Fields A and B exhibited any potential deficiencies in essential nutrients (Table 2; Donohue and Heckendorn, 1994) or abnormally elevated soil levels of potentially toxic trace elements (Kabata-Pendias and Pendias, 1984). Only the pH of the Field B soil (5.40) was considered to pose growth-limiting conditions for soybean. This was corrected with lime for the greenhouse experiment.

### Sludge and Biosolids Characteristics

The characteristics of the PVSC primary sludge and Zimpro™-processed biosolids are presented in Table 3. The Zimpro™ process apparently caused the loss of organic C and N. None of the measured macro or trace elements were present at concentrations that would be expected to cause phytotoxicity, although the USEPA 503 ceiling concentration limits for Mo (75 mg/kg) were exceeded in the primary sludge.

### Experiment 1—Soybean Growth in Previously Zimpro™-amended Soils

*Germination and Seedling Development*

There were no observable differences in germination rate and early seedling development that could be attributed to treatment. Within two weeks after emergence, marginal necrotic spotting appeared on the first true leaves in the Reclamation Site and Field A treatments and marginal chlorosis appeared on the older leaves in the Field B treatment. Toxicity symptoms increased in rate and intensity in the order Reclamation Site > Field A > Field B.

*Symptom Progression Before Flowering*

Between 15 and 30 days after emergence, Reclamation Site treatment plant leaves, which had been small, puckered, and crinkled during the emergence period, developed total necrosis. The earlier-described marginal necrotic spotting spread from the leaf tip to the entire leaf margin, and then moved inward. This effect progressed most rapidly in the oldest leaves (Figure 1), which eventually senesced and dropped. These leaves first showed dark brown necrotic spotting at the margin near the leaf tip, which then developed into a band of dark brown marginal necrosis which eventually extending around the entire leaf. The band then moved inwards, leaving dead tissue. The inside of the leaf then became almost completely yellow, and the leaf dropped off. All of the leaves on the Reclamation Site treatment plants, except for the newest trifoliolates, had marginal necrosis throughout the rest of the experiment.

The identical progression of symptoms occurred in the Field A plants, except the severity was less than in the Reclamation Site treatment. The Field B plants were

***Figure 1.*** *Close-up of marginal necrosis, crinkling and puckering, and overall growth stunting in soybeans growing in the Reclamation Site treatment with adequate moisture in Experiment 1. Approximately 78 Mg/ha Zimpro™-processed biosolids had been applied to the mine soil in the field 18 months previously.*

generally smaller than the Field A and Control plants. Many of the first true leaves and first trifoliolates on Field B plants exhibited diffuse, marginal chlorosis but not the marginal necrotic spotting observed in the Field A and the Reclamation Site treatments. Only a few leaves were slightly puckered and crinkled. Field B plants generally had fewer trifoliolate leaves than the Field A and Control plants.

Subsequent imposition of drought stress to the Control, Field A, and Field B treatments at 30 days after emergence reduced the overall growth of plants in all DRY treatments but did not exacerbate the previously observed leaf symptoms.

*Symptom Progression After Flowering*

Flowers appeared on all but one of the Reclamation Site treatment plants between 52 and 57 days after emergence. The final Reclamation Site treatment plant flowered at 72 days after emergence. The order of observed flowering among treatments was Control = Field A < Field B < Reclamation Site, with drought inducing earlier flowering. We removed lower and upper trifoliolates from all plants at 56 to 58 days after emergence for tissue analysis. At this time, approximately one-third of the older leaves in the Field A treatment in the WET moisture regime and one-half of the older leaves in the DRY moisture regime had some marginal necrosis. All leaves on the Field B plants under both moisture regimes exhibited more interveinal chlorosis than the Control and Field A plants. Chlorosis was more pronounced in plants in the Field B DRY treatment. The leaves of the Field B plants became progressively chlorotic after flowering but the plants set pods. The proportion of symptom-affected to non-symptomatic leaves was higher in plants in the Field A and Field B DRY moisture regime than in the WET moisture regime throughout the experiment. The Reclamation Site plants dropped many leaves during the experiment but rebounded from near death after flowering to produce new leaves that exhibited severe marginal necrosis and few or no small pods.

The symptoms on the Field A plants were similar to those that appeared on the Reclamation Site plants and unlike those that appeared on Field B plants. It is thus possible that the symptoms on the Field B plants may not have been caused by the same agent as that which caused necrosis in the Reclamation Site and Field A plants.

*Observations at Harvest*

We harvested pods at 145 days after emergence, at which time all plants had set seed. The DRY plants were shorter and reached the full pod stage earlier than the WET plants. The combined leaf and stem weights and the height of plants in the WET soil moisture regime were greater in the Control than in any Zimpro™-amended treatment (Table 4). The Field A and Field B plants accumulated more biomass and grew taller than Reclamation Site plants. There were no treatment differences among the growth characteristics of

***Table 4. Effects of Experiment 1 Treatments on Soybean Weight, Height, Pod Number, and Pod Weight.***

| | Leaf + Stem wt. (g) | | Height (cm) | | Pod Number | | Pod wt. (g) | |
|---|---|---|---|---|---|---|---|---|
| **Treatment** | **WET*** | **DRY** | **WET** | **DRY** | **WET** | **DRY** | **WET** | **DRY** |
| Control | 36.02a | 18.60a | 96.3a | 71.4a | 40a | 26a | 13.5a | 10.3a |
| Field A | 24.95b | 14.38a | 84.1b | 68.6a | 38a | 23a | 15.8a | 10.5a |
| Field B | 28.50b | 17.67a | 83.9b | 67.5a | 44a | 31a | 13.6a | 10.8a |
| Reclamation Site** | 5.80c | — | 47.3c | — | 19b | — | 7.4b | — |

*Means followed by the same letter within columns are not significantly different (p = 0.05).
**The reclamation site soil did not undergo a DRY moisture regime.

the DRY Control, Field A, and Field B plants, indicating that moisture stress was more growth limiting than any biosolids constituent(s). The mean number of soybean pods per plant and total pod weight did not differ among the Control, Field A, and Field B treatments in either soil moisture regime, but the Reclamation Site treatment (WET moisture regime only) produced lower values for these variables.

*Root Observations*

The root mass of the DRY plants appeared to be smaller than that of the WET plants. Within the WET soil moisture regime, roots of the plants in the Control, Field A, and Field B treatments had many nodules, while those in the Reclamation Site plants had none. The Reclamation Site plant roots were observed to be smaller and less fibrous than those in all of the other treatments. When the sizes of the root masses of the WET regime plants were compared visually, the root masses of the Control were larger than those of the Field A plants, which in turn were larger than those of the Field B plants. In the DRY regime plants, the same relative size relationship was observed.

*Post Harvest Soil Analysis*

At the completion of the experiment, none of the elements measured in the soil were present at a concentration that would be considered detrimental to plant growth (Table 5, Donohue and Heckendorn, 1994). Soluble salts and B in the Reclamation Site soil were elevated above the values in the other treatments, but B and salt concentrations were not high enough to have caused yield reductions in soybean (Maas, 1984).

*Plant Analysis Data*

We used sufficiency ranges for soybeans grown in the southern United States (Sabbe et al., 2000) to assess the effects of treatment on tissue nutrient levels. Tissue analysis of samples taken from older (lower), symptomatic soybean leaves was used to compare the Reclamation Site treatment with other treatments because the Reclamation Site treatment did not produce enough upper trifoliolates for sampling.

The upper trifoliolate leaves of the soybeans growing in the WET moisture regime Zimpro™-amended soils were higher in Zn and P (Table 6). Plant tissue Zn in the Field B, but not Field A, treatment was slightly above sufficiency levels (>80 mg/kg), from which we inferred that Zn toxicity was not the cause of the Zimpro™ related symptoms. Plant tissue grown in both the Control and Field B soils had slightly elevated Mn levels (>100 mg/kg), likely because of high native soil Mn levels, but all other macro- and micro-nutrient concentrations in plants grown in the Zimpro™-amended soils were within sufficiency ranges.

The lower trifoliolate leaves of the soybean plants (Table 7) showed no clear trends of deficiency or toxicity. Tissue Zn was higher in plants grown in Zimpro™-amended soils and exceeded the sufficiency range in the adequately watered soils, and tissue B was slightly below sufficiency levels in most treatments under both moisture regimes. Tissue analysis levels for older soybean leaves, however, cannot be correlated with average values for sufficiency, because these values were established for the most recently fully-expanded leaves. Tissue Mn levels were much higher in lower trifoliolates from both the control and Field B

***Table 5. Effects of Experiment 1 Treatments on pH, Soluble Salts and Mehlich I Extractable Soil Elements.***

| Treatment | pH | Soluble Salts (dS/m) | Mehlich I Extractable Elements (mg/kg) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Ca | Mg | P | K | Mn | Zn | Fe | Cu | B |
| **WET Moisture Regime*** | | | | | | | | | | | |
| Control | 6.38c | 0.31b | 660c | 41d | 21c | 41a | 13b | 3c | 10b | 1.3a | 0.10b |
| Field A | 6.25d | 0.30b | 737bc | 46c | 98b | 30ab | 5d | 11b | 38a | 1.3a | 0.09b |
| Field B | 6.52b | 0.67b | 767b | 59b | 49c | 22b | 15a | 9b | 10b | 1.2a | 0.10b |
| Reclamation site | 7.30a | 2.13a | 5,780a | 111a | 159a | 24b | 10c | 34a | 7c | 1.1a | 0.21c |
| *P < F* | *0.0001* | *<0.0001* | *<0.0001* | *<0.0001* | *<0.0001* | *0.0236* | *<0.0001* | *<0.0001* | *<0.0001* | *0.26271* | *<0.0001* |
| **DRY Moisture Regime** | | | | | | | | | | | |
| Control | 6.45a | 0.34b | 701b | 42b | 24c | 43a | 13b | 3c | 10b | 1.1a | 0.11a |
| Field A | 6.22a | 0.25b | 748ab | 47b | 102a | 36a | 5c | 11a | 38a | 1.3a | 0.10a |
| Field B | 6.50a | 1.08a | 821a | 64a | 57b | 49a | 16a | 10b | 10b | 1.2a | 0.11a |
| *P < F* | *0.1719* | *0.019* | *0.030* | *0.0005* | *<0.0001* | *0.6321* | *0.0002* | *<0.0001* | *<0.0001* | *0.4521* | *0.5676* |

*Means followed by the same letter within columns by moisture regime are not significantly different (p = 0.05).

***Table 6. Effects of Experiment 1 Treatments on Elemental Composition of Soybean Upper Trifoliolate Leaves. (Upper Trifoliolates of Reclamation Treatment Plants were Not Analyzed).***

| | Element (mg/kg) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Treatment** | **Zn** | **Cu** | **B** | **Mn** | **Fe** | **P** | **Mg** | **Ca** | **K** |
| | | | | **WET Moisture Regime*** | | | | | |
| Control | 47c | 8b | 26a | 104a | 82a | 2.86c | 3.22a | 12.5b | 17.3b |
| Field A | 68b | 7b | 30a | 50b | 80a | 3.23b | 3.13a | 10.6c | 16.7b |
| Field B | 91a | 11a | 23a | 102a | 93a | 3.79a | 3.51a | 15.3a | 19.4a |
| *P < F* | *<0.0001* | *<0.0001* | *0.6478* | *0.0002* | *0.2556* | *0.0004* | *0.1322* | *0.0002* | *0.0248* |
| | | | | **DRY Moisture Regime** | | | | | |
| Control | 48b | 6a | 34a | 103b | 103a | 2.39b | 2.68b | 11.1b | 15.8b |
| Field A | 69a | 7a | 20a | 45a | 119a | 2.85a | 3.08a | 9.2c | 15.8b |
| Field B | 60ab | 7a | 17a | 104b | 113a | 3.04a | 3.13a | 15.6a | 19.0a |
| *P < F* | *0.0501* | *0.6713* | *0.3664* | *0.0006* | *0.2079* | *0.0327* | *0.0456* | *0.0006* | *0.0545* |

*Means followed by the same letter within columns by moisture regime are not significantly different (p = 0.05).

treatments than in the Field A and the Reclamation site treatments which indicates that Mn did not cause the phytotoxic symptoms in the Field A and Reclamation Site treatments.

*Physiological Interpretation*

Based on the occurrence of symptoms in plant parts, the toxicant appeared to be xylem-mobile (water soluble) and caused effects similar to soluble salts, boron, and a variety of known herbicides and other bio-active compounds (personal communication, Dr. Larry Foy, Virginia Tech). Excessive soluble salts and B damage were eliminated as possible causes based on soil and plant tissue analyses. Isolation and identification of other compound(s) was not possible without extensive screening analyses of numerous organic and additional inorganic compounds in the Zimpro™-processed product.

*Overall Results*

In Experiment 1, a reduction of soybean yield was only observed in the Reclamation Site treatment. The marginal necrosis, with some increased leaf puckering, tended to appear on a higher percentage of the leaves of soybeans growing in the DRY (moisture stressed) Zimpro™-amended treatments, but the yield-suppressing effect of any toxicant was not exacerbated by drought as evidenced by the lack of differences in soybean growth among the Control, Field A and Field B plants. We were not able to correlate soil or tissue analytical data with the soybean leaf symptoms.

***Table 7. Effects of Experiment 1 Treatments on Elemental Composition of Soybean Lower Trifoliolate Leaves.***

| | Element (mg/kg) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Treatment** | **Zn** | **Cu** | **B** | **Mn** | **Fe** | **P** | **Mg** | **Ca** | **K** |
| | | | | **WET Moisture Regime*** | | | | | |
| Control | 74c | 7b | 17a | 121b | 89a | 1.80a | 4.80b | 27.3b | 5.5b |
| Field A | 94b | 6b | 18a | 28c | 97a | 2.36a | 4.77b | 24.2bc | 7.5b |
| Field B | 111a | 7b | 18a | 226a | 91a | 2.20a | 6.76a | 40.7a | 8.3b |
| Reclamation site | 122a | 13a | 74a | 18c | 95a | 2.92a | 4.26b | 22.0c | 14.4a |
| *P < F* | *0.0002* | *<0.0001* | *0.0661* | *<0.0001* | *0.293* | *0.2946* | *0.0032* | *<0.0001* | *0.0003* |
| | | | | **DRY Moisture Regime** | | | | | |
| Control | 43b | 5a | 16a | 124a | 109a | 1.70c | 3.68a | 22.6a | 9.7a |
| Field A | 66a | 5a | 37a | 32a | 120a | 2.19a | 4.21a | 20.5a | 10.6a |
| Field B | 64a | 4a | 19a | 207a | 108a | 2.00b | 5.80a | 37.0a | 10.5a |
| *P < F* | *0.0172* | *0.5329* | *0.1753* | *0.0002* | *0.0652* | *0.0005* | *0.0041* | *0.0069* | *0.326* |

*Means followed by the same letter within columns by moisture regime are not significantly different (p = 0.05).

## Experiment 2—Effects of PVSC Primary Sludge and PVSC Zimpro™-processed Biosolids on Soybean Growth

*Germination and Seedling Development*

There were no observable effects of treatment on germination and emergence rate, but plants in the Control treatment were uniformly taller than those in both the Pre- and Post-Zimpro™ amended soils one week after emergence. Many of the first true leaves in the Pre- and Post-Zimpro™ treatments exhibited crinkling symptoms.

Marginal necrosis similar to that observed in the Reclamation Site and Field A plants in Experiment 1 was visible on almost all of the first true leaves of the Post-Zimpro™ plants approximately three weeks after emergence. The symptom progression was similar to that observed in Experiment 1, i.e., necrotic spotting at the leaf tip which spread to form a band of dark necrotic tissue around the entire leaflet. Interveinal chlorosis and leaf puckering also occurred. Younger leaves on the Pre-Zimpro™ plants exhibited puckering but not necrosis. The size of the leaves decreased according to treatment in the order Control > Post-Zimpro™ > Pre-Zimpro™. Marginal necrosis of the older trifoliolates was more extensive on Post-Zimpro™ than Pre-Zimpro™ plants, but the Pre-Zimpro™ older trifoliolates additionally exhibited white spotting, and more extensive interveinal chlorosis, puckering, and crinkling. Drought exacerbated the crinkling/puckering effect caused by both the Pre-Zimpro™ and Post-Zimpro™ treatments.

*Observations at Harvest*

Both Pre- and Post-Zimpro™ plants flowered earlier than the Control plants, which did not begin to flower until harvest (65 days after emergence). The Post-Zimpro™ plants in both moisture regimes were larger than the Pre-Zimpro™ plants at harvest, despite exhibiting marginal necrosis on approximately 50% to 75% of the leaves. The Post-Zimpro™ plants grown under the DRY moisture regime resembled the Reclamation Site plants from Experiment 1 in expression and extent of symptoms.

Only above-ground biomass and height were measured because the plants were harvested at the time of flowering (Table 8). There were no differences in plant biomass or height in the DRY treatment, but the biomass and height of Pre-Zimpro™ and Post-Zimpro™ plants were lower than Control plants in the WET moisture regime. This indicated to us that the addition of both PVSC Zimpro™ and the primary sludge from which the Zimpro™ biosolids were produced retarded soybean growth up to at least early flowering.

Growth was reduced in the order Control > Post-Zimpro™ > Pre-Zimpro™ indicating that either (1) the plant growth limiting factor(s) was present in the primary sludge, (2) Zimpro™ processing did not exacerbate the detrimental effect of the toxicant, and/or (3) the symptoms and reduced plant growth were caused by different factors in the two PVSC materials. The Pre-Zimpro™ sludge caused some symptoms in soybean seedlings (e.g., white spotting) that were morphologically different than symptoms in seedlings grown in the Post-Zimpro™ treatment or in soybeans observed in the field.

*Root Observations*

Plant root mass was longer and more branched under WET than DRY soil moisture conditions in all treatments. In both the WET and DRY moisture regimes, the root mass of the Control plants was larger than the root mass of the Post-Zimpro™ and Pre-Zimpro™ plants. As with the biomass response, the root mass of the Control treatment under both moisture regimes was greater than either the Pre- and Post-Zimpro™ treatments. Nodulation was only observed on plants in the WET regime of the Control treatment.

*Soil Properties*

Both primary sludge and Zimpro™-processed biosolids additions reduced soil pH and increased concentrations of soluble salts and nearly every macro- and micro-nutrient (Table 9). The addition of the Pre-Zimpro™ primary sludge resulted in a lower pH than that generated by the Post-Zimpro™ biosolids, possibly because the primary sludge contained oxidizable acidity-producing organic N and S compounds and/or had higher soluble salt concentrations that

***Table 8. Effect of PVSC Primary Sludge (Pre-Zimpro™) and Zimpro™–processed Biosolids (Post-Zimpro™) on Soybean Biomass and Height in Experiment 2.***

| | Biomass (g) | | Height (cm) | |
|---|---|---|---|---|
| **Treatment** | **WET*** | **DRY** | **WET** | **DRY** |
| Control | 11.99a | 1.70a | 66.5a | 26.6a |
| Pre-Zimpro™ | 2.80b | 1.01a | 32.2c | 21.5a |
| Post-Zimpro™ | 5.62c | 1.25a | 46.2b | 24.4a |

*Means followed by the same letter within columns are not significantly different (p = 0.05).

***Table 9. Effect of PVSC Primary Sludge (Pre-Zimpro™) and Zimpro™–processed Biosolids (Post-Zimpro™) on pH, Soluble Salts, and Mehlich I Extractable Soil Nutrients in Experiment 2.***

| Treatment | pH | Soluble Salts (dS/m) | Mehlich I Extractable Elements (mg/kg) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Ca | Mg | P | K | Mn | Zn | Fe | Cu | B |
| **WET Moisture Regime*** | | | | | | | | | | | |
| Control | 6.20a | 0.30c | 702bc | 41c | 24b | 61c | 19c | 3c | 10b | 0.9b | 0.13a |
| Pre-Zimpro™ | 5.20c | 2.26a | 767a | 68a | 43a | 121a | 46a | 4b | 14a | 1.6a | 0.15a |
| Post-Zimpro™ | 5.62b | 1.12b | 743a | 55b | 38a | 99b | 33b | 5a | 13a | 1.1b | 0.15a |
| *P < F* | *<0.0001* | *<0.0001* | *0.0202* | *<0.0001* | *0.0003* | *<0.0001* | *<0.0001* | *<0.0001* | *<0.0001* | *0.0073* | *0.2187* |
| **DRY Moisture Regime** | | | | | | | | | | | |
| Control | 6.50a | 0.70c | 749a | 46c | 30c | 107b | 20c | 3c | 10b | 0.9b | 0.14b |
| Pre-Zimpro™ | 5.25c | 2.17a | 779a | 69a | 39b | 129a | 49a | 4b | 14a | 1.4a | 0.16a |
| Post-Zimpro™ | 5.45b | 1.37b | 762a | 56b | 48a | 118ab | 39b | 6a | 14a | 1.1ab | 0.14b |
| *P < F* | *<0.0001* | *0.0007* | *0.396* | *0.0001* | *0.0019* | *0.0688* | *<0.0001* | *0.0012* | *0.0007* | *0.03* | *0.0016* |

*Means followed by the same letter within columns by moisture regime are not significantly different (p = 0.05).

would lower pH measured in water by displacing potential acidity from the solid matrix. Chloride concentrations in the Pre-Zimpro™ primary sludge were much higher than in the Post-Zimpro™ biosolids, but the soluble salt concentration in the Pre-Zimpro™-amended soil would not have been expected to reduce soybean growth (Maas, 1984). A combination of increased Mn concentration, reduced soil pH, and, perhaps, reducing conditions may have promoted Mn toxicity (Kabata-Pendias and Pendias, 1984).

*Plant Analysis Data*

Manganese and Zn concentrations in plant tissue grown on the Pre- and Post-Zimpro™ treatments were higher than in the Control, presumably due to the soil enrichment of Mn and Zn from primary sludge or biosolids application coupled with lower soil pH (Table 10). Tissue Zn concentration under the WET moisture regime appeared to be inversely related to soil pH.

Tissue Mn concentrations (745 and 861 mg/kg) of whole plants grown in the Pre-Zimpro treatment was greater than published phytotoxicity threshold values of 400–500 mg/kg for leaf tissue by Kabata-Pendias and Pendias (1984) and 720 mg/kg for whole plant by Fageria (2001). Soybeans grown in this treatment may have suffered Mn toxicity, symptoms which include stunting and crinkled young leaves, Fe chlorosis, necrotic spotting, and uneven chlorophyll distribution in older leaves (Kabata-Pendias and Pendias, 1984). Manganese uptake by plants is positively correlated with soil organic matter concentration and negatively correlated with soil pH and redox. Symptoms of Mn toxicity

***Table 10. Effect of PVSC Primary Sludge (Pre-Zimpro™) and Zimpro™-processed Biosolids (Post-Zimpro™) on Soybean Whole Plant Tissue Elemental Composition at Flowering in Experiment 2.***

| Treatment | Element (mg/kg) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Zn | Cu | B | Mn | Fe | P | Mg | Ca | K |
| **WET Moisture Regime*** | | | | | | | | | |
| Control | 34b | 10a | 27a | 119b | 85a | 2.79a | 3.35b | 15.8c | 23.9a |
| Pre-Zimpro™ | 91a | 22a | 31a | 861a | 104a | 1.98a | 4.09a | 26.3a | 22.5a |
| Post-Zimpro™ | 64a | 9a | 25a | 289b | 90a | 2.48a | 3.96a | 20.4b | 23.2a |
| *P < F* | *0.0989* | *0.6106* | *0.1289* | *0.01* | *0.4844* | *0.1115* | *0.0292* | *0.0007* | *0.1736* |
| **DRY Moisture Regime** | | | | | | | | | |
| Control | 48a | 4a | 30a | 110c | 82a | 1.37a | 3.33a | 20.1b | 23.9a |
| Pre-Zimpro™ | 43a | 3a | 33a | 745a | 69a | 1.19a | 3.64a | 27.6a | 22.5a |
| Post-Zimpro™ | 47a | 5a | 38a | 460b | 74a | 1.73a | 3.17a | 18.7b | 23.2a |
| *P < F* | *0.0281* | *0.1414* | *0.5877* | *0.0014* | *0.2814* | *0.3453* | *0.1573* | *0.0195* | *0.687* |

*Means followed by the same letter within columns by moisture regime are not significantly different (p = 0.05).

are generally observed in younger leaves, but there is some evidence that Mn can be translocated to older plant tissue when present at high soil levels (Kabata-Pendias and Pendias, 1984). Drought-stressed plants in the Post-Zimpro™ treatment also accumulated high concentrations of Mn (460 mg/kg).

Whole plant Zn and Cu levels in the WET moisture regime of the Pre-Zimpro™ treatment were also high, but the variability between plants in this treatment was so great that the difference was not statistically significant. These differential metal levels may also have been the result of the acidic soil pH levels discussed earlier.

*Overall Results*

Amendment of soil with the Pre-Zimpro™ sludge resulted in soybean plants that were smaller than soybeans grown in soil amended with resultant Post-Zimpro™ biosolids. The soybeans grown in the Pre-Zimpro™-amended soil exhibited symptoms such as white spotting, crinkling and puckering, and chlorosis, but did not exhibit the distinctive continuous marginal necrosis typical of the leaves of soybeans grown in Zimpro™-amended soil. Soil testing and plant analysis indicated that yield and morphology of plants grown in soil amended with the primary Pre-Zimpro™ sludge may have been caused by Mn toxicity. Soil and plant tissue analyses did not reveal any elemental toxicity or soluble salt injury that might have directly caused the plant symptoms in either the Pre-Zimpro™ or Post-Zimpro™ treatments.

## CONCLUSIONS

We were able to successfully replicate the 1999 field-observed phytotoxicity symptoms in soybeans grown in soils amended with Zimpro™-processed PVSC biosolids in the greenhouse. We observed the identical progression of symptoms (i.e., leaf puckering/crinkling, marginal necrosis and necrotic spotting, interveinal chlorosis, stunted growth and, finally, full necrosis) that was reported for soybeans in Zimpro™-amended soils in Essex County, VA. These symptoms appear to be specifically and consistently associated with Zimpro™-processed biosolids from the PVSC treatment plant. The symptoms were most evident in plants growing in the treatment which had received the highest rate of Zimpro™-processed biosolids (Reclamation Site). Moisture stress reduced plant size, increased the number of leaves with phytotoxic symptoms, and exacerbated symptoms in some treatments, but did not cause any symptoms that were not also observed in soybeans which received adequate moisture.

Poor plant growth and phytotoxicity symptoms were observed in soybeans growing in soils amended with both the Pre-Zimpro™ sludge and the Post-Zimpro™ biosolids from the PVSC wastewater treatment plant. The appearance of the symptoms and the extent of plant growth reduction indicated that either (1) the toxic agents in the two materials were different or (2) the detrimental effects of the Pre-Zimpro™ sludge masked the symptoms observed in the field and in the first greenhouse experiment. The plant tissue analysis and symptoms expressed by the plants grown in the Pre-Zimpro™-amended soils were indicative of Mn toxicity. The composition of the primary sludge (high organic C content, high soluble salt content) may have induced the low soil pH and redox conditions that favor solubilization of Mn. The soil treated with Pre-Zimpro™ sludge had a lower pH, higher electrical conductivity, and higher concentration of Mehlich I extractable Mn than those treated with the Post-Zimpro™ biosolids although the concentration of total Mn was lower in the sludge than in the resulting biosolids.

It is not clear from our work whether or not the toxic agent was a product of the Zimpro™ process. The agent may have been present in the raw sludge and, subsequently concentrated and/or altered by the Zimpro™ process. Some symptoms in the Essex County field soybeans were similar to the Mn toxicity-like symptoms induced by the Pre-Zimpro™ sludge in the greenhouse experiment. In the field soils, however, the causative agent persisted for several years after application of Zimpro™-processed biosolids. Such persistence, coupled with the lower than toxic concentrations of Mn in plant tissue and soil samples, likely eliminate Mn toxicity as the cause of the phytotoxic symptoms in the Essex County field soybeans.

The toxicant may be an organic compound which exhibits species-specific plant growth regulator activity because soybean, but not corn or wheat, were affected, and may be slowly mineralized or released/desorbed into solution for plant uptake. Similar phytotoxicities have been documented following the application of other waste by-products onto land. Several pyridine carboxylic acid herbicides persisted at concentrations that caused phytotoxicity in sensitive crops grown in soils amended with compost produced from turfgrass that had been treated with clopyralid or with compost

produced from manure excreted by livestock that had ingested picloram-treated tall grass hay (Bezdicek et al., 2001; Houck and Burkhart, 2001; Rynk, 2002). Despite the mineralization and subsequent reduction in activity in these herbicides during the biological degradation that occurred in the compost windrows, these compounds remained bioactive at concentrations of less than 10 ppb. These herbicides mimic plant growth regulators and cause symptoms similar to those described in the field soybeans in Virginia, such as crinkling, puckering, chlorosis, and necrosis.

Further analysis of a wide range of organic chemical constituents would be required to determine the exact cause of the phytotoxicity that might initially be present in the wastewater treatment plant sludge or created during the wet air oxidation processing of the biosolids. An exhaustive review of industrial inputs and analyses of wastewater sludge and finished product, which was beyond the scope of this investigation, would be needed.

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# Chemical, Physical and Leaching Studies of Bottom Ash from a Medium-sized (32 MW) Municipal District Heating Plant for Assessing its Suitability for an Earth Construction Agent and for a Fertilizer used in Agriculture and in Forestry

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**ABSTRACT:** In Finland, the new limit values for the maximal allowable heavy metal concentrations in agricultural and forestry fertilizers came into force in March 2007 and for materials used as an earth construction agent in July 2006. From the utilization point of view, it was notable that the total heavy metal concentrations in the bottom ash originating from the 32 MW municipal district heating plant of Kemin Energia Oy at Kemi, Northern Finland, did not exceed the maximal allowable heavy metal concentrations for ash used as a fertilizer in agriculture and in forestry. Furthermore, the total heavy metal as well as the leachable heavy metal and the leachable DOC, fluoride, sulphate and chloride concentrations in the extractant were lower than the limit values for materials used as an earth construction agent. According to a five-stage sequential leaching study, in which the distribution of heavy metals in the bottom ash between the water soluble ($H_2O$), exchangeable ($CH_3COOH$), easily reduced ($NH_2OH$-HCl), oxidizable ($H_2O_2$ + $CH_3COONH_4$), and residual fractions (HF + $HNO_3$ + HCl) were evaluated, the highest concentrations of most of the heavy metals occurred in the residual fraction, which means that heavy metals in ash are tightly bound to the matrix. The results indicated that, the bottom ash is a potential material to be used especially as an earth construction agent.

## INTRODUCTION

UTILIZATION of forestry and sawmill residues (e.g. bark, wood chips, sawdust), as well as peat in heat and power production is a common and environmentally friendly alternative to other energy production (e.g. fuel oil, coal) technologies. Especially, the incineration of sawmill residues plays an increasingly important role in the transition towards a $CO_2$-lean energy system since, the use of wood bioenergy is that it could significantly reduce the use of fossil fuels and the formation of carbon dioxide emissions. In addition, the use of sawmill residues and peat for energy production has a number of advantages over other sources—they are domestic and usually local, their use creates jobs, and promotes silviculture in the forests. However, energy generation from wood waste and peat produces a considerable amount of ash. In Finland, the pulp and paper industry produces alone ca. 240,000 tons of ash per year. Other significant ash sources are municipal district heating plants, as well as agriculture and households where ash is generated in small (< 50 MW) power plants. Most of the ash is still dumped in landfills. Only less than 10% of the ashes are recycled into back to forest ecosystems [1].

The ash usually contains plant nutrients, especially base cations, and has a strongly alkaline pH. It would be ecologically beneficial if the solid residues, e.g. ash, that contain nutrients could be returned back to the forest ecosystem [2]. However, it is well know that, ashes can contain compounds that may give rise to pollution on disposal or during reuse. Ash is known to contain a variety of heavy metals which, under certain conditions, can leach out. Because the mobile elements are

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non-degradable or conservative, their long-term fate is a matter of concern from the viewpoint of pollution and sustainability. This is especially so for ashes derived from combustion of fossil fuels, because this leads to the mobilization of elements that were previously sequestered geochemically [3]. Therefore, the estimation of leaching (extraction) potential especially for heavy metals is important in assessing the possible environmental impacts associated with the ash reuse and disposal.

Information about environmental impacts essential when disposal or utilization decisions are made about solid waste. Decisions made regarding the appropriate waste management options (i.e. disposal, beneficial reuse) of solid waste frequently rely on the assessment of risk. In order to evaluate the risk, many extraction tests have been development and are widely use under a vast range of waste management scenarios. In the European Union (EU), the properties of solid wastes, especially when they are utilized or taken to a landfill, have to be known. From the environmental point of view, it is not the total element concentrations in ash, which is of prime importance, but rather how easily the elements are mobilized in the environment. The total elemental concentration represent a source term only for the unrealistic environmental scenario in which the entire mineral structure of the solid material is dissolved. Thus, measurement of the total concentration of metals provides relatively misleading information for assessing the bioavailability, toxicity and mobility of metals. In order to estimate the bioavailability of metals and their potential mobility it is necessary not only to determine the total concentrations but also the different chemical forms or the processes binding the heavy metals to the solid phase of the sample [4].

Sequential leaching (extraction) is a way to determine the actual metal activity in the environment, and provide a new perspective on analytical control. In the sequential leaching procedure, chemical extractants of various type are applied to the sample, each successive treatment being more drastic than the previous one [5]. The aim of this method is to divide the total leachable concentrations of metals into fractions in order to assess the form in which the metals occur in the waste material. Leaching is by no means equivalent to the total decomposition, and the leachable recoveries of analytes are generally lower than the total concentrations. Recoveries can only reach the total values if an element is completely soluble in the extraction solvent. Leaching studies are often applied in assessing worst case environmental scenarios, in which the individual components of the sample become soluble and mobile [6].

Although a large number of different methodological approaches have been developed and adapted to the sequential leaching procedures, most of them mimic the basic method initially developed by Tessier et al. [7]. They used the procedure to fractionate metals into the following fractions: (1) exchangeable fraction, representing the most easily available metals, (2) acid-soluble (carbonate bound) fraction, (3) reducible (Fe—Mn oxide bound) fraction, (4) oxidizable (organically + sulphide bound) fraction, and (5) residual fraction, tightly bound to the silicate matrix of the sample. If the heavy metals are present in the loosely bound fractions, such as the water soluble or exchangeable fraction, then they tend to be readily mobile and dispersed, whereas metals associated with the silicate matrix are not easily separated or mobilized [8].

## BACKGROUND

### Aims of the Study and Previous Work

The aims of this study was to investigate the physical and chemical properties (i.e. plant nutrient concentrations, dry matter content, neutralizing and reactivity values, LOI, TOC, pH and electrical conductivity), as well as the total concentrations of Cd, Cu, Pb, Cr, Zn, As, V, Ni, Ba, Mo and Hg in the bottom ash originating from the fluidized bed boiler at the medium sized (32 MW) municipal district heating plant of Kemin Energia Oy, at Kemi, Northern Finland. These are the heavy metals for which the European Union (EU) and Finnish legislation set the maximal allowable concentrations if the ash is utilized as a fertilizer or as an earth construction agent. In addition, when ash is used as an earth construction agent, the Finnish waste legislation sets the maximal concentrations for certain heavy metal, as well as for the maximal concentrations for fluoride, sulphate, chloride and DOC in extractant. In order to assess the mobility and potential bioavailable of heavy metals in the bottom ash, we used a five-stage sequential leaching procedure to determine the distribution of Cu, Pb, Cr, Zn, As, V, Ni and Ba in bottom ash into the following fractions: (1) water soluble fraction ($H_2O$), (2) exchangeable fraction ($CH_3COOH$), (3) easily reduced fraction ($NH_2OH$-HCl), (4) oxidizable fraction ($H_2O_2 + CH_3COONH_4$), and (5) residual fraction (HF + $HNO_3$ + HCl). This study is a part of a major project in

which the sequential leaching procedure is being used for assessing the leachability of heavy metals in bottom ash and fly ash [9] originating from the large-scale municipal district heating plants (266/315 MW), as well as in grate-fired boiler wood ash [10] and cyclone fly ash [11] from a small scale (6 MW) municipal district heating plant.

### Sampling

The bottom ash investigated in this study was obtained from the municipal district heating plant of Kemin Energia Oy at Kemi, Northern Finland. The plant has a 32 MW bubbling fluidized bed boiler (BFB), and it started to operate at the end of 2006. At the present time, approximately 80% of the energy produced by the BFB boiler originates from the incineration of peat, and ca. 20% from the incineration of forest residues (i.e. bark, wood chips, sawdust).

The sampling was carried out over a period of 5 days, and the individual samples were combined to give one composite sample with a of 5 kg (wet weight). The sampling period represented normal process conditions for the plant, e.g. $O_2$ content and temperature. A coning and quartering method was applied repeatedly to reduce the wet ash sample to a size suitable for the laboratory analyses. After sampling, the samples were stored in polyethylene bottles in a refrigerator (+4°C).

## EXPERIMENTAL

### Determination of the Physical and Chemical Properties

The pH and electrical conductivity (EC) of the bottom ash was determined by a combination pH/EC analyser equipped with a Thermo Orion Sure Flow pH electrode (Turnhout, Belgium) and a Phonix conductivity electrode with a cell constant of 1.0. pH and EC were determined according to the SFS-EN 13037 European standard [12] at a solid to liquid (S/L) ratio of 1:5

Determination of the dry matter content of the bottom ash was carried out according to the SFS-EN European standard 12880 [13], in which a sample is dried overnight to a constant mass in an oven at 105°C. The organic matter content, as measured by the loss-on-ignition (LOI), was determined according to the SFS-EN 12879 European standard [14], in which an oven-dried (105°C) sample is heated overnight in a muffle furnace (Box Furnace, Lindberg, Blue M, Asheville, USA) at 550°C. The total organic carbon (TOC) content was determined according to the SFS-EN 13137 European standard [15] using a Leco CHN-600 analyser (Leco Inc., USA), in which a sample is combusted and the evolved carbon dioxide is measured by infrared spectrometry.

The neutralizing (liming effect) value and reactivity value were determined according to the SFS-EN 12945 European standard [16] and the SFS-EN 13971 European standard [17], respectively. In determining the neutralizing value, the dried sample was dissolved in a specific quantity of a standard hydrochloric acid solution. Determination of the excess acid was performed by titration with a standard sodium hydroxide solution. Determination of the reactivity value is based on the decomposition of carbonates with acids.

### Determination of the Easily Soluble Plant Nutrient Concentrations

The determination of easily soluble nutrients such as Ca, Mg, Na, K, S, P, Mn, Cu and Zn in the bottom ash was carried out according to the procedures of MTT Agrifood Research Finland [18]. Easily soluble Ca, Mg, Na, K, S and P were extracted with 0.5 M acidic ammonium acetate (pH 4.65). In the extraction of Mn, Cu and Zn, the acidic (pH 4.65) ammonium acetate extract contained 0.02 M ethylenediaminetetra-acetic acid disodium salt ($Na_2EDTA$). In both extraction procedures, one volume part of a dry sample (i.e. ash) was shaken with ten parts of extraction solution for 1 h. Before determination, the extract was separated from the solid residue by filtration through a Schleicher & Schuell 589 blue ribbon filter paper (12.5 mm diameter).

The concentration of P in the extract was determined spectrophotometrically by the molybdenum blue method [19] using an automatic Foss–Tecator FIAStar 5000 Flow Injection Analyser (Högnes, Sweden). The concentrations of Ca, Mg, Na, K and S were determined by inductively coupled plasma optical emission spectrometer (ICP/OES, Thermo Elemental Iris Interepid II XDL, Franklin, USA), and the concentrations of Mn, Cu and Zn by flame atomic absorption spectrometer (FAAS, Perkin Elmer Aanalyst 700, Norwalk, USA). Before the nutrient determination on the bottom ash, the sample was dried overnight to constant mass at 105°C in a drying oven (Termaks) according to the SFS-EN 12880 European standard [13].

### Determination of the Total Element Concentrations

For the determination of total nutrient concentrations (Ca, Mg, Na, K, S, P, Mn, Cu and Zn), as well as total heavy metal concentrations (Cd, Cu, Pb, Cr, Zn, As, V, Ni, Ba, Mo and Hg) in the bottom ash, the dried sample was digested with a mixture of HF (3 mL) and $HNO_3$ (9 mL) in a CEM Mars 5 microprocessor controlled microwave oven with CEM HP 500 Teflon vessels (CEM corp., Matthews, USA) using US EPA method 3052 [20]. After cooling the digestion vessels, 5 mL of $H_3BO_3$ (27.5 g/ 500 mL) was added and heated for 7 min in the microwave oven. The cooled solutions were transferred to 100 mL volumetric flasks and the solutions were diluted to volume with ultrapure water. The ultrapure water was generated by an Elgastat Prima reserve osmosis and Elgastat Maxima ion exchange water purification system. All reagents and acids were suprapure or pro analysis quality.

Except for Hg, the total element concentrations in the ash were determined with a Thermo Elemental IRIS Intrepid II XDL Duo inductively coupled plasma optical emission spectrometer (Franklin, USA). The concentration of Hg in the ash were determined with a Perkin Elmer Aanalyst 700 cold-vapour atomic absorption spectrometry (Norwalk, USA) equipped with a Perkin Elmer FIAS 400 and AS 90plus autosampler.

### Leaching Procedures and Determination of Leachable Concentrations in Extractants

For the determination of the leachable concentrations of Sb, As, Ba, Cd, Cr, Cu, Hg, Pb, Mo, Ni, V, Zn, Se, DOC, fluoride, sulphate and chloride in the bottom ash, the SFS-EN 12457-3 [21] European standard was used. This procedure is a two stage batch test at a liquid-to-solid ratio (L/S) of 2 L/kg and 8 L/kg, and the sum of leachable concentrations (i.e. L/S 10 L/kg) is compared to the maximal allowable concentrations, which in turn, together with the total element concentrations determine whether the ash may be used as an earth construction agent. The metal (i.e. Sb, As, Ba, Cd, Cr, Cu, Hg, Pb, Mo, Ni, V, Zn and Se) concentrations in the extracts were determined with a Thermo Elemental IRIS Intrepid II XDL inductively coupled plasma optical emission spectrometer (Franklin, USA). Determination of the dissolved organic carbon (DOC) content in the extractant was carried out according to the SFS-EN 1484 European standard [22] using a Leco CHN-600 analyser (Leco Inc., USA). Determination of the fluoride and chloride concentrations in the extractant were carried out according to the SFS-EN ISO 10304-1 European standard [23] using a Dionex ICS 2000 ion chromatography with conductivity detection (Dionex Corp., USA).

For the partitioning of heavy metals (i.e Cu, Pb, Cr, Zn, As, V, Ni and Ba) in the bottom ash between the water soluble ($H_2O$), exchangeable ($CH_3COOH$), easily reduced ($NH_2OH$-HCl), oxidizable ($H_2O_2$ + $CH_3COONH_4$) and residual fractions (HF + $HNO_3$ + HCl), we used a five-stage sequential leaching procedure, which is fully described in our previous studies [10,11]. In this procedure, leaching stages 2–4 follow the protocol proposed by the Measurement and Testing Program (formerly BCR Program) of the European Union. The BCR procedure has been widely applied for heavy metal fractionation in various matrices, e.g. ash, soil, sediment and sludge [24].

However, in the first stage (i.e. leaching stage 1) we estimated the leaching of elements in acidified ultrapure distilled water (pH = 4.0). Water extraction is recommended as the first step before the BCR procedure because the extraction of water soluble species yields very important information required in evaluating the risk of environmental pollution by dumping waste [25]. Distilled water, acidified with $HNO_3$, has a higher ionic strength than demineralized water. This type of water is, in fact, what the waste material comes into contact with under normal conditions in a landfill, as well as during transport and storage, and it simulates acidic rainwater [26].

Leaching was carried out by shaking 4 g of the bottom ash. The leaching was carried out in polypropylene bottles. In order to minimize possible chemical and/or microbiological changes in the ash, the leaching was carried out using ash the sample as such, instead of a dried sample since, according to Kosson et al. [27], it is preferable to avoid sample drying before extraction. After each leaching step the extracts were separated from the solid residue by filtration through a 0.45 μm membrane filter (47 mm diameter). In order to avoid losses between the leaching stages, the filters and adhering ash particles from the previous leaching stage were also included in the next stage. After addition of 200 μL of 65% $HNO_3$ in the supernatant phase (not in stage 5 because it was already strongly acidic), it was stored in a refrigerator (+4°C) until the element determinations. The heavy metal (i.e. Cu, Pb, Cr, Zn, As, V, Ni and Ba) concentrations in the extracts (i.e. leaching

stages 1–5) were determined with a Thermo Elemental IRIS Intrepid II XDL inductively coupled plasma optical emission spectrometer (Franklin, USA).

## RESULTS AND DISCUSSION

### The Physical and Chemical Properties

The most important physical and chemical properties of the bottom ash are given in Table 1 The results are means of triplicate samples and are expressed on a dry weight (d.w.) basis. However, the standard deviations are not given to all elements, because the triplicate samples had exactly the same element concentrations. As seen in Table 1, the pH of the bottom ash was strongly alkaline (pH 10.4), which means that it has a liming effect. The low loss-on-ignition (LOI) value (< 1.0%; d.w.) indicates that the bottom ash contains almost no organic matter. The low total organic concentration (i.e. TOC) value (2.4 mg $kg^{-1}$; d.w.) supports this. The almost complete combustion of organic matter in the fluidized bed boiler is reasonable due to the fact that the incineration temperature in the bed sand varies between 810 and 830°C and in the upper zone of the boiler between 1100 and 1200°C.

Although loss-on-ignition (LOI) is a common and widely used method to estimate the the organic content of waste material, it does not necessarily represent well the amount of unburned carbon in ash, but rather the volatile fraction. According to Heiri et al. [28], other reactions than the burning of organic matter can take place at 550°C, e.g. dehydration of metal oxides and loss of volatile salts. Thus, LOI in an indirect measure of the organic matter content of ash.

The neutralizing value (NV) of 5.3% expressed as Ca equivalents (d.w.) indicates that 7.2 tonnes of bottom ash would be required to replace 1 tonne of a commercial ground limestone produced by SMA Saxo Mineral Ltd, the neutralizing value of which is 38% (Ca equivalents; d.w.). The reactivity value ($r_{ac}$) was determined in order to assess the speed and effectiveness of the neutralizing potential of the ash. The $r_{ac}$/NV ratio indicates that the so-called "fast acting" capacity of the ash is ca. 47%. This means that the bottom ash has a relatively good liming effect and is therefore a potential soil conditioner agent.

The easily soluble Ca concentration of 9.0 g $kg^{-1}$ (d.w.) was ca. 5.6 times higher than the typical Ca value of 1.6 g $kg^{-1}$ (d.w.) in a coarse mineral soil in Finland. The concentrations of easily soluble Mg (0.4 g $kg^{-1}$; d.w.), P (0.2 mg $kg^{-1}$; d.w.) and Zn (20.6 g $kg^{-1}$; d.w.) were correspondingly ca. 2, 20 and 6 times higher than the typical value of Mg, P and Zn in the coarse mineral soil in Finland. The elevated Ca, Mg and P concentrations in the bottom ash indicate that it is also a potential agent for soil remediation and for improving soil fertility. However, the low concentration of K (0.1 g $kg^{-1}$; d.w) in the bottom ash does not improve soil fertility.

***Table 1. Concentrations (mean ± standard deviation; n = 3; d.w.) of easily soluble nutrients in the bottom ash, as well as the physical and chemical properties of the bottom ash, and the average nutrient concentrations in the coarse mineral soil in Finland.***

| Nutrient/Parameter | Unit | Bottom Ash | Mineral Soil |
|---|---|---|---|
| Ca | g $kg^{-1}$; (d.w.) | 9.0 | 1.6 |
| Mg | g $kg^{-1}$; (d.w.) | 0.4 | 0.2 |
| Na | g $kg^{-1}$; (d.w.) | 0.1 | 0.02 |
| K | g $kg^{-1}$; (d.w.) | 0.1 | 0.10 |
| S | g $kg^{-1}$; (d.w.) | 0.2 | 0.06 |
| P | g $kg^{-1}$; (d.w.) | 0.2 | 0.01 |
| Mn | mg $kg^{-1}$; (d.w.) | 18.0 ± 0.8 | 50 |
| Cu | mg $kg^{-1}$; (d.w.) | 0.8 | 3.4 |
| Zn | mg $kg^{-1}$; (d.w.) | 20.6 ± 1.2 | 3.5 |
| TOC | mg $kg^{-1}$; (d.w.) | 2.4 ± 0.3 | |
| LOI (550°C) | % (d.w.) | < 1.0 | |
| Dry matter content (105°C) | % | 99.9 | |
| Neutralizing value (NV) | % (Ca; d.w.) | 5.3 ± 0.2 | |
| Reactivity value ($r_{ac}$) | % (Ca; d.w.) | 2.5 ± 0.1 | |
| pH | — | 10.4 ± 0.1 | |
| Electrical conductivity (EC) | mS $cm^{-1}$ | 0.3 | |

### Total Heavy Metal Concentrations in the Bottom Ash

The total heavy metal concentrations in the bottom ash (Table 2) are expressed on a dry weight (d.w.) basis. The results are means of triplicate samples. However, the standard deviations are not given to all elements, because the triplicate samples had exactly the same element concentrations. According to the results in Table 2, the total heavy metal concentrations in the bottom ash were lower than the current Finnish limit values for the maximal allowable heavy metal concentrations in agricultural and forestry fertilizers, as well as for materials used as an earth construction agent e.g. in roads, cycling paths, pavements, car parks, sport fields etc. In Finland, the limit values for the heavy metal concentrations for fertilizers used in agriculture and in forestry came into force in March 2007, and the limit values for the total heavy metal concentrations for material used

*Table 2. Total heavy metal concentrations (mg kg$^{-1}$; d.w., n = 3) in the bottom ash and the current Finnish limit values (mg kg$^{-1}$; d.w.) for maximal allowable heavy metal concentrations for agricultural and forestry fertilizers, and for materials used as an earth construction agent.*

| Element | Bottom Ash | Limit Value (agricultural fertilizer) | Limit Value (forestry fertilizer) | Limit Value (earth construction agent) |
|---|---|---|---|---|
| Cd | < 0.3 | 1.5 | 17.5 | 15 |
| Cu | 16.9 ± 0.4 | 600 | 700 | 400 |
| Pb | 5.4 ± 0.2 | 100 | 150 | 300 |
| Cr | 14.1 ± 0.1 | 300 | 300 | 400 |
| Zn | 172 ± 2.0 | 1500 | 4500 | 2000 |
| As | 5.8 ± 0.1 | 25 | 30 | 50 |
| V | 30.7 ± 1.3 | | | 400 |
| Ni | 12.8 ± 0.2 | 100 | 150 | |
| Ba | 347 ± 15 | | | 3000 |
| Mo | < 1.0 | | | 50 |
| Hg | < 0.03 | 1.0 | 1.0 | — |

as an earth construction agent in July 2006. The Finnish limit values are based on the European Union (EU) directives and regulations.

Although ashes are rarely used in agriculture, we wanted to compare the total heavy metal concentrations in the bottom ash to the maximal allowable heavy metal concentrations for agricultural fertilizer, set on the basis of the Finnish legislation. This comparison is made only in order to obtain information about the heavy metal concentrations in ash, not for recommendation purposes. It is worth noting that the physical and chemical quality of ashes varies significantly depending on e.g. the ratio of the fuels burnt, tree species, growing site, climate and tree component (e.g. bark, wood, leaves). Other factors which affect on the physical and chemical quality of the ash are size and age of the tree, logging technique, collection and storage, as well as the burning technique such as the combustion temperature and the type of boiler [29]. Therefore according to Aronsson and Ekelund [30] caution must always be exercised if application of the ash is to occur in the natural environment. The utilization of ash and other industrial residues always requires approval by the competent authority. In this context it is worth noting that we have not analysed the toxicity properties of the bottom ash in this study. This is due to the fact that according to the Finnish legislation, if ash is utilized, the knowledge of toxicity properties are not necessary needed; however in some cases the competent authority may decide that the toxicity properties have to be determined before the ash is utilized.

The very low total Hg concentration (< 0.03 mg kg$^{-1}$; d.w.) in the bottom ash is a favourable phenomenon. Recently, concern has been raised about solid waste disposal and the potential release of mercury from solid waste landfills. If the mercury is not stable in the waste and industrial by-products, e.g. ash, it will eventually enter the global mercury cycle. It is recognized, however, that the long-term storage of ash containing sorbed or bound mercury might eventually become a host to micro-organisms that could form methylated mercury species or could cause the reduction of mercury compounds to elemental mercury. This could enhance vapour transport and some forms of mobility, such as liquid transport with colloids. However, if the ash is strongly alkaline, as it is in our case (pH 10.4), the biotransformation of mercury and of mercury compounds to methylated species is unlikely [31].

## The Concentrations of Leachable Compounds in Extractants

The leachable concentrations of Sb, As, Ba, Cd, Cr, Cu, Hg, Pb, Mo, Ni, V, Zn, Se, DOC, fluoride, sulphate and chloride in the bottom ash are shown in Table 3. According to the results, the concentrations of leachable compounds in the bottom ash were lower than the Finnish waste legislation requirements for

*Table 3. Leachable concentrations of heavy metals, DOC, fluoride, sulphate and chloride at a liquid-to-solid (L/S) ratio of 10 L/Kg (mg kg$^{-1}$; d.w., n =1) in the bottom ash and the current Finnish limit values (mg kg$^{-1}$; d.w.) of these compounds for the unpaved and paved materials used as an earth construction agent.*

| Compound | Bottom Ash (L/S 10 L/kg) | Limit Value (unpaved) (L/S 10 L/kg) | Limit Value (paved) (L/S 10 L/kg) |
|---|---|---|---|
| Sb | < 0.05 | 0.06 | 0.18 |
| As | < 0.2 | 0.5 | 1.5 |
| Ba | 3.1 | 20 | 60 |
| Cd | < 0.02 | 0.04 | 0.04 |
| Cr | 0.2 | 0.5 | 3.0 |
| Cu | < 0.1 | 2.0 | 6.0 |
| Hg | < 0.01 | 0.01 | 0.01 |
| Pb | < 0.2 | 0.5 | 1.5 |
| Mo | 0.3 | 0.5 | 6.0 |
| Ni | < 0.1 | 0.4 | 1.2 |
| V | 0.7 | 2.0 | 3.0 |
| Zn | < 0.1 | 4.0 | 12 |
| Se | < 0.1 | 0.1 | 0.5 |
| DOC | < 10 | 500 | 500 |
| Fluoride | < 0.5 | 10 | 50 |
| Sulphate | 259 | 1000 | 10,000 |
| Chloride | 18 | 800 | 2,400 |

waste acceptance as an earth construction agent. Therefore, due to the low total metal concentrations (Table 2) and due to the low concentrations of leachable compounds (Table 3) in the bottom ash, it is recommended that the bottom ash from Kemin Energia Oy is used as an earth construction agent instead of dumping it in landfills; the utilization of industrial residues always saves natural raw materials.

## Partitioning of Heavy Metals in the Bottom Ash

If inorganic materials and by-products, e.g. wastes, are utilized in earth construction, the content of harmful compounds must be low and the harmful components must be tightly bound to the matrix [32]. Leaching test are widely used as a tool for estimating the release potential of constituents from waste materials over a range of possible waste management activities, including recycling or reuse, for assessing the efficacy of a waste treatment process, and after disposal. When the sequential leaching procedure is applied for the partitioning of heavy metals in environmental samples (e.g. ash, sludge, sediment, soil etc.), the ability of different extraction agents to release metal ions depends on their association with specific fractions in the sample. Extractants like electrolytes, weak acids and chelating agents release metals from the coordination sites, while strong acids and redox agents are capable of releasing additional quantities of metals as a result of the decomposition of the solid matrix. Thus, consecutive leaching techniques allow us to obtain information about the mobility of major and trace elements under different environmental conditions, such as acidic or alkaline, oxidizing or reducing behaviour, the action of chelating agent etc. [25].

The distribution of heavy metals (i.e. Cu, Pb, Cr, Zn, As, V, Ni and Ba) in the bottom ash after a five-stage leaching procedure between water soluble fraction ($H_2O$), exchangeable fraction ($CH_3COOH$), easily reduced fraction ($NH_2OH$-HCl), oxidizable fraction ($H_2O_2$ + $CH_3COONH_4$), and residual fraction (HF + $HNO_3$ + HCl) are shown in Figure 1; Cd, Mo and Hg are not fractionated due to their low total concentrations (see Table 2).

The residual fraction (F5) was the predominant matrix for most of the heavy metals in the bottom ash. This means that if the bottom ash is utilized, e.g. as an earth construction agent, the proportion of heavy metals partitioned in the fraction F5 are not easily to leach out. This residual fraction is the non-mobile, and is potentially the least harmful. The metals associated with this fraction are retained within the crystal lattice of minerals and inside crystallized oxides and can only be mobilised as a result of weathering. Although fraction 5 can be dissolved under laboratory conditions, this fraction is not likely to be dissolved under the conditions normally found in nature, and it is therefore called the "inert phase". Thus, this fraction is an assessment of the "worst case environmental scenario", in which the components of the bottom ash become soluble and mobile [33].

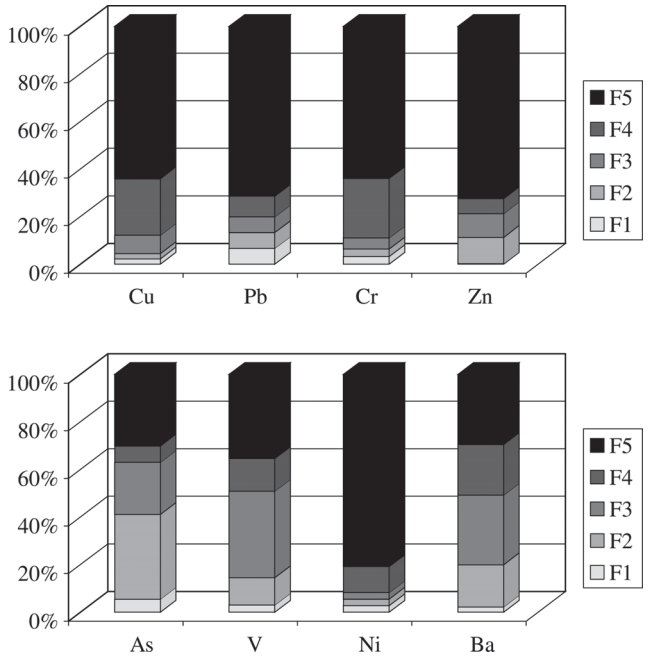


***Figure 1.*** *Partitioning of heavy metals in the bottom ash between water-soluble fraction (F1), exchangeable fraction (F2), easily reduced fraction (F3), oxidizable fraction (F4), and residual fraction (F5).*

The oxidizable fraction (F4), in which the combination of $H_2O_2$/$NH_4OAc$ is used, corresponds to metals that are organically bound or occur as oxidizable minerals, e.g. sulphides. Metals bound to this fraction can be released under oxidising conditions [34]. Besides the leaching of cations by ammonium ions, partial removal of some elements may occur due to complexation with acetate anions [34]. According to the total organic carbon (TOC) value of 2.4 g $kg^{-1}$ (d.w.), the amount of organic matter in the bottom ash is relatively low (Table 1), and it is therefore not very likely that the degradation of organic matter under oxidizing conditions can lead to release of the metals bound to these organic components. From utilization point of view, the low organic matter in ash is a favourable phenomenon since, if present, the organic matter may form complexes with toxic heavy metals [35]. However, according to Smichowski

et al. [36], the organic fraction released in the oxidizable step is not considered to be very mobile and available.

The use of hydroxylamine hydrochloride ($NH_2OH$-HCl), which is a strong reducing agent, as extractant in the easily reduced fraction (F3) simulates anoxic conditions that are likely to occur in a natural medium [37]. The use of $NH_2OH$-HCl as leachant in this fraction influences the complexation of metals with chloride. The elements in this fraction are thermodynamically unstable in anoxic conditions [35]. The metals in this fraction can be mobilised with increasing reducing or oxidising conditions in the environment. This means, that potentially toxic metals such as As, V and Ba, which showed clear tendency to be partitioned in this fraction, are potentially bioavailable.

The exchangeable fraction (F2), that is leachable with $CH_3COOH$, gives an indication of the amount of metals bound on the surface of the particles, as well as metals that are released as acid-soluble salts such as carbonates. Among the potentially toxic metals such as As, V and Ba showed a clear partitioning in this fraction. This fraction is bioavailable and corresponds to the form of metals that are most available for plant uptake, and can be released by merely changing the ionic strength of the medium. The use of acetic acid as an leachant emulates the organic acids produced from decomposing waste in anaerobic environments such as landfill since, in the acetogenesis phase during the anaerobic degradation of organic matter, carboxylic acids (e.g. acetic acid), volatile fatty acids and ethanol are produced and transformed into acetate, carbon dioxide and hydrogen by acidogeneous bacteria [38].

The metals that are leachable in the water-soluble fraction (F1) are relatively mobile and thus may be readily extractable and potentially bioavailable. In water extraction, the driving forces influencing the partial dissolution of matrix components are solubility, the diffusion rate of the element forming part of the matrix, and the wash off of compounds on the surface of the matrix. This fraction is the most readily available metal fraction from the point of view of the environment, and thus the extraction of metals in this fraction is a major environmental concern. Water soluble species yield very important information required in evaluating the risk of environmental pollution by dumping waste since, in nature, water is an important vector of harmful compounds into the environment [33]. It is interesting to note, that only low proportions of Cu (2.2%), Pb (6.6%), Cr (3.2%), Zn (0.2%), As (5.5%), V (3.0%), Ni (2.7%) and Ba (2.2%) were partitioned in this fraction. This indicates that the above mentioned metals are not easily extracted in the water-soluble fraction.

## CONCLUSIONS

In Finland, the new limit values for the maximal allowable heavy metal concentrations in agricultural and forestry fertilizers came into force in March 2007. The total concentrations of metals in the bottom ash originating from the medium-sized (32 MW) municipal district heating plant of Kemin Energia Oy at Kemi, Northern Finland, were lower than the heavy metal limit values for the maximal allowable heavy metal concentrations in agricultural and forestry fertilizers. The total metal concentrations in the bottom ash were also clearly lower than the Finnish limit values for material used as an earth construction agent, which came into force in July 2006. Furthermore, the leachable heavy metal as well as DOC, fluoride and chloride concentrations in the extractant were lower than the limit values for material used as an earth construction agent.

According to a five-stage leaching study, the residual fraction (F5), in which the combination of HF + $HNO_3$ + HCl is used, was the predominant matrix for most of the heavy metals in the bottom ash. This means that if the bottom ash is utilized, the proportion of heavy metals partitioned in this fraction are not easily to leach out. From the environmental point of view, the mineral acid mixture of HF + $HNO_3$ + HCl is never found in nature. Although the heavy metals in the bottom ash can be dissolved totally under laboratory conditions, the heavy metals associated in this fraction are not likely to be dissolved under the conditions normally found in nature, and it is therefore called the inert phase. Therefore, it is recommended that the bottom ash is used as an earth construction agent instead of dumping it in landfills.

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# Microelements in Food Materials Consumed by Children in Boarding Schools and Day Nurseries

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**ABSTRACT:** The basic resources of microelements that are essential for human life are food materials. However, in larger or lower amounts, they may become more important and critical. They may be accumulated in biological systems and pose a significant hazard to health. Biochemical and physical adaptation of microorganisms are related to geophysical properties and the environmental factors in the surrounding habitat. Therefore, many research activities have focused on microelements and their effect on ecosystem. In the Republic of Azerbaijan specialized research activities have been carried out since 1950s.

This study has been carried out in the Kuba-Khachmas and Sheki-Zaqatala regions where endemic goiter is a critical health disease. In order to compare the effect of microelements, their level in a blank region, Absheron peninsula, was also investigated. 160 samples of consumed food materials in the regions were collected from boarding and nursery schools. Iodine, fluorine, copper, cobalt, manganese, zinc and molybdenum levels were determined for each sample Although for almost each microelements a large range was observed between minimum and maximum levels, the results showed that the soil character in the studied areas had lower microelement amount levels than required for vital activities.

## INTRODUCTION

MANY studies have been conducted on heavy metal and microelement contamination in soil, plants, waters, and sediments throughout the world [1–9].

Micronutrients play an important role for human-being. In particular, a deficiency of micronutrients such as iron, zinc, iodine, has been shown to affect growth, maximal work capacity, mental function, visual acuity [10]. Fluoride occurs naturally in soil, water, plants and animals in trace quantities. When fluoride is ingested, some is taken up by body tissues, with long-term deposition in teeth and bones [11]. Meanwhile, assessments of excessive fluoride intake from public water supplies, other beverages and foods, and non-food sources are also critical in developing an understanding of enamel and skeletal fluorosis [12].

As well as being toxic at high levels, the fact that the human body also needs microelements increases the importance of heavy metals and the need for control mechanisms [13]. For example, it has been revealed in the monitoring studies carried out in Kola Peninsula in Russia that environment systems in the vicinity of the sites where metallurgical processes are carried out are in big danger [14–17].

Food materials are one of the most important resources for beneficial microelements that are essential for human and animal. Biochemical and physical adaptation of organisms depend on the geophysical properties of the surrounding environment. Therefore, numerous studies have been conducted on this subject [18–20].

Vegetables are the main resources of microelements for humans. The biological characteristics of plants, type of plant and microelement amount are important factors for transportation of microelements from soil to plants [21, 22]. In this case, the type of consumed food, age of animal and class of food materials are important as well as taxonomy of microelements for each specific organism [23].

The main objective of this study was to determine daily levels of microelements in food materials consumed by children in different regions with different characteristics in the Azerbaijan Republic. The reason of determining the amount of iodine, fluorine, cobalt,

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copper and zinc in some regions of the Azerbaijan Republic is that resources in these regions are endemic biochemical. The relation between these 7 elements ( I, F, Cu, Co, Mn, Zn, Mo) is determined by taking into account that these elements are an additional factor for occurrence of endemic illnesses (for example, correlation of iodine with other microelements in goitre).

## MATERIALS AND METHODS

The Kuba-Khachmas and Sheki-Zaqatala regions, where goiter is an endemic disease, were the main areas where this study was carried out. In order to compare the results, a control region was also taken into consideration. Absheron was selected as the control area where goiter is not an endemic disease. The data relating to the health diseases were taken from regional health institutions.

Absheron is a peninsula where Baku, the capital city of the Republic of the Azerbaijan, is located. One of the biggest industrial zones, Sumgayit, is also located in the peninsula. The surface area of the peninsula is around 4365 $m^2$ that is equal to 4.5% of total surface area of the Republic. The region is rich in natural resources such as petroleum and natural gas reserves. Clay is the dominant soil form in the peninsula and agricultural activities are wide-spread and it has economic value for people. The Sheki-Zaqatala region is located in the northwest of the Republic and southwest of Caucasus Mountains. The surface area is 8227 $m^2$. The natural environment has a characteristic property depending on regional climatic conditions. Agriculture is an important activity for local people. Walnuts, hazelnuts and fruit cultivation are wide-spread and has economic value. Stockbreeding is also an important activity in the lands of Sheki-Zaqatala.

The Kuba-Khachmas region is located in the northwest side of the Azerbaijan. The surface area is about 8500 $m^2$. The climate in the region has unstable characteristics. The region is rich in surface water resources; therefore, agriculture is also wide-spread. Soil has different structures. It varies depending on the distance from the coastal zone. Sand and clay are the dominant soil forms in the region.

In this study, the microelement contents of both regions with endemic goiter disease and region without endemic disease were determined and the results were compared in order to reveal a clear and understandable data background concerning the present situation in the Absheron peninsula.

Samples for determination of contents in daily food materials were collected from boarding schools and day nurseries in Sheki, Kuba and Baku provinces. Iodine, fluorine, manganese, cobalt, zinc, copper and molybdenum were analyzed in 24 composite samples.

In this study, EPA 3050 Method was used for extraction (6 ml HCl and 3 ml $HNO_3$ was added on 0.5 gr sample). Extracted samples were kept in plastic vessels. Colorimetric measurements were carried out according to Standard Methods [16] in the laboratories of the National Medical Research Institute of The Republic of Azerbaijan. Iodine was determined after free iodine transformation by bromide addition in acidic pH. Flourine was analyzed following sodiumflouride transformation. In order to control interferences during analyzes, chlorine, sulfate, nitrate, phosphate parameters were determined. In the case of high interference by these parameters, liquid samples were analyzed by colorimetric methods after a distillation process. For all other parameters, chemicals and solutions were prepared according to Standard Methods. Cu, Co, Mn, Zn and Mo concentrations were determined via ICP-OES (Optical Emission Spectrophotometre) (Perkinelmer, Optima 2100DV).

## RESULTS AND DISCUSSION

Some specific boarding schools and day nurseries were selected as sampling sites. In the samples taken from selected sites, microelements such as iodine, phosphorus, manganese, cobalt, copper, zinc, molybdenum were analyzed. Microelement levels were determined for each sample. Tables 1, 2 and 3 show the analyzed values for the samples.

Data obtained from the boarding schools and day nurseries (for cooked food materials) are given in Table 4, 5, 6, 7. A statistical approach was also used in order to make a reliable evaluation of the results. Cooking and keeping methods that led to microelement loss in food materials were not considered in this statistical approach.

Statistical analyses of the data obtained during this study are given in Table 8.

### Iodine

The Kuba-Khachmas, Sheki-Zaqatala and Absheron regions had different soil structures with respect to iodine content. The results confirmed that much higher

***Table 1. Microelement Content in (Uncooked) Food Materials in Absheron (Average).***

| Food Type | Iodine | Fluorine | Manganese | Cobalt | Copper | Zinc | Molybdenum |
|---|---|---|---|---|---|---|---|
| | μg/kg or μg/l (for liquids) | | | | | | |
| 1. Wheat Flour (Quality 1) | 50.7 | 15.0 | 720.0 | 13.0 | 160.0 | 480.0 | 5.3 |
| 2. Wheat Flour (Quality 2) | 33.8 | 82.5 | 1200.0 | 16.0 | 360.0 | 360.0 | 5.3 |
| 3. Macaroni Types | 35.9 | 104.2 | 860.0 | 16.0 | 240.0 | 324.0 | 5.3 |
| 4. Pounded wheat | 63.4 | 15.0 | 940.0 | 17.0 | 200.0 | 192.0 | 4.0 |
| 5. Dark pounded wheat | 63.4 | 157.5 | 1300.0 | 16.0 | 280.0 | 240.0 | 4.0 |
| 6. Bread | 44.0 | 86.0 | 920.0 | 9.0 | 800.0 | 4320.0 | 28.0 |
| 7. Dark bread | 64.9 | 292.0 | 2010.0 | 9.65 | 580 | 1760 | 32 |
| 8. Pakistan rice | 219.9 | 621.0 | 1700.0 | 8.0 | 400.0 | 1000.0 | 24.0 |
| 9. Rice (Lenkaran) | 215.7 | 22.5 | 1888.0 | 7.0 | 480.0 | 6600.0 | 48.0 |
| 10. Kidney bean (Lenkaran) | 261.0 | 1275 | 1940.0 | 14.0 | 480.0 | 1800.0 | 640.0 |
| 11. Haricot bean | 182.9 | 195 | 2200.0 | 9.0 | 720.0 | 6480.0 | 536.0 |
| 12. Pea | 169.1 | 320 | 2960.0 | 41.5 | 720.0 | 4800.0 | 80.0 |
| 13. Potatoes | 29.4 | 21.4 | 278.6 | 7.6 | 131.6 | 527.0 | 5.7 |
| 14. Tomatoes | 8.4 | – | – | 2.4 | 7.0 | 82.0 | 0.8 |
| 15. Aubergine | 4.9 | – | 913.0 | 17.0 | 5.2 | 703.0 | 10.3 |
| 16. Bulgarian Pepper | 7.7 | 0.9 | 746.0 | 16.5 | 41.0 | 498.0 | 16.5 |
| 17. Green Pepper | 4.2 | – | 1976.0 | 18.0 | 23.0 | 558.0 | 32.0 |
| 18. Green Onion | 73.3 | 182.5 | 1108.0 | 12.1 | 80.0 | 1613.3 | 15.1 |
| 19. Green leaf plants | 109.0 | 18.0 | 2120.0 | 19.0 | 27.0 | 1730.0 | 33.0 |
| 20. Cabbage | 28.3 | – | – | – | – | – | – |
| 21. Carrots | 42.3 | – | 1302.0 | 6.8 | 171.0 | 2440.0 | 4.2 |
| 22. Cow milk | 4.4 | 3.8 | 17.0 | 0.45 | 1.3 | 160.0 | 0.9 |
| 23. Milk powder | 52.8 | 45.6 | 35.5 | 2.25 | 9.1 | – | 3.6 |
| 24. Veal | 22.7 | 53.4 | 13.0 | 6.0 | 80.0 | 160.0 | 13.0 |
| 25. Lamb | 52.8 | 42.8 | 27.0 | 16.0 | 40.0 | 160.0 | 27.0 |

***Table 2. Microelement Content in (Uncooked) Food Materials in Kuba-Khachmas (Average).***

| Food Type | Iodine | Fluorine | Manganese | Cobalt | Copper | Zinc | Molybdenum |
|---|---|---|---|---|---|---|---|
| | μg/kg or μg/l (for liquids) | | | | | | |
| 1. Wheat | 67.4 | 157.2 | 3400.0 | 3.38 | 24.0 | 4200.0 | 22.0 |
| 2. Bread | 33.8 | – | 2250.0 | 3.75 | 35.0 | 1980.0 | 17.5 |
| 3. Tomatoes | 7.3 | – | 313.7 | 0.9 | 195.0 | 923.7 | 3.1 |
| 4. Onion | 22.2 | 121.3 | 1318.0 | 1.4 | 29.8 | 546.2 | 5.9 |
| 5. Cabbage | 28.3 | 70.7 | 1418.0 | 1.7 | 13.0 | 1215.0 | 5.5 |
| 6. Potatoes | 71.8 | 67.3 | 1137.0 | 3.25 | 90.5 | 756.2 | 6.7 |
| 7. Red beet | 63.1 | 149.1 | 1710.0 | – | 124.0 | 1330.0 | 6.6 |
| 8. Apple | 25.2 | 37.2 | 1313.2 | 2.15 | 37.6 | 1069.5 | 12.1 |
| 9. Pear | 16.9 | 48.3 | 980.0 | 1.45 | 151.5 | 131.2 | 8.8 |
| 10. Plum | 17.2 | – | 867.0 | 1.0 | 33.0 | 153.0 | 2.0 |
| 11. Lamb | 14.8 | – | 1390.0 | 2.1 | 8.0 | 1408.0 | 2.1 |
| 12. White cheese | 8.8 | 131.0 | 28.0 | 2.6 | 4.0 | 624.0 | 1.9 |
| 13. Milk | 6.65 | 4.25 | 44.0 | 1.3 | 3.0 | 384.0 | 1.0 |
| 14. Egg | 64.0 | 139.2 | 64.0 | 0.4 | 120.0 | 760.0 | 6.3 |

***Table 3. Microelement Content in (Uncooked) Food Materials in Sheki-Zaqatala (Average)***

| Food Type | Iodine | Fluorine | Manganese | Cobalt | Copper | Zinc | Molybdenum |
|---|---|---|---|---|---|---|---|
| | μg/kg or μg/l (for liquids) | | | | | | |
| 1. Wheat | 67.4 | 157.2 | 3400.0 | 3.38 | 24.0 | 4200.0 | 22.0 |
| 2. Bread | 33.8 | – | 2250.0 | 3.75 | 35.0 | 1980.0 | 17.5 |
| 3. Tomatoes | 7.3 | – | 313.7 | 0.9 | 195.0 | 923.7 | 3.1 |
| 4. Onion | 22.2 | 121.3 | 1318.0 | 1.4 | 29.8 | 546.2 | 5.9 |
| 5. Cabbage | 28.3 | 70.7 | 1418.0 | 1.7 | 13.0 | 1215.0 | 5.5 |
| 6. Potatoes | 71.8 | 67.3 | 1137.0 | 3.25 | 90.5 | 756.2 | 6.7 |
| 7. Red beet | 63.1 | 149.1 | 1710.0 | – | 124.0 | 1330.0 | 6.6 |
| 8. Apple | 25.2 | 37.2 | 1313.2 | 2.15 | 37.6 | 1069.5 | 12.1 |
| 9. Pear | 16.9 | 48.3 | 980.0 | 1.45 | 151.5 | 131.2 | 8.8 |
| 10. Plum | 17.2 | – | 867.0 | 1.0 | 33.0 | 153.0 | 2.0 |
| 11. Lamb | 14.8 | – | 1390.0 | 2.1 | 8.0 | 1408.0 | 2.1 |
| 12. White cheese | 8.8 | 131.0 | 28.0 | 2.6 | 4.0 | 624.0 | 1.9 |
| 13. Milk | 6.65 | 4.25 | 44.0 | 1.3 | 3.0 | 384.0 | 1.0 |
| 14. Egg | 64.0 | 139.2 | 64.0 | 0.4 | 120.0 | 760.0 | 6.3 |

***Table 4. Determination of Microelement Content in (Cooked) Food Materials Consumed by Children (Baku, Shuvelan Small town, Boarding school).***

| No. p/p | Iodine | Fluorine | Manganese | Cobalt | Copper | Zinc | Molybdenum |
|---|---|---|---|---|---|---|---|
| | μg/portion | | | | | | |
| 1 | 260.1 | 2741.2 | 1410.0 | 70.0 | 88.0 | 31610.0 | 140.0 |
| 2 | 269.3 | 5569.2 | 2120.0 | 127.0 | 106.0 | 38200.0 | 127.0 |
| 3 | 179.8 | 3925.0 | 2000.0 | 53.0 | 100.0 | 5400.0 | 60.0 |
| 4 | 412.0 | 4970.7 | 2100.0 | 210.0 | 158.0 | 12620.0 | 63.0 |
| 5 | 254.9 | 4244.4 | 1640.0 | 52.0 | 104.0 | 2510.0 | 84.0 |
| (M) | 275.2 | 4290.1 | 1854.0 | 102.4 | 111.2 | 18068.0 | 94.8 |
| δ | 84.4 | 1074.7 | 314.5 | 67.5 | 27.1 | 15976.0 | 36.8 |
| $m^+$ | 37.7 | 480.6 | 140.6 | 30.2 | 12.1 | 7144.7 | 16.5 |

***Table 5. Determination of Microelement Content in (Cooked) Food Materials Consumed by Children (Kuba, Boarding school).***

| No. p/p | Iodine | Fluorine | Manganese | Cobalt | Copper | Zinc | Molybdenum |
|---|---|---|---|---|---|---|---|
| | μg/portion | | | | | | |
| 1 | 177.6 | 2447.0 | 4960.0 | 64.0 | 40.0 | 7200.0 | 40.0 |
| 2 | 160.7 | 2021.2 | 3490.0 | 40.0 | 38.0 | 2740.0 | 38.0 |
| 3 | 152.2 | 2021.2 | 3740.0 | 57.0 | 108.0 | 2070.0 | 21.0 |
| 4 | 179.8 | 2447.0 | 5200.0 | 52.0 | 42.0 | 2690.0 | 25.0 |
| 5 | 186.0 | 2021.2 | 3680.0 | 40.0 | 120.0 | 2560.0 | 44.0 |
| 6 | 175.0 | 2447.0 | 3710.0 | 5102 | 64.0 | 1820.0 | 19.0 |
| (M) | 172.0 | 2234.0 | 4130.0 | 50.7 | 68.7 | 3180.0 | 31.2 |
| δ | 12.84 | 233.2 | 744.9 | 9.46 | 36.5 | 2003.0 | 10.8 |
| $m^+$ | 5.2 | 95.2 | 304.1 | 3.9 | 14.9 | 817.7 | 4.4 |

***Table 6. Determination of Microelement Content in (Cooked) Food Materials Consumed by Children (Sheki, Boarding School).***

| | Iodine | Fluorine | Manganese | Cobalt | Copper | Zinc | Molybdenum |
|---|---|---|---|---|---|---|---|
| **No. p/p** | | | | **μg/portion** | | | |
| 1 | 186.2 | 1214.5 | 1704.0 | 34.1 | 298.0 | 5110.0 | 34.0 |
| 2 | 189.2 | 1086.3 | 2130.0 | 16.0 | 170.4 | 8180.0 | 29.0 |
| 3 | 198.2 | 1240.0 | 3919.0 | 16.0 | 426.0 | 8520.0 | 42.0 |
| 4 | 144.2 | 985.5 | 3919.0 | 16.0 | 384.0 | 5340.0 | 26.0 |
| 5 | 151.1 | 1190.0 | 3260.0 | 25.0 | 462.0 | 5390.0 | 16.8 |
| 6 | 124.4 | 1260.0 | 2100.0 | 21.0 | 495.0 | 6720.0 | 33.6 |
| 7 | 198.0 | 1188.0 | 2475.0 | 25.0 | 227.0 | 7130.0 | 29.7 |
| 8 | 188.0 | 1188.0 | 2475.0 | 19.8 | 272.0 | 5690.0 | 39.6 |
| 9 | 165.2 | 1503.6 | 3178.0 | 9.1 | 210.0 | 4630.0 | 27.0 |
| 10 | 169.3 | 1498.0 | 2360.0 | 36.0 | 200.0 | 5450.0 | 27.0 |
| (M) | 171.4 | 1229.4 | 2752.1 | 21.8 | 314.4 | 6216.0 | 30.4 |
| δ | 25.1 | 205.9 | 773.1 | 8.43 | 118.5 | 1346.0 | 7.1 |
| $m^+$ | 7.9 | 65.1 | 244.5 | 2.7 | 37.5 | 425.7 | 2.3 |

***Table 7. Determination of Microelement Content in (Cooked) Food Materials Consumed by Children (Sheki, Day Nursery).***

| | Iodine | Fluorine | Manganese | Cobalt | Copper | Zinc | Molybdenum |
|---|---|---|---|---|---|---|---|
| **No. p/p** | | | | **μg/portion** | | | |
| 1 | 167.2 | 967.5 | 750.0 | 9.4 | 38.0 | 1320.0 | 15.0 |
| 2 | 124.7 | 976.5 | 671.0 | 26.0 | 52.0 | 2490.0 | 20.0 |
| 3 | 121.2 | 846.0 | 800.0 | 26.0 | 210.0 | 730.0 | 20.0 |
| (M) | 137.7 | 930.0 | 74.0 | 20.4 | 100.0 | 1513.0 | 18.4 |
| δ | 25.6 | 72.9 | 656.6 | 9.5 | 95.5 | 895.9 | 2.6 |
| $m^+$ | 14.8 | 42.1 | 37.9 | 5.5 | 55.2 | 517.2 | 1.5 |

***Table 8. Statistical Evaluation of the Results of Microelement Analyses in Baku, Kuba and Sheki.***

| | | Parameters | | | |
|---|---|---|---|---|---|
| | **Provinces** | **M** | **m+** | **t** | **p** |
| **Microelements** | **1** | **2** | **3** | **4** | **5** |
| Iodine | Baku | 275.2 | 37.7 | | Control |
| | Kuba | 171.9 | 5.3 | 2.71 | 0.05 |
| | Sheki | 171.4 | 7.9 | 2.69 | 0.02 |
| Fluorine | Baku | 4290.1 | 480.6 | | Control |
| | Kuba | 2234.1 | 95.1 | 4.2 | 0.01 |
| | Sheki | 1229.1 | 65.1 | 6.31 | 0.0001 |
| Manganese | Baku | 1854.1 | 140.6 | | Control |
| | Kuba | 4130.0 | 304.1 | 6.8 | 0.0001 |
| | Sheki | 2752.1 | 244.5 | 6.3 | 0.01 |
| Cobalt | Baku | 102.4 | 30.2 | | Control |
| | Kuba | 50.7 | 3.86 | 1.7 | 0.2 |
| | Sheki | 21.8 | 2.7 | 2.7 | 0.02 |
| Copper | Baku | 111.2 | 12.1 | | Control |
| | Kuba | 68.7 | 14.9 | 2.2 | 0.1 |
| | Sheki | 314.4 | 37.5 | 5.2 | 0.001 |
| Zinc | Baku | 18068.0 | 7144.7 | | Control |
| | Kuba | 68.7 | 14.9 | 2.1 | 0.1 |
| | Sheki | 6216.0 | 425.7 | 1.7 | 0.2 |
| Molybdenum | Baku | 94.8 | 16.5 | | Control |
| | Kuba | 31.2 | 4.4 | 3.7 | 0.01 |
| | Sheki | 30.5 | 2.3 | 3.9 | 0.002 |

M : Average value of repeated analyses
+m : Values higher than M
−m : Values lower than M
T : Mathematical average
P : Probability (*t* and *p* real mathematical values of study results)
Control : Blank (Comparison-area with endemic goiter and area where goiter is not endemic disease)
δ: Standard deviation

iodine was determined in the blank region than other two sampling sites. Iodine values in rice samples in Absheron were found very high as 215–220 μg/kg. In addition, iodine contents of kidney bean and bean in the region were determined as 183–261 μg/kg, for pea it was 169 μg/kg, for green leaf vegetables the value was 109 μg/kg.

Sheki-Zaqatala showed a lower iodine content. The iodine content of rice was determined as 80 μg/kg, for beans 44 μg/kg and it was 72 μg/kg for green leaf vegetables in the region. The lowest iodine level was analyzed in onion samples as 5.5 μg/kg. The content of iodine in carrot samples was found to be 5.17 μg/kg in Sheki-Zaqatala while it was 42.3 μg/kg in Absheron. In the same period of time, samples taken from Kuba-Khachmas were also analyzed. Iodine values were found to be 22 μg/kg in onions while it was 73 μg/kg in Absheron.

Animal food materials also showed different iodine content variations in comparison with each other. Iodine in veal samples were determined as 22.73 μg/kg in Absheron and as 12.3 μg/kg in Sheki-Zaqatala. In lamb samples the level was 53 μg/kg in Absheron, 15 μg/kg in Sheki-Zaqatala and 0.88 μg/kg in Kuba-Khachmas.

When it comes to wheat samples, in Kuba-Khachmas iodine levels in wheat samples were found in a large range as 21–192 μg/kg. In Khachmas the values were between 21–46.5 μg/kg, in Gusar 107.8–192.4 μg/kg and in Kuba it was 23.2–91 μg/kg. Based on both previous regional studies and this study, it was observed that iodine in food materials was below the desired levels in the regions with endemic goiter.

Values in the tables above reveal the iodine amount that was included in the food materials consumed by children in studied regions. Average values were determined as 275.2 μg, 172 μg and in the range of 137–171 μg in Baku, Kuba and Sheki, respectively.

## Fluorine

In the Republic, fluorine levels varied over a large range. For instance, fluorine in wheat samples in Khachmas varied in the range of 127–170 μg/kg with average value of 157.2 μg/kg. Fluorine amount in bread was found as 53 μg/kg and 86 μg/kg in Sheki-Zaqatala and Absheron, respectively.

Leguminosae was known to be a good fluorine accumulator. The iodine level was determined between 127.5–320 μg/kg in Absheron and was found as 247.5 μg/kg in Sheki-Zaqatala. The highest value was determined in Pakistan rice with 621 μg/kg. Native rice samples contained fluorine in the range of 22.5–172.5 g/kg in Lenkaran, Absheron, Sheki-Zaqatala regions.

Experiments revealed that fluorine content of potatoes in 3 regions was similar. The range was determined as 21.4–67.3 μg/kg. Apple, pear and grape samples had a low amounts of fluorine.

Previous studies reported that the fluorine content in cow milk was dependent on the fluorine amount in cow feeding materials and the water they consumed. In this study, the fluorine amount in cow milk was found as 3.7 μg/kg, 3 μg/kg and 4.25 μg/kg in Sheki, Absheron and Kuba, respectively. Fluorine in milk powder and milk produced in the factory was determined as 10–12 times higher than the amounts determined in milk. Fluorine in egg samples was around 139.2–143.3 μg/kg while yolk contained 100 μg/kg fluorine. The fluorine content of cow meat and lamb samples varied over the range of 42.8–72 μg/kg. Experimental results among 3 sites showed no significant differences.

## Manganese

Manganese amounts determined in the vegetable food materials was in the range of 720–2960 μg/kg. In the Absheron peninsula, green leaf vegetables, green onion, pepper and carrot samples contained 2120 μg/kg, 1108 μg/kg, 1976 μg/kg and 1302 μg/kg fluorine, respectively.

In Sheki-Zaqatala, the manganese values in green leaf vegetables and carrots were around 68.3 μg/kg and 147 μg/kg. In the same region hazelnut and walnut samples contained relatively higher amounts, 416 μg/kg and 720 μg/kg.

In Kuba-Khachmas region manganese in apple, grape and pear samples was determined over a range between 867–1313 μg/kg.

The manganese amount in animal based food materials was found relatively low during elemental analyses. Manganese in milk was 17 μg/kg in Absheron, 44 μg/kg in Kuba-Khachmas and 40 μg/kg in Sheki-Zaqatala.

When it comes to milk samples, the manganese level was around 13–28 μg/kg. Evaluation of experimental data showed that there was not a significant manganese variation among the samples except some vegetables.

The manganese content of food materials for children in day nurseries also showed a large variation between 2.3–9.8 g/portion.

**Cobalt**

In Kuba-Khachmas, chickpea and leguminosae cobalt content was found as 41.5 μg/kg and 13–17 μg/kg, respectively. The cobalt content in aubergine samples was 17 μg/kg and in pepper 16–18 μg/kg, in green leaf vegetables 8–19 μg/kg and in walnut samples 8–12 μg/kg.

Cobalt in cabbage, potato, tomato and onion samples taken from area with endemic goiter was determined to be relatively low in comparison with control site cobalt levels. In Kuba-Khachmas cobalt in tomatoes was found around 0.9 μg/kg while it was 2.4 μg/kg in Absheron. Cobalt in cabbage, potato and onion samples was 1.7–2.56 μg/kg, 2–3.5 μg/kg and 1.4–2 μg/kg in Kuba-Khachmas and in Sheki-Zaqatala, respectively. Cobalt levels in animal food materials were determined to be relatively low. For cow milk, cobalt was found as 1.1–1.3 μg/kg in Absheron and around 0.45 μg/kg for both Kuba and Sheki. The cobalt level in milk powder samples was determined to be 2.25 μg/kg. In Kuba-Khachmas and Sheki-Zaqatala, the cobalt content of meat and meat products was around 1.6–2.1 μg/kg and it was 6–8 μg/kg in Absheron.

**Copper**

Copper in hazelnut and walnut samples in Sheki-Zaqatala region was determined as 1280–1920 μg/kg. The copper content in cereals and cereals products was around 160–180 μg/kg. Copper values of leguminosae and rice were relatively high at 480–720 μg/kg. In Sheki-Zaqatala copper content in kidney bean and rice was found to be 40 μg/kg and 100 μg/kg, respectively.

Copper values in milk samples were analyzed as 4.3–13μg/kg. The lowest values were analyzed in Absheron. In milk powder this value was determined as 9.1 μg/kg. In the same region meat products had 40û80 μg/kg copper. In the same period, in Kuba-Khachmas and Sheki-Zaqatala, values for meat and meat products varied in the range of 4–16 μg/kg. Egg samples contained 120 μg/kg and 95 μg/kg in Kuba-Khachmas and Sheki-Zaqatala regions, respectively. Reported copper values in national research activities in The Republic and the results in the present study are similar.

**Zinc**

In Lenkaran, the zinc content in Pakistan rice was 1000 μg/kg and in pepper and aubergine samples it was found as 1730–2156 μg/kg and in carrots it was in the range of 370–2440 μg/kg. When compared to other microelements, animal food materials accumulated zinc to a much higher level.

According to the data obtained in this study, the zinc content in chicken eggs and cow meat samples were found around 765 μg/kg and 160 μg/kg, respectively. In Sheki-Zaqatala, the zinc content in cow milk was around 260 μg/kg. The zinc content in lamb was relatively high both for Sheki-Zaqatala and Kuba-Khachmas regions with average value of 528–1408 μg/kg. In the same region, 160–384 μg/kg zinc content was determined in cow milk samples and it was 624–1240 μg/kg in cheese samples.

**Molybdenum**

The molybdenum content in chickpeas in the same region was 80 μg/kg. Molybdenum was analyzed to be 4.2–6.7 μg/kg in potato samples in Sheki-Zaqatala while it was 2,4 μg/kg in onion samples. Meanwhile, it was 15.1 μg/kg in Absheron and 519 μg/kg in Kuba-Khachmas for the same food materials, respectively. Cabbage and carrot samples in the region did not exceed a value of 5.5 μg/kg

In milk samples, the molybdenum content was around 0.8–1 μg while it was much higher, 3.6 μg/kg, in milk powder samples. Lamb samples contained 0.8 μg/kg, 2.1 μg/kg and 27 μg/kg molybdenum in Sheki-Zaqatala, Kuba-Khachmas and Absheron, respectively. High amount of molybdenum level was determined in chicken eggs. The range was between 6.3–7.4 μg/kg. The white of an egg and yolk samples contained almost similar amount, 5.4 μg/kg. 9 μg/kg, 8.8 μg/kg and 12.2 μg/kg molybdenum were reported in walnut, pear and apple samples, respectively. Significant differences between endemic and blank sites were not determined during experimental analyses.

**CONCLUSIONS**

The microelement content in animal and vegetable food materials were analyzed and relation between soil structure, food material and endemic goiter was evaluated on the basis of the experimental data in the Republic of Azerbaijan.

Results showed that plant food material type, its accumulation potential and its existence in soil structure are dependent on each other. Especially, it was observed

that leguminosae and cereals are the richest products in element accumulation. Meanwhile, animal food products have less potential to accumulate microelements.

Experiments and analyses showed that there was a good relation between goiter and iodine deficiency. Not only iodine deficiency but also the existence of other microelements in soil had a significant effect on accumulation properties.

In the literature, the iodine level for an adult was reported to be 200–220 μg. Depending on this reality, food material in Baku requires an adequate level of iodine for children in the region. However, in Sheki and Kuba, an iodine deficiency was observed in the samples taken from day nurseries. This resulted in lower uptake of iodine for children in these regions. The iodine in food materials supplied only 62–78% of the total requirement of daily uptake.

It was reported that physiological fluorine requirement for children between ages 7–16 was 0.5–1.8 mg/day in the literature. For this reason, in Kuba and Sheki fluorine uptake by food materials and water resources was not adequate.

When experimental data was evaluated it was observed that in Baku (1854 μg), in Kuba (4130 μg) and in Sheki (740–2752 μg) measured values were below the literature data of 7–8 mg. The manganese content of food materials for children in day nurseries also showed a large variation between 2.3–9.8 mg. Generally, intensive consumption of vegetable food materials was assumed to be one of the reasons for the low manganese uptake in the Republic.

In this study 102.4 μg, 50.7 μg and 20 μg cobalt was determined in the samples collected in Baku, Kuba and Sheki, respectively. In Absheron, optimum uptake levels were observed.

Previous studies reported that an adult needs 10–16 mg zinc per day. The zinc requirement for a growing child is 0.3–0.6 mg/kg. With respect to this information, the daily zinc uptake of 18068 μg for children by food materials in Baku was accepted to be adequate. In Sheki and Kuba. the zinc amount in food materials and the uptake levels were not adequate for a child. 6216 μg and 2752 μg zinc was determined in food materials, respectively.

In the literature there are many reports related to molybdenum and its adequate uptake amount. Reported values were 75 μg in minimum and 100 μg in maximum. Observed values were around 95 μg/day in Baku. The daily uptake level for children in research areas was relatively low. 31.1 μg, 18–31 μg molybdenum levels were analyzed in the samples collected from day nurseries in Kuba and Sheki, respectively.

An iodine deficiency was observed in the Republic of Azerbaijan. Not only iodine but also other microelement uptakes by food materials and drinking water were also reported to be below the required values in the literature and previous studies. Especially, iodine deficiency and regions with endemic goiter showed a strong relation. Additional iodine reinforcement is needed in the endemic regions. Interaction among the microelements in the soil structure was another important point to be considered.

Not only for the Republic of Azerbaijan but also for each country monitoring the relation between soil structure, food materials and microelement uptake by food materials, such as animal and vegetable products, will be inevitable in order to evaluate the adverse affects of rapid industrialization and uncontrolled agricultural activities. Undesired effects of uncontrolled human activities may result in irreversible health diseases.

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# Effects of Long Term Irrigation with Polluted Water and Sludge Amendment on Some Soil Enzyme Activities

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**ABSTRACT:** The objective of this study was to determine the effects of wastewater sludge-fly ash mixtures on urease, dehydrogenase, alkaline phosphatase and β-glucosidase activities in soils. In order to evaluate the probable effects of previous soil management practices (irrigation with polluted water) on soil enzymes, two different soil samples which were similar in physical properties, but different in irrigation practice were used. The application of wastewater sludges supplemented with varying doses of fly ash increased potential enzyme activities for a short period of time (3 months) in comparison to unamended soils. However, the activity levels generally showed a decreasing trend with increasing ash ratios indicating the inhibitory effect of fly ash. The urease and dehydrogenase activities were particularly lower in soils irrigated from a polluted stream, indicating the negative effects of the previous soil management on soil microbial activity.

## INTRODUCTION

SOIL as a biological system contains numerous enzymes derived from the microbial community, soil animals, plant residuals and root systems. Soil enzymes are accepted as a possible integrative measure of soil quality to reflect biological status of soils since they are involved in microbial cycling of nitrogen, phosphorus and carbon. The rate of enzyme production, and the activity-stability of enzymes are controlled by environmental conditions and ecological interactions [1]. Therefore human activities such as cultivation, cropping, lime application, fertilization and waste disposal affect the levels and distribution of enzyme activities in soils.

Land application of organic wastes such as wastewater sludge to agricultural soils has received considerable attention in recent years. It is known that wastewater sludge is a source of available organic C and nutrients and serves to increase microorganism numbers and enzyme activities in soils [6].

Fly ash is the mineral residue resulting from the combustion of coal that enters the flue gas stream. The high CaO content of alkaline fly ash makes it potentially useful as an additive in stabilisation processes for sludge originating from wastewater treatment plants. The use of wastewater sludge-fly ash mixtures as organic fertilizers or soil conditioners may be beneficial means of disposal that permits recycling of plant nutrients in both sludge and fly ash. Different studies have been conducted on the influence of fly ash on biological status of soil media. They indicated that the addition of alkaline power plant fly ash to soils increased microbial respiration [35], N mineralization and nitrification [8], but inhibited the activities of some soil enzymes [29]. However, studies on the effects of wastewater-fly ash mixtures on the activity of enzymes are scarce [19]. High doses of fly ash in wastewater sludges may have detrimental effects (high pH, soluble salts, toxic components) on microorganisms in soil system thus reducing or totally removing the beneficial effects of wastewater sludge. There exists a need to investigate the effects of fly ash added sludge application on enzyme activities in soils. In the present study, urease, alkaline phosphatase and β-glucosidase were selected to represent nitrogen, phosphorus and carbon cycles, respectively in the micro-ecosystem of the amended soil [11]. Dehydrogenase, which is involved in the electron transport system to remove the oxidative substrate was also selected due to its high correlation with the oxygen uptake and organic substrate removing rates in activated sludge [25].

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The objective of this study was to determine the effects of wastewater sludges with varying doses of alkaline fly ash (40%, 80% and 120%, on dry weight bases) on the selected enzyme activities in incubated soils for 360 days at 28°C. In order to evaluate the probable effects of previous soil management practises (irrigation with polluted water) on soil enzymes, two different soil samples which were similar in physical properties, but different in irrigation practice were used in the incubation study.

## MATERIALS AND METHODS

### Sample Collection and Preparation

Dewatered sludge samples were collected from the treatment plant of a canned food company where the domestic and industrial wastewaters of the firm were treated together by an activated sludge system. The fly ash used in this study was obtained from Orhaneli Power Station (located at Orhaneli suburb which is 55 km south of Bursa city in Turkey) where lignite is used for fuel. Soil samples (order: Entisol, suborder: Fluvent) were collected from the top 20 cm of soil layer in Bursa-Nilüfer region (5 km west of Bursa city). Soil samples which were similar in physical properties but different in irrigation practice were taken from the adjacent arable fields in Bursa-Nilüfer region (5 km west of Bursa city). One field has been irrigated with polluted river (Nilüfer-Ayvalı Stream) water for more than 20 years whereas no irrigation was applied on the other. It was stated in many studies that Nilüfer-Ayvalı Stream is heavily polluted owing to untreated wastewater discharges especially from textile and metal industries [16, 9].

Raw sludge was mixed with 0%, 40%, 80% and 120% of fly ash on dry weight basis and the mixtures were then air-dried to 90% dry solids content prior to incubation study. General characteristics of soil, sludge and fly ash samples are presented in Table 1.

***Table 1. General Characteristics of Soil Samples, Sludge and Fly Ash.***

| Parameters* | Soil Samples: Non-Irrigated | Soil Samples: Irrigated | Sludge | Fly Ash |
|---|---|---|---|---|
| Sand, % | 30.0 | 28.0 | – | – |
| Silt, % | 29.0 | 30.0 | – | – |
| Clay,% | 40.0 | 42.0 | – | – |
| Texture | clay | clay | – | – |
| pH (1:5, solid:water) | 7.12 | 7.08 | 6.60 | 12.0 |
| $EC_{25°C}$ (1:5, solid:water), µS | 212 | 539 | 6390 | 3690 |
| Kjeldahl-N, $mg.kg^{-1}$ | 1200 | 1500 | 40500 | 120 |
| Ammonium-N, $mg.kg^{-1}$ | 8.49 | 10.4 | 1030 | 8.95 |
| Nitrate-N, $mg.kg^{-1}$ | 3.64 | 31.0 | 46.0 | 4.48 |
| Total-P, $mg.kg^{-1}$ | 1302 | 3378 | 5127 | 3603 |
| Available-P, $mg.kg^{-1}$ | 21.1 | 25.7 | 190 | 25.9 |
| Total Organic-C, % (w/w) | 1.17 | 1.65 | 25.3 | <0.10 |
| Acid soluble heavy metals, $mg.kg^{-1}$ | | | | |
| Cu | 25.4 | 55.3 | 36.5 | 71.2 |
| Zn | 27.4 | 68.3 | 218 | 88.4 |
| Ni | 3.30 | 10.3 | 14.7 | 25.6 |
| Cd | <1.00 | 2.80 | 4.50 | 4.25 |
| Pb | 5.50 | 16.4 | 30.7 | 65.8 |
| Cr | 2.50 | 7.70 | 20.4 | 32.5 |

*on dry weight base.

### Incubation Study

Wastewater sludge containing varying doses of fly ash were amended to soils at rates equivalent to 100 t (dry weight) raw sludge $ha^{-1}$ soil. Amended and control soils were placed in plastic pots and the pots were then arranged in a randomized block design with three replicates in a controlled chamber at 28°C in the dark. The samples were incubated aerobically under a non-leaching procedure for 360 days. The soil moisture content was maintained at 70% of field capacity by the addition of distilled water as required. Sub samples were removed at time intervals of 3 and 12 months to determine the short term and long term variations in enzyme activities.

### Chemical and Physical Analyses

Electrical conductivity ($EC_{25°C}$) and pH of the soil and sludge samples were measured in sample extracts obtained by shaking the material with distilled water at 1:5 (w/v) sample: water ratio using a conductivity meter and pH meter, respectively [30, 23]. The soil particle size distributions were determined by the hydrometer method [14].

Nitrate nitrogen and $NH_4^+$-N concentrations were determined in samples which were extracted using 2 M KCl. The concentrations in extracts were analysed by steam distillation with MgO and Devarda alloy [17]. Total Kjeldahl N content of samples was measured by Kjeldahl digestion method [7]. The $NO_3^-$-N values were added to Kjeldahl values in order to determine actual total nitrogen content. A 0.5 M $NaHCO_3$ solution was used to extract available phosphorus. Nitric acid-sulphuric acid digestion was used to determine total phosphorus. Phosphate-P in extracts was measured

by ascorbic acid method [4]. Total organic carbon in samples was determined by potassium dichromate oxidation followed by spectrophotometric measurement at 590 nm [24].

The acid soluble heavy metal contents of sludge and fly ash samples were measured by ATI/UNICAM 929 atomic absorption spectrophotometer (AAS) following digestion with $HNO_3$ [4]. The heavy metal content of soil samples was also determined by AAS after digestion with aqua regia (HCl:$HNO_3$, 3:1, v/v).

### Analyses of Enzyme Activities

The potential enzymatic activity of urease, dehydrogenase, alkaline phosphatase and β-glucosidase were determined according to the methods described by Tabatabai [33].

The assay for urease activity was based on the determination of $NH_4^+$ released by urease when soil was incubated with THAM buffer (pH = 9), urea solution (0.02 M) and toluene at 37°C for 2 hours. The formation of ammonium was determined by steam distillation and results were expressed as μg $NH_4^+$-N $g^{-1}$ $h^{-1}$.

In order to determine the dehydrogenase activity, triphenyl tetrazolium chloride solution (3%) was added to soil and the suspension was incubated at 37°C for 24 hours. The formation of TPF (triphenyl formazan) was determined spectrophotometrically at 485 nm and the results were expressed as μg TPF $g^{-1}$ $h^{-1}$.

Alkaline phosphatase activities were performed by addition of modified universal buffer (pH = 11), toluene and p-nitrophenyl phosphate solution (0.025 M) to soil and incubation of the soil suspension at 37°C for 1 hour. Released (PNP) p-nitrophenol was determined spectrophotometrically at 410 nm and the results were expressed as μg PNP $g^{-1}$ $h^{-1}$.

β-glucosidase activity test was based on colorimetric determination of p-nitrophenol released by the enzyme when soil was incubated with buffered PNG (p-nitrophenyl-β-D-glucoside) solution as substrate and toluene. Released PNP (p-nitrophenol) was determined spectrophotometrically at 410 nm and the results were expressed as μg PNP $g^{-1}$ $h^{-1}$.

### Statistical Analyses

Factorial ANOVA test was carried out in order to test whether the amendments, soil type and incubation time caused any variation in enzyme activities. When significant main effects were indicated by ANOVA, post hoc analysis was performed using the Tukey's HSD multiple comparison test.

## RESULTS

Considerable variations in all enzyme activities were observed for the different fly ash ratios in sludge at different sampling times. The results of the factorial ANOVA test revealed that urease, dehydrogenase and alkaline phosphatase activities were significantly dependent on soil type, amendments and incubation time. The soil type was not found to affect β-glucosidase activity, whereas the effect of amendments and incubation time were found to be highly significant (Table 2).

### Urease Activity

Figure 1 shows the short term and the long term effects of sludge amendments on urease activity of soils. According to the results of short term incubation, application of sludge and sludge-fly ash mixtures inclined to increase urease activity in both types of soils. Addition of S0, S40 and S80 to NI soils increased the urease activity approximately 2.5 fold in comparison to C soil. However, the recorded activity in S120 amended soil was significantly lower ($p < 0.05$) than the value recorded in soil amended with sludge only (S0), indicating the short-term inhibitory impact of higher dose of fly ash on urease activity.

***Table 2. Results of Factorial ANOVA Test (Main Effects).***

| Sources of Variation | df | MS | *F* | *p* |
|---|---|---|---|---|
| Dependent variable: Urease | | | | |
| Soil | 1 | 1781 | 37.81 | <0.001 |
| Time | 1 | 4445 | 94.35 | <0.001 |
| Amendment | 4 | 2553 | 54.19 | <0.001 |
| Error | 40 | 47.11 | | |
| Dependent variable: Dehydrogenase | | | | |
| Soil | 1 | 372.9 | 384.5 | <0.001 |
| Time | 1 | 2140 | 2207 | <0.001 |
| Amendment | 4 | 196.4 | 202.5 | <0.001 |
| Error | 40 | 0.969 | | |
| Dependent variable: Alkaline Phosphatase | | | | |
| Soil | 1 | 3644 | 36.05 | <0.001 |
| Time | 1 | 274841 | 2718 | <0.001 |
| Amendment | 4 | 9861 | 97.55 | <0.001 |
| Error | 40 | 101.1 | | |
| Dependent variable:β-Glucosidase | | | | |
| Soil | 1 | 97.92 | 1.995 | n.s.* |
| Time | 1 | 10792 | 219.9 | <0.001 |
| Amendment | 4 | 733.9 | 14.95 | <0.001 |
| Error | 40 | 49.08 | | |

*n.s.: not significant

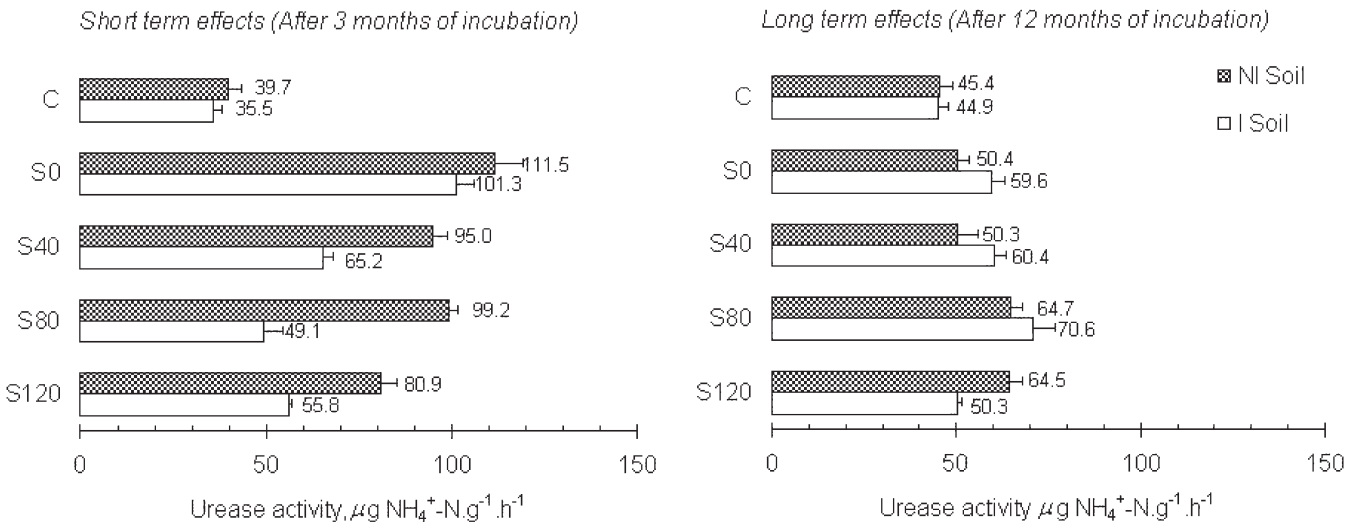


***Figure 1.*** *The effects of sludge amendments on urease activity of soils (C: control; S0: sludge without fly ash; S40: sludge+40% fly ash; S80: sludge+80% fly ash; S120: sludge+120% fly ash; I: irrigated soil; NI: nonirrigated soil).*

Although similar urease activities were observed in unamended NI and I soils (39.7 and 35.5 μg $NH_4^+$-N $g^{-1}$ $h^{-1}$, respectively), the general trend of the effects of sludge and fly ash-sludge amendments on I soils was rather different. Urease activities in polluted (I) soils were significantly lower than those observed in NI soils ($p < 0.05$).

In addition, the recorded activity values for, S40, S80 and S120 amendments on I soil (65.2, 49.1 and 55.8 μg $NH_4^+$-N $g^{-1}$ $h^{-1}$, respectively) were also significantly ($p < 0.05$) lower than that for S0 amendment (101.3 μg $NH_4^+$-N $g^{-1}$ $h^{-1}$).

The long term effects of amendments clearly indicated that urease activities in all sludge amended soils (S0, S40, S80 and S120) significantly decreased and generally approximated to control values. Tukey's HSD test ($p < 0.05$) indicated that no significant difference was observed between NI and I soils.

## Dehydrogenase Activity

Figure 2 shows the short term and the long term effects of sludge amendments on dehydrogenase activity of soils. Application of sludge and sludge-fly ash mixtures to I and NI soils significantly increased dehydrogenase activities ($p < 0.05$). However, there

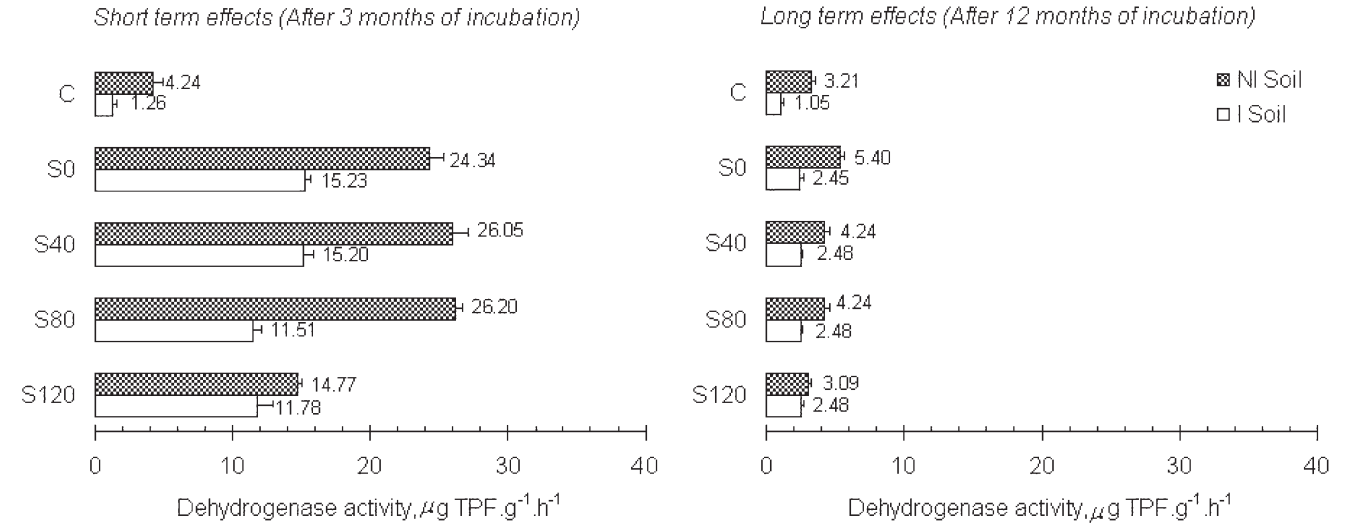


***Figure 2.*** *The effects of sludge amendments on dehydrogenase activity of soils (C: control; S0: sludge without fly ash; S40: sludge+40% fly ash; S80: sludge+80% fly ash; S120: sludge+120% fly ash; I: irrigated soil; NI: nonirrigated soil).*

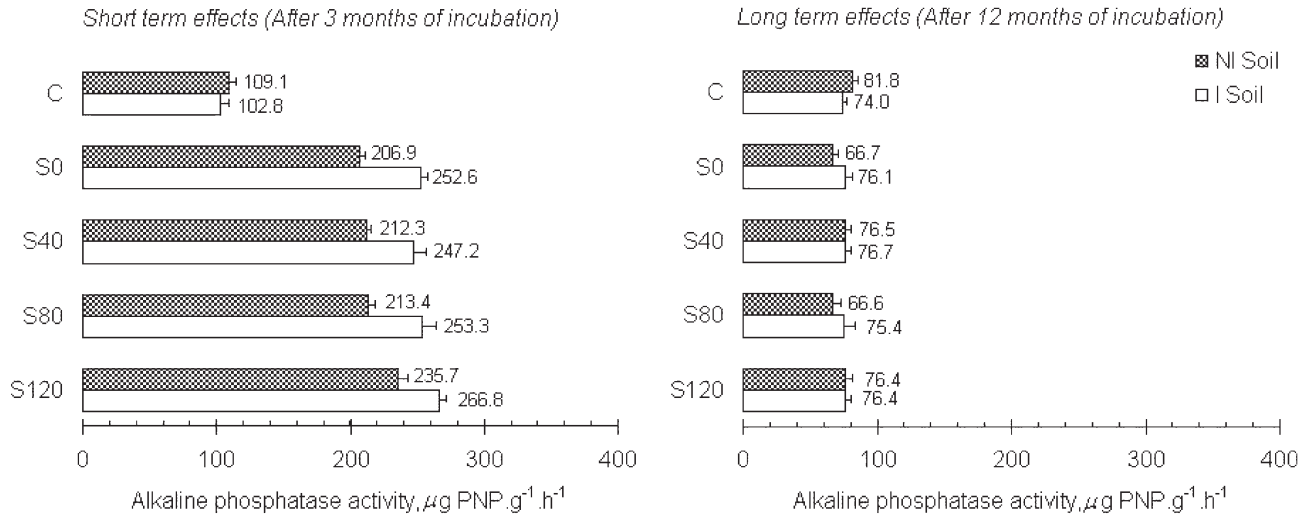


***Figure 3.*** *The effects of sludge amendments on alkaline phosphatase activity of soils (C: control; S0: sludge without fly ash; S40: sludge+40% fly ash; S80: sludge+80% fly ash; S120: sludge+120% fly ash; I: irrigated soil; NI: nonirrigated soil).*

were apparent differences between I and NI soils during the short term incubation. S0, S40, S80 and S120 amendments increased dehydrogenase activity of NI soils to 24.34, 26.05, 26.20 and 14.77 μg TPF $g^{-1}$ $h^{-1}$, respectively from a value of 4.24 μg TPF $g^{-1}$ $h^{-1}$. On the other hand, corresponding values for I soils were 15.23, 15.20, 11.51 and 11.78 μg TPF $g^{-1}$ $h^{-1}$.

After an incubation period of 12 months, the dehydrogenase activities in all amended soils decreased and approximated to control values. Activity values were recorded to be between 3.09–5.40 mg TPF $g^{-1}$ $h^{-1}$ in amended NI soils and between 2.45–248 μg TPF $g^{-1}$ $h^{-1}$ in amended I soils. No long term inhibition was observed in dehydrogenase activity of sludge/sludge-fly ash amended soils with respect to unamended control values.

## Alkaline Phosphatase Activity

The short term and the long term effects of sludge amendments on alkaline phosphatase activity of soils were presented in Figure 3. After the short term incubation, alkaline phosphatase activities significantly increased in both soils supplemented with sludge/sludge-fly ash mixtures ($p < 0.05$). Unparallel to the results obtained for urease and dehydrogenase activities, alkaline phosphatase activity appeared to be higher in I soil than that for NI soil. Slight increases were observed

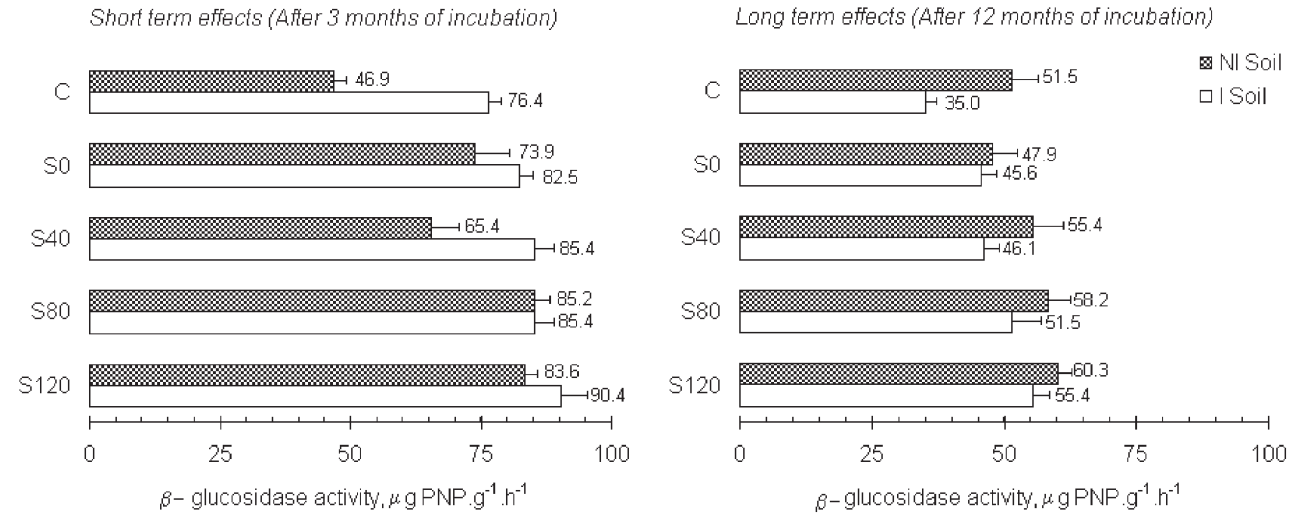


***Figure 4.*** *The effects of sludge amendments on β-glucosidase activity of soils (C: control; S0: sludge without fly ash; S40: sludge+40% fly ash; S80: sludge+80% fly ash; S120: sludge+120% fly ash; I: irrigated soil; NI: nonirrigated soil).*

in both types of amended soils with increasing fly ash ratios.

The long term effects showed that the alkaline phosphatase activities in all sludge amended soils significantly decreased and approximated to control values. Tukey's HSD test ($p < 0.05$) indicated that no significant differences occurred between NI and I soils after the incubation period of 12 months.

### β-glucosidase Activity

Figure 4 depicts the short term and the long term effects of sludge amendments on β-glucosidase activity of soils. The results of short term incubation indicated that the recorded β-glucosidase activity in unamended NI soil was significantly lower than that in I soil. However, sludge amendments brought the β-glucosidase activity of NI and I soils to similar levels except for S40 amendment. Higher activity was measured in S40 amended I soils. No short term inhibition was observed regarding fly ash content of sludge in both soils.

After the incubation period of 12 months, the activity level in unamended I soil decreased whereas it remained stable in unamended NI soil. The results of long term incubation indicated that there was again no significant difference ($p < 0.05$) between sludge amended NI and I soils with respect to β-glucosidase activity . The activities varied between 45.6 and 60.3 μg PNP $g^{-1}$ $h^{-1}$.

## DISSCUSSION

Significant increases in enzymatic activities were observed in all sludge and sludge-fly ash amended soils in short term, which were followed by progressive decreases. The highest activity values were generally recorded in sludge-only amended soils. High microbial biomass content and the high level of substrates in sludge were thought to activate the enzyme synthesis [5]. Other researchers have also observed important increases in enzyme activities in soils treated with organic materials [2].

In the present study, the sludge amendments increased soil urease activity by 40% to 100% in the short term. Stimulated urease activity in amended soils reflects the enhanced hydrolyzing capacity of C-N bonds of amide and urea. The urease activity of all treated soils showed similar trends. High levels of urease activity were observed after the short-term incubation followed by a decline to a relatively constant level at the end of long-term incubation. This decline may be attributed to the binding of urease inside the sludge floc [28]. In addition during the long term incubation, free urease would be attacked by soil protease as well as re-bound into the microbial cellular components [32]. Humification processes during the incubation period would also inactivate the urease by forming complexes with the functional group of the enzyme [18].

All sludge/sludge-fly ash amendments also caused initial increases in soil dehydrogenase activity. Sludge is composed of highly oxidizable organic substrates and a large biomass which explains the high intracellular dehydrogenase activity in soil receiving sludge amendment only [13]. A strong increment of dehydrogenase activity after the application of municipal solid waste compost has also been reported by Serra-Wittling et al. [31]. Martens et al. [21] also found similar increases after the use of sewage sludges. However, the high pH and salinity of fly ash were found to reduce the cell growth and dehydrogenase activity [36]. Pichtel and Hayes [29] reported that the activity of dehydrogenase were significantly inhibited after treatments of soil with alkaline power plant fly ash. Accordingly, increasing the amount of fly ash in sludge-fly ash mixtures in this study caused an apparent decrease in short-term dehydrogenase activities with respect to sludge only treatment. The final dehydrogenase activities (after 12 months of incubation) generally approximated to the control values. This appears to occur due to the depletion of organic substrates, accumulation of metabolic toxins and the decrease in pH after the peak of mineralization [26]. In addition, the encouraged nitrification process and the produced $NO_2^-$ and $NO_3^-$ may have an inhibitory effect on dehydrogenase activity [18].

Phosphatase is an enzyme of great agronomic value because it hydrolyzes compounds of organic phosphorus and transforms them into different forms of inorganic phosphorus, which are assimilable by plants [3]. Phosphatases catalyse reactions leading to the hydrolysis of both esters and anhydrides of $H_3PO_4$ [33]. Among these enzymes, phosphomonoesterases have been extensively studied in terrestrial ecosystems. Indeed, they are considered as the predominant phosphatases in most types of soil and litter [34] probably because of the low substrate specificity of these enzymes. The results of our short term incubation clearly showed that alkaline phosphatase activities were significantly increased in both soils supplemented with sludge/sludge-fly ash

mixtures probably due to high nutrient supply from the sludge. The activity levels slightly increased with increasing ash ratios in amended soils. Similarly, Lai et al. [19] found that fly ash-sludge mixture amendment showed a higher phosphatase activity than the amendment of sludge alone. They proposed that fly ash amendments were likely to improve soil texture and reduce binding of the enzyme to the clay particles. The increase in soil pH by the addition of high amounts of fly ash may also have activated the alkaline phosphatase activity. In various ecosystems, the hydrogen ion concentration may have an important influence on the production and activity of phosphatases. For example, Kang and Freeman [15] showed that pH was a dominant factor for phosphatase activities in wetland soils. Dick et al. [10] also pointed out the importance of pH by showing that the ratio of alkaline phosphatase to acid phosphatase increased after soil liming.

The results of short term incubation indicated that sludge amendments increased the β-glucosidase activity of soils significantly. Glucosidases are widely distributed in nature and their hydrolysis products as low molecular weight sugars are important source of energy for soil microorganisms. β-glucosidase catalyzes the hydrolysis of β-D-glucopyranoside and is one of the three or more enzymes involved in the saccharification of cellulose [5].

The present study also demonstrated that the responses of enzymes to sludge/sludge-fly ash amendments were apparently dependent on soil properties. The measured enzyme activities in two types of amended soils were rather different. The potential urease and dehydrogenase activities recorded at the end of 3 months of incubation were apparently lower in soil samples that were previously irrigated from polluted Nilüfer stream. The long term irrigation appears to have caused accumulation of toxic metabolites and heavy metals and resulted in the alteration of biochemical processes occurring in soil. When metal concentrations were high enough, inhibition was reported for enzyme activities [22]. Eizavi and Tabatabai [12] assumed that metal ions might inhibit enzyme reactions by complexing the substrate, combining with the protein active group of the enzymes or reacting with the enzyme-substrate complex. In addition, the increase in soil salinity due to irrigation from polluted Nilüfer stream may have a negative effect on soil's microbiological activity. Okur et al. [27] stated that the reductions in soil respiration, phosphatase and β-glucosidase activities were 70%, 61.5% and 61%, respectively when the salinity of the irrigation water was increased from 0.65 to 6.5 dS $m^{-1}$.

On the contrary, alkaline phosphatase activities were found to be higher in irrigated soils in this study. The enhanced alkaline phosphatase activity in irrigated soils may reflect a stress response to unfavourable conditions related to irrigation from polluted stream [20]. The overall evaluation of the study indicated that there was a remarkable difference between the responses of enzymes in two types of soils. Previous irrigation with polluted water appeared to reduce the activity of soil dehydrogenases and ureases.

## CONCLUSIONS

The following conclusions may be drawn from the present study:

1. The application of 100 t/ha wastewater sludge supplemented with fly ash doses of 40%, 80% and 120% (dry weight base) increased urease, dehydrogenase, alkaline phosphatase and β-glucosidase activities during short term incubation. However, the activity levels generally showed a decreasing trend with increasing ash ratios indicating the inhibitory impact of fly ash.
2. Enzyme activities generally approximated to the control values at the end of 12-month incubation.
3. The probable negative effects of fly ash on enzymatic activities were thought to be masked by the counteracting effect of wastewater sludge.
4. The previous management practices in soils may also have a significant effect on the responses of urease and dehydrogenase activities to sludge/sludge-fly ash amendments. The measured activities were found to be significantly lower in soils irrigated from a polluted stream.
5. Alkaline phospatases and β-glucosidases appear to be less sensitive to soil contamination created by the past management practices.

## ACKNOWLEDMENTS

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# Kinetics of Thermo Chemical Hydrolysis of Biomass: A Comparative Study of Waste Activated Sludge and Yeast

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**ABSTRACT:** The kinetics of thermo-chemical hydrolysis of waste activated sludge from wastewater treatment plant and yeast has been studied under different environmental conditions. In order to determine the effect of thermo-chemical hydrolysis, the experiments have been performed at different temperatures, time intervals and pHs. It has been observed that increasing temperature and hydrolysis time increased the rate and extent of hydrolysis. Under these conditions, the extent of solubility increased for values between pH (2–3) and between pH (10–11). The increased temperature has also increased the efficiency of hydrolysis, and the solubility of COD in biomass has also increased up to 18–85% of mass for activated sludge and yeast under different experimental conditions. The kinetics of hydrolysis has been found to fit first order kinetics.

## 1. INTRODUCTION

WASTEWATER treatment capacity is expanding quickly around the world because of more stringent effluent criteria and due to the construction of new wastewater treatment plants. Therefore, managing the excess sludge from wastewater treatment plants will be one of the most challenging tasks. Coupled with social and environmental concerns, processes aimed at reduction and minimization of excess sludge are receiving increasing attention.

Sludge in treatment plants is either produced by physical/mechanical pretreatments, chemical treatments (i.e. precipitation) or biological treatment. There are in principle three main strategies to reduce excess sludge in biological treatment plants: 1-enhanced pretreatment (reduced load) [1], 2-yield reduction using various strategies such as by increasing maintenance and endogenous metabolism either using un-couplers or controlling sludge retention time in activated sludge process [2,3], and 3-sludge disintegration by which greater part of sludge becomes biodegradable and re-assimilated by biomass.

In order to disintegrate the structure of waste activated sludge, physicochemical pretreatment methods are used and particulate compounds in waste sludge are transformed into soluble compounds [4]. The studies on pretreatment of waste sludge are mainly focused on thermal and thermo-chemical processes. Chang et al. (2002) studied the hydrolysis of activated sludge by adding NaOH at 25°C. They found 45% of solublized COD (sCOD) for 10 hours reaction time and 40 meq/l NaOH dosages at 1% TSS concentration. Stuckey and McCarty (1979a) and Stuckey and McCarty (1984b) reported that 40–55% of total COD was solubilized at a temperature range of 175–200°C, using HCl, NaOH, and $Ca(OH)_2$ at one hour retention time. In another study it has been reported that at 20–40°C temperatures and 0.5–24 hours retention times, 45% of total COD has been solubilized using NaOH [7,8].

The aim of this study is the optimization and kinetics of thermo-chemical hydrolysis of waste activated sludge and yeast. Yeast was chosen as eukaryotic model organism. The waste activated sludge and yeast have been subject to various temperatures, pH, and disintegrated into soluble compounds. The kinetics of the process has been assessed as a function of operational conditions.

### 1.2. KINETICS OF HYDROLYSIS

The rate of hydrolysis of biomass into soluble sub-

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strates is a first order function of particulate biomass at constant temperature and pH [9,10]:

$$\frac{d(\mathrm{COD})}{dt} = k_1(\mathrm{COD}_\infty - \mathrm{COD}) \quad (1)$$

where COD = soluble COD concentration ($mgL^{-1}$); $COD_\infty$ = maximum theoretical soluble *COD* concentration ($mgL^{-1}$) defined as $COD_p - COD_{nh}$; $COD_p$ = particulate COD concentration; $COD_{nh}$ = non-hydrolysable $COD_p$ concentration; $k_1$ = first order rate constant ($h^{-1}$). Integration of Equation (1);

$$\int_0^{\mathrm{COD}} \frac{d(\mathrm{COD})}{\mathrm{COD}_\infty - \mathrm{COD}} = \int_0^t k_1 dt \quad (2)$$

yields;

$$\ln\left(\frac{\mathrm{COD}_\infty}{\mathrm{COD}_\infty - \mathrm{COD}}\right) = k_1 t \quad (3)$$

or

$$\mathrm{COD} = \mathrm{COD}_\infty(1 - e^{-k_1 t}) \quad (4)$$

or changing natural logarithm;

$$\mathrm{COD} = \mathrm{COD}_\infty(1 - 10^{-k_1 t}) \quad \text{where} \quad k = 0.434\, k_1 \quad (5)$$

$$F_1 = 1 - 10^{-kt} \quad (6)$$

$$F_2 = (2.3kt)\left[1 + \frac{2.3kt}{6}\right]^{-3} \quad (7)$$

Equations (6) and (7) can be transformed in series as follows [11];

$$F_1 = (2.3kt)\left[1 - \frac{1}{2}(2.3kt) + \frac{1}{6}(2.3kt)^2 - \frac{1}{24}(2.3kt)^3 + \ldots\right] \quad (8)$$

$$F_2 = (2.3kt)\left[1 - \frac{1}{2}(2.3kt) + \frac{1}{6}(2.3kt)^2 - \frac{1}{21.6}(2.3kt)^3 + \ldots\right] \quad (9)$$

The first three terms of the above series of equations are similar and small differences exist for the rest of the terms that will affect $F_1$ and $F_2$ very little. Hence $F_1 = F_2$ written using Equations (5), (6) and (7);

$$\mathrm{COD} = (2.3kt)\left[1 + \frac{2.3}{6}kt\right]^3 \mathrm{COD}_\infty \quad (10)$$

By rearranging Equation (10) the following expression is obtained;

$$\left(\frac{t}{\mathrm{COD}}\right)^{1/3} = \frac{1}{2.3k(\mathrm{COD})^{1/3}} + \frac{(2.3k)^{2/3}}{6\mathrm{COD}^{1/3}}t \quad (11)$$

Equation (11) is linear if $(t/\mathrm{COD})^{1/3}$ is plotted versus $t$. The parameters A and B are determined from Equation (11) using linear regression and their relationship with $k$ and $COD_\infty$ are given by Equation (12) and (13) as;

$$A = [2.3k(\mathrm{COD}_\infty)]^{-1/3} \quad (12)$$

$$B = \frac{(2.3k)^{2/3}}{6\mathrm{COD}_\infty^{1/3}} \quad (13)$$

$$(t / \mathrm{COD})^{1/3} = A + Bt \quad (14)$$

From Equation (12) and (13)

$$k_1 = 2.61\frac{B}{A} \quad \text{or} \quad k = 4.8387\frac{B}{A} \quad (15)$$

$$\mathrm{COD}_\infty = \frac{1}{6A^2B} \quad (16)$$

## 2. MATERIALS AND METHODS

Thermochemical hydrolysis of the sludge and yeast taken from an industrial biological wastewater treatment plant was investigated by using $H_2SO_4$ and NaOH. Variables considered in the hydrolysis experiments were pH, temperature and time (pH = 2–12, temperature = 70–100°C in a water bath, 120°C maintained in an autoclave and 1.5 atm pressure, duration 30 min–8 hours). The operation was carried out in 1000 mL flask stirring of reactors by magnetic device was on a continuous basis.

The total solid concentration is expressed as dry weight. After the samples were kept at certain temperature, time and pH, they are centrifuged by NÜVEFÜJ 815 (4000 rotation/minute) for ten minutes to separate the dissolved part. Then, it is filtered by 0.40 µm filter

paper with known weight and then dried. The COD measurement in supernatant is performed according to Standard Methods (Method No 5220-D). The Total Kjeldahl nitrogen concentrations in the supernatant were determined after combustion at 400°C according to Standard Methods (Method No 4500-$N_{org}$ B). Supernatant $NH_4^+$-N measurements were also performed by distillation and titration methods (Methods No 4500-$NH_3$ B. Preliminary Distillation Step and 4500-$NH_3$ C. Titrimetric Method). The material on the filter is dried at 105°C for one day according to Standard Methods (Method No 2540 D Total suspended Solids Dried at 103–105°C), and then a known amount of it is incinerated at 550°C for 30 minutes for the analysis of organic matter according to Standard Methods (Method No 2540 E Fixed and Volatile Solids Ignited at 550°C) [12]. The empirical formula of the activated sludge is given as $C_5H_7NO_2$ in the literature and the molecular weight is 113 g $mol^{-1}$ [13]. The empirical formula of the yeast is given as

$$CH_{1.613}O_{0.557}N_{0.158}P_{0.012}S_{0.003}K_{0.022}Mg_{0.003}Ca_{0.001}$$

in the literature and the molecular weight is 26.175 g $mol^{-1}$ [14]. The oxidation of the sludge cell and yeast is given as (17, 18);

$$C_5H_7NO_2 + 5O_2 \rightarrow 5CO_2 + 2H_2O + NH_3 \quad (17)$$

$$\begin{aligned} &CH_{1.613}O_{0.557}N_{0.158}P_{0.012}S_{0.003}K_{0.022}Mg_{0.003}Ca_{0.001} + \\ &1.151O_2(g) \rightarrow 1.000CO_2(g) + 0.806H_2O(l) + \\ &0.079N_2(g) + 0.003P_4O_{10}(cr) + 0.003SO_3(g) + \\ &0.011K_2O(cr) + 0.003MgO(cr) + 0.001CaO(cr) \end{aligned} \quad (18)$$

In Equation (17), the COD value for 1 g sludge is 1.42 $gO_2$. In Equation (18), the COD value for 1 g yeast is 1.41 $gO_2$. In the hydrolysis studies, the suspended volatile solids content is calculated and the dissolved COD efficiency is calculated over the sludge and yeast COD.

## 3. RESULTS AND DISCUSSION

### 3.1. Effect of Environmental Conditions on Soluble COD

The effect of environmental conditions on soluble COD (sCOD) has been studied at different pH values, reaction times and temperature for both activated sludge and yeast.

The experiments were carried out were at pH 2–12 and temperatures 70, 100, 120°C for yeast. The samples were taken at the end of 30, 60, 120 minutes and 5 hours. The results of hydrolysis experiments for yeast are shown in Figure 1 (a, b, c, d) for different reaction times. The initial COD concentration of yeast to be hydrolyzed was 57330 mg $L^{-1}$. As can be seen from this figure, the sCOD increases at extreme pH's both on the acidic and alkaline side. The solubility of yeast dramatically increased up to 70–80% above pH 11. The extent of hydrolysis is also a function of temperature and reaction time and increases with raised pH and reaction time. At the 5 hours reaction time and 120°C the extent of hydrolysis reached 80% and this value shows that hydrolysis is continuing.

The effect of environmental conditions on the extent of hydrolysis of excess sludge produced from a wastewater treatment plant was studied at 2, 4, and 8 hours reaction time and similar pH and temperatures to yeast [15]. The results are shown in Figure 2 (a, b, c). According to the results, temperature and pH have a more positive effect on the extent of hydrolysis than reaction time. Even at the 2 hours of reaction time, almost 90% of hydrolysis has been achieved at alkaline and acidic pH's.

### 3. 2. Effect of Environmental Conditions on Soluble N

The change in soluble nitrogen in yeast and sludge was investigated by thermochemical hydrolysis.

Soluble Total Kjeldahl Nitrogen (sTKN) and free ammonia were measured and monitored during hydrolysis experiments. The sTKN values for yeast and activated sludge are shown in Figures 3 (a, b, c, d) and 4 (a, b, c) respectively for different reaction time, pHs and temperatures.

The maximum amount of nitrogen present in the yeast sample was 3437 $mgL^{-1}$. Figure 3 (a, b, c, d) shows that soluble nitrogen concentration increases as the temperature pH and reaction time increase. Almost half of total nitrogen has been solubilized at 5 hours reaction time and 120°C temperature. Similar results were observed for the solubilization of total nitrogen for activated sludge as shown in Figure 4(a, b, c).

The initial maximum amount of dissolved N is 3437 $mgL^{-1}$. From the Figure 3(a) it can be seen that as pH and temperature increase, dissolved TKN concentration increases. While under conditions of pH 2–3 the N solubility is lower, between pH 4–9 N solubility in-

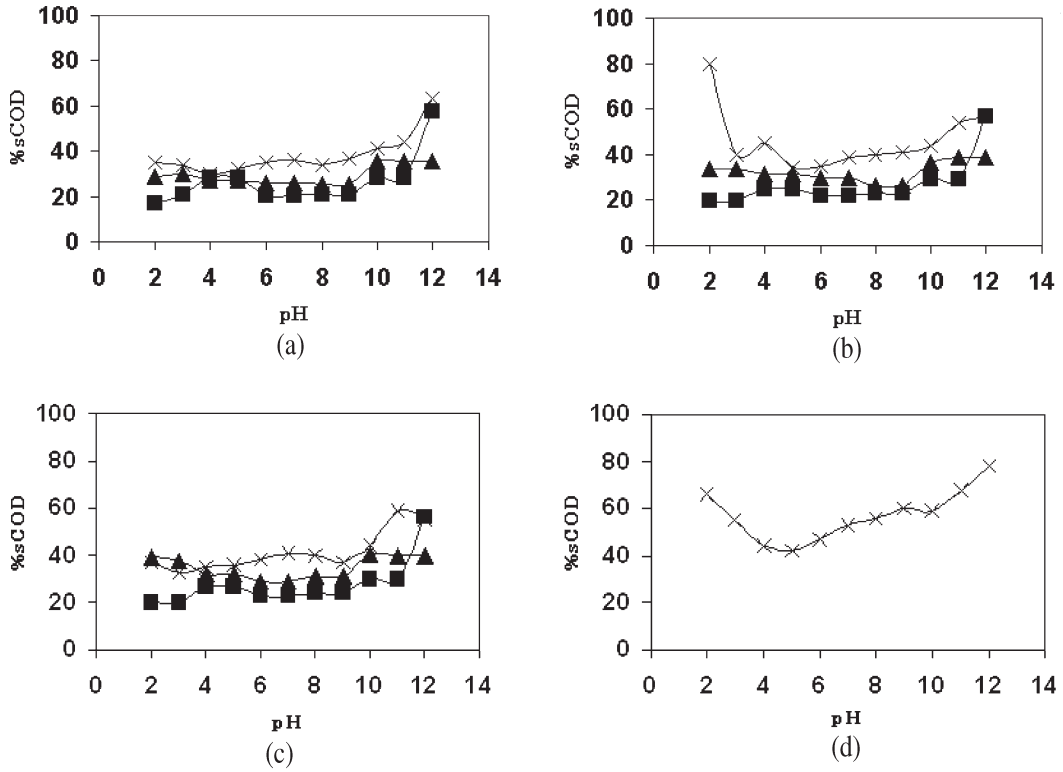


***Figure 1.*** *The extent of hydrolysis of yeast as a function of pH and temperature at different reaction time; (a) 30 minutes (b) 60 minutes (c) 120 minutes (d) 5 hours. (■) 70°C, (▲) 100°C, (×) 120°C.*

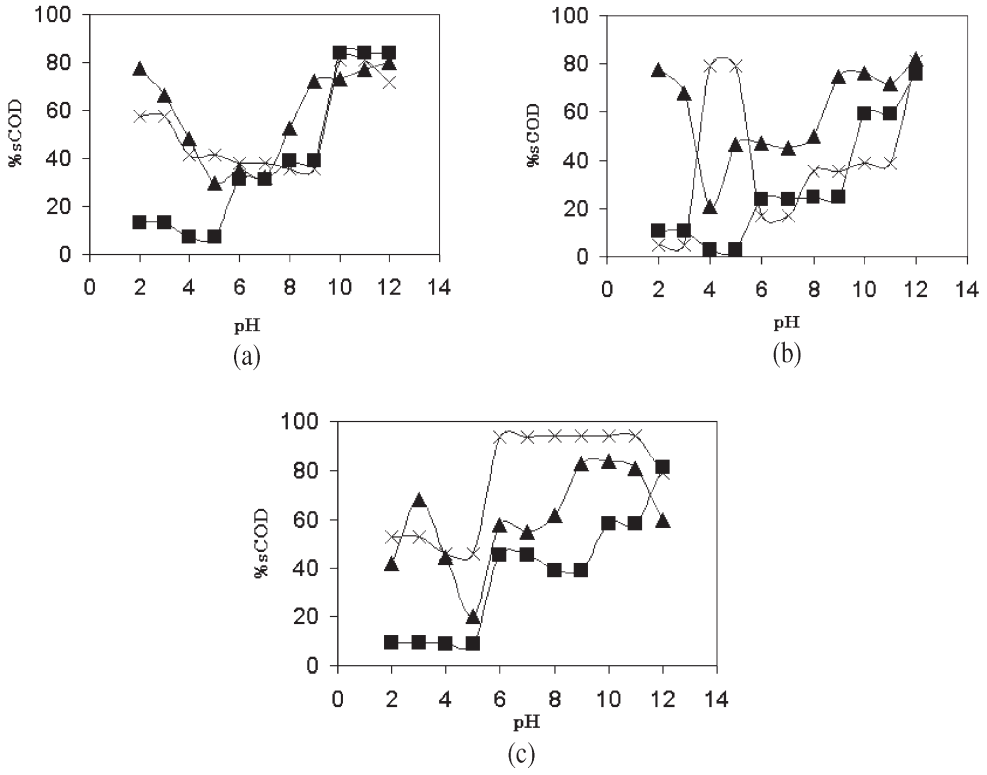


***Figure 2.*** *The extent of hydrolysis of sludge as a function of pH and temperature at different reaction time; (a) 2 hours (b) 4 hours (c) 8 hours. (■) 70°C, (▲) 100°C, (×) 120°C.*

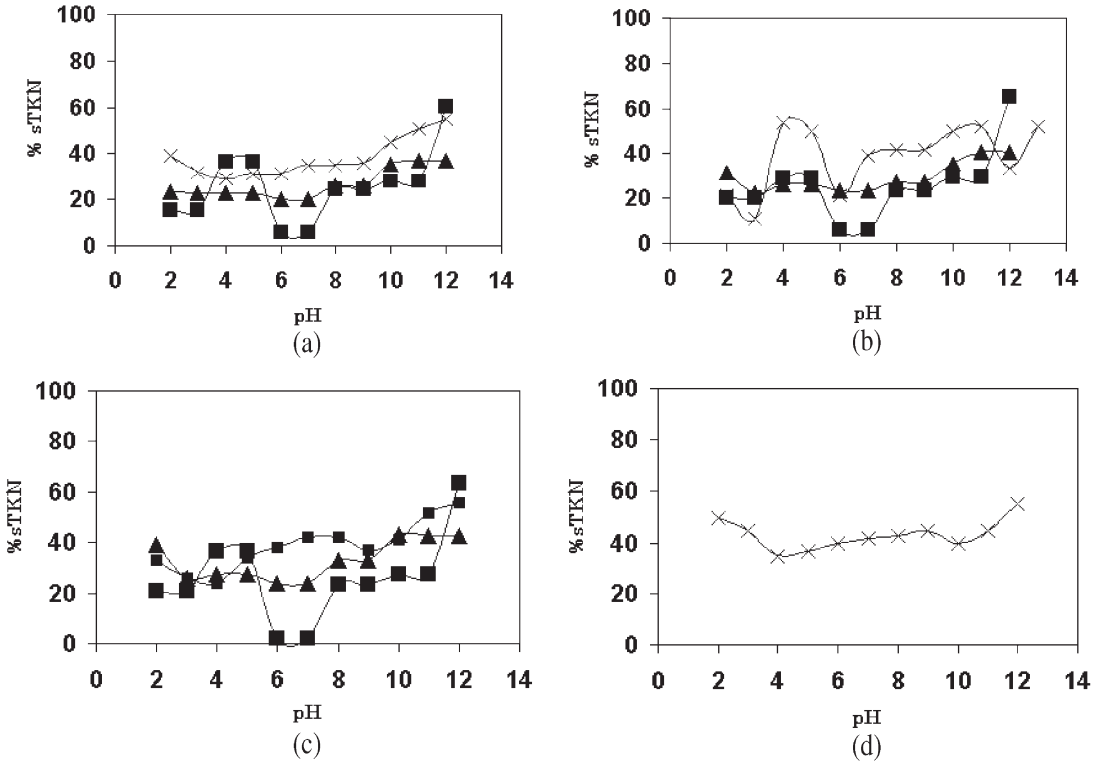


***Figure 3.*** *Solubilization Total Kjeldahl Nitrogen of yeast as a function of pH and temperature at (a) 30 minutes (b) 60 minutes (c) 120 minutes (d) 5 hours reaction times. (Total initial nitrogen 3437 mgL$^{-1}$). (■) 70°C, (▲) 100°C, (×) 120°C.*

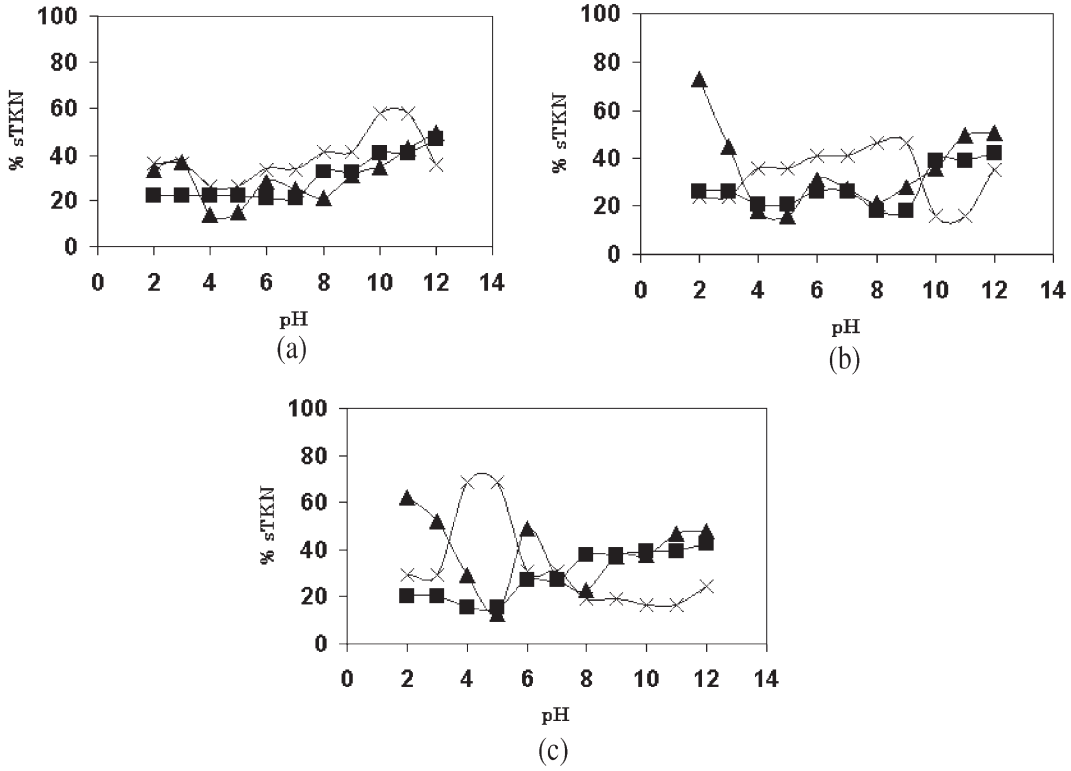


***Figure 4.*** *Solubilization Total Kjeldahl Nitrogen of sludge as a function of pH and temperature at (a) 2 hours (b) 4 hours (c) 8 hours reaction times. (Total initial nitrogen 2125 mgL$^{-1}$). (■) 70°C, (▲) 100°C, (×) 120°C.*

creases. Also, above pH 9 a sharp increase in the N solubility is observed. At pH 9–12 and high temperatures, the amount of TKN dissolved increases as seen from the Figure 3(a).

According to Figure 3(b), N solubility at pH 2–3 and 100°C is higher than that at the other temperatures. Also, at 120°C rapid N solubility is observed just after pH 3, but then at pH 4–6 it decreases and at pH 6–11 increases again. It is seen that at pH 10–11 the dissolved amount of TKN at 120°C is much more than at 70°C and 100°C.

As it can be seen from the Figure 3 (c) the N solubility increases above pH 11. Figure 3(d) indicates that as the hydrolysis time increases the amount of dissolved TKN increases. As a result, increasing hydrolysis of time affects the efficiency of TKN solubility positively. For activated sludge the dependency of dissolved TKN amount to pH at different temperatures are shown for 2, 4, 8 hours reactions times on Figure 4(a, b, c) respectively.

### 3.3. Effect of Environmental Conditions on Soluble $NH_4^+$-N

In order to determine whether soluble nitrogen is ammonia ($sNH_4^+$-N) or organic nitrogen, ammonia concentrations were measured and monitored during hydrolysis experiments. These results are shown in Figures 5 (a, b, c, d) and 6 (a, b, c) for yeast and sludge, respectively. As can be seen from Figure 5, one fifth to one tenth of total soluble nitrogen is in the form of ammonia nitrogen and the rest is in the form of organic nitrogen. The highest soluble ammonia was obtained at 120°C. Similar results were obtained for the solubilization of ammonia nitrogen for activated sludge as shown in Fig. 6 (a, b, c). Again, the maximum solubility of ammonia was obtained at 120°C for sludge hydrolysis experiments.

## 4. KINETICS OF HYDROLYSIS

### 4.1. Yeast Hydrolysis

The data obtained from hydrolysis experiments presented in previous sections were analyzed for the kinetics of biomass hydrolysis. The data is plotted using Equation (14) for experimental data of yeast hydrolysis in Figure 7 (a, b, c) at different temperatures and pHs.

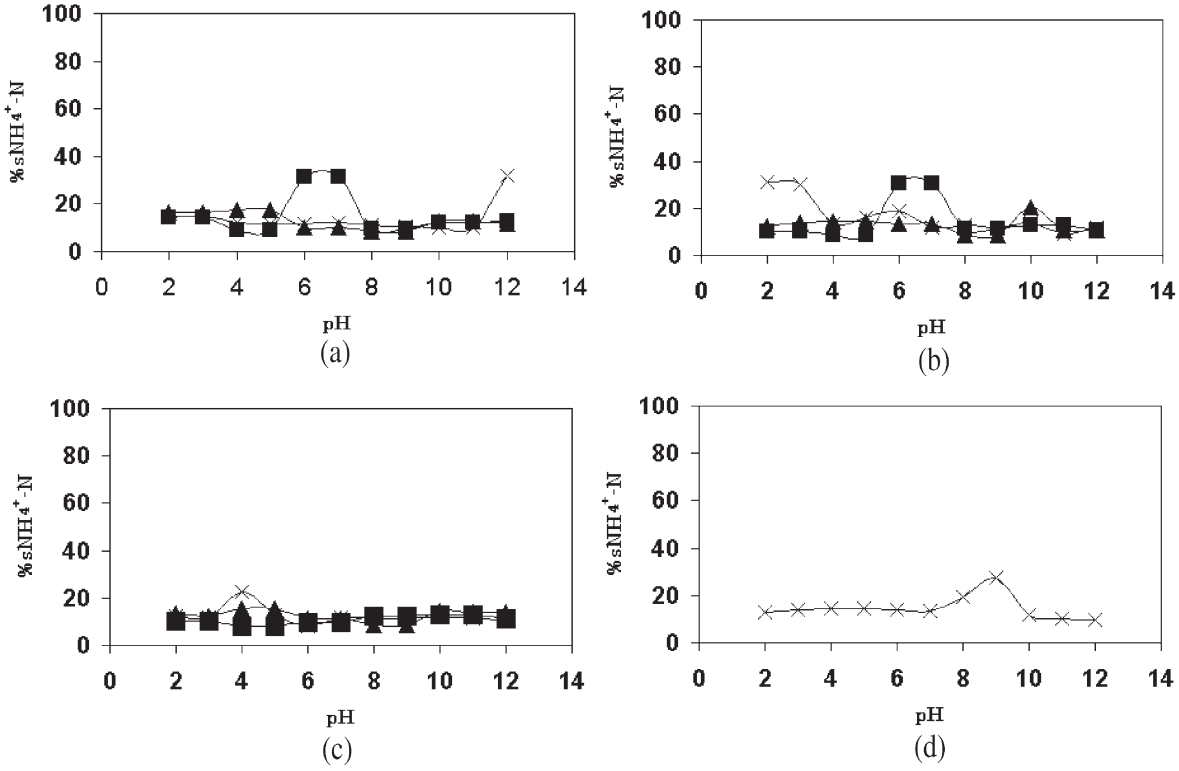


***Figure 5.*** *Soluble ammonia concentration of yeast as a function of pH and temperature at (a) 30 minutes (b) 60 minutes (c) 120 minutes (d) 5 hours reaction times. (Total initial nitrogen 3437 mgL$^{-1}$). (■) 70°C, (▲) 100°C, (×) 120°C.*

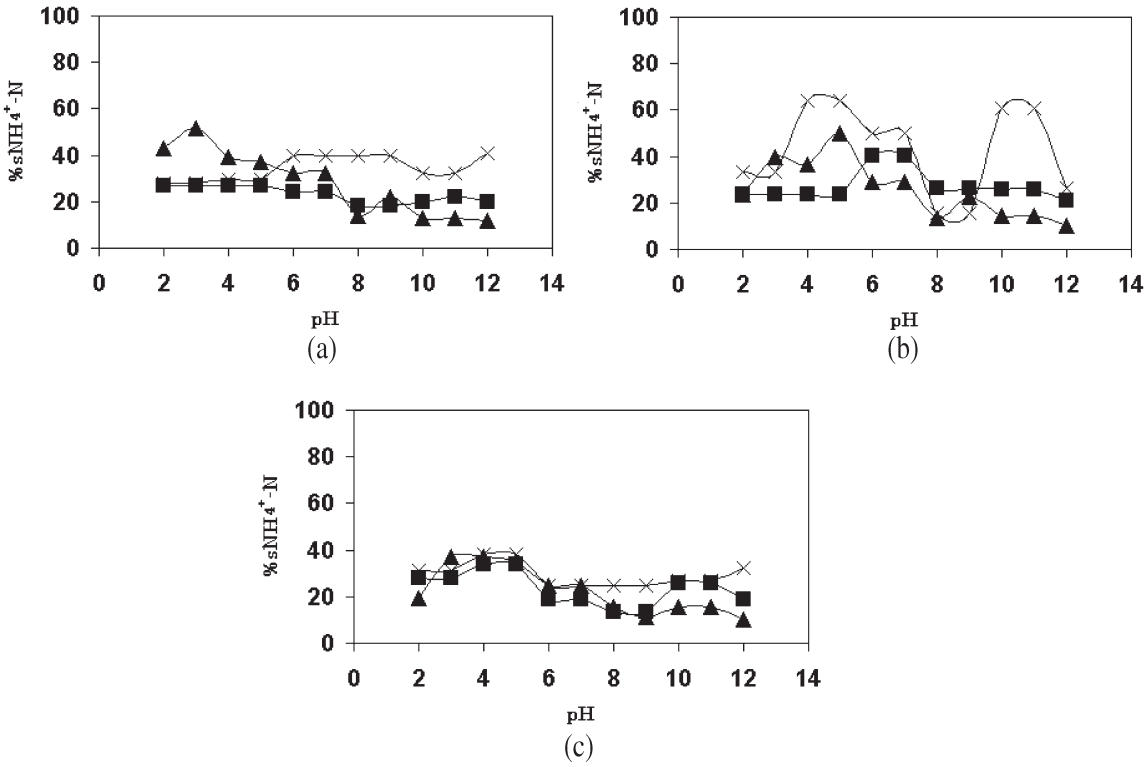


***Figure 6.*** *% Soluble ammonia concentration of sludge as a function of pH and temperature at (a) 2 hours (b) 4 hours (c) 8 hours reaction times. (Total initial nitrogen 2125 mgL$^{-1}$). (■) 70°C, (▲) 100°C, (×) 120°C.*

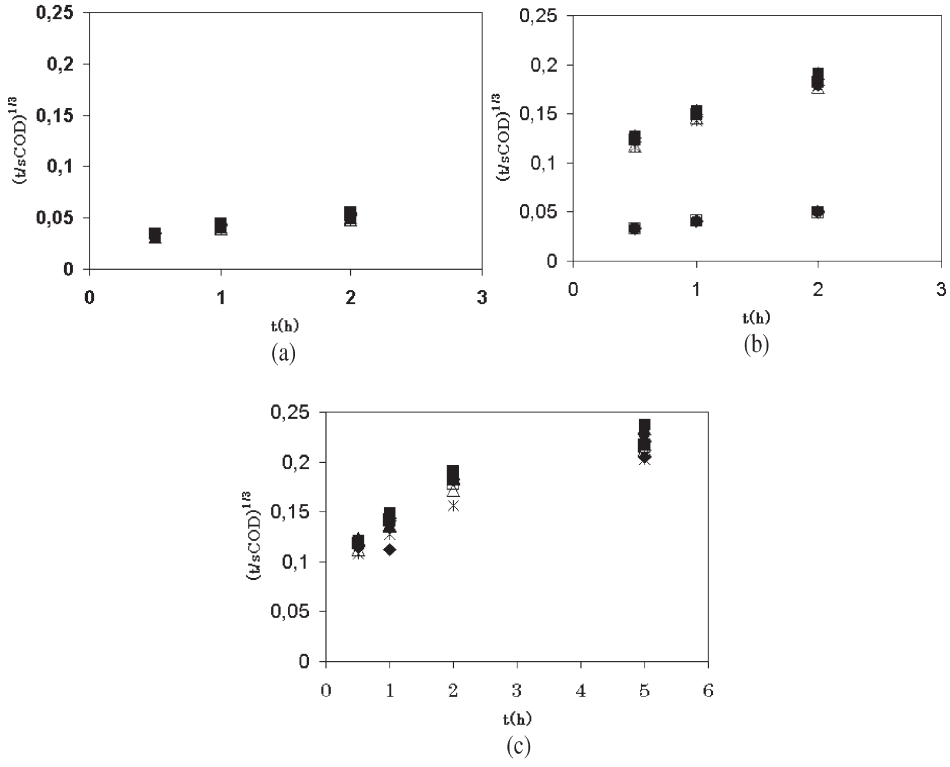


***Figure 7.*** *The rate of hydrolysis kinetics for yeast. (a) 70°C, (b) 100°C, (c) 120°C, (◆) pH 2, (■) pH 3, (▲) pH 4, (■) pH 5, (✱) pH 6, (–) pH 7, (□) pH 8, (◇) pH 9, (△) pH 10, (×) pH 11, (○) pH 12.*

### 4.2. Hydrolysis of Waste Activated Sludge

The data obtained from hydrolysis experiments presented in previous section were analyzed for the kinetics of biomass hydrolysis. The data is plotted using Equation (14) for experimental data of waste activated sludge hydrolysis in Figure 8 (a, b, c) at different temperatures and pHs.

Also, in Figure 9, the dependency of hydrolysis rate constant on pH at different temperatures of the sludge is shown. It is seen that the values of hydrolysis rate constant ($h^{-1}$) at 70–120°C are between 0 and 50 $h^{-1}$. According to Figure 9 (b), the highest hydrolysis rate constant was obtained at 100°C for pH 9 and at 120°C for pH 4.

As it can be seen from Figure 9, the hydrolysis of activated sludge occurred more rapidly than that of yeast. While the values of hydrolysis rate constants in sludge reach 150 and 200 $h^{-1}$, the biggest value of that in yeast is 29 $h^{-1}$. Also, decrease in hydrolysis rate at natural sludge (pH 6–7) is observed. The dependence of activations energy for yeast and activated sludge as a function of pH and temperature is shown in Figure 10.

The relationship between temperature and reaction rate is given by Arrhenius equation (19) [16].

$$\ln k = -\frac{\Delta H}{RT} + \ln C \tag{19}$$

where $k$ = rate coefficient, $R$ = universal gas constant (8.314 J/Kmol), $T$ = Temperature (K), $\Delta H$ = activation energy ($kJmol^{-1}$).

The activation energy for the breakdown of yeast is relatively higher than that for excess sludge as shown in Figure 10. This also indicates that hydrolysis of yeast is more difficult than breaking down bacteria.

## 5. CONCLUSIONS

Following conclusions can be drawn from this study:

- The Raised temperature increases the hydrolysis efficiency and the efficient biodegradation is observed at 120°C.
- The increase in retention time makes the hydrolysis more efficient.
- Under different experimental conditions the amount of sCOD reaches 18–85% for the yeast and the activated sludge.
- When TKN and $NH_4^+$-N are compared, there is always more TKN in the supernatant than that of $NH_4^+$-N.

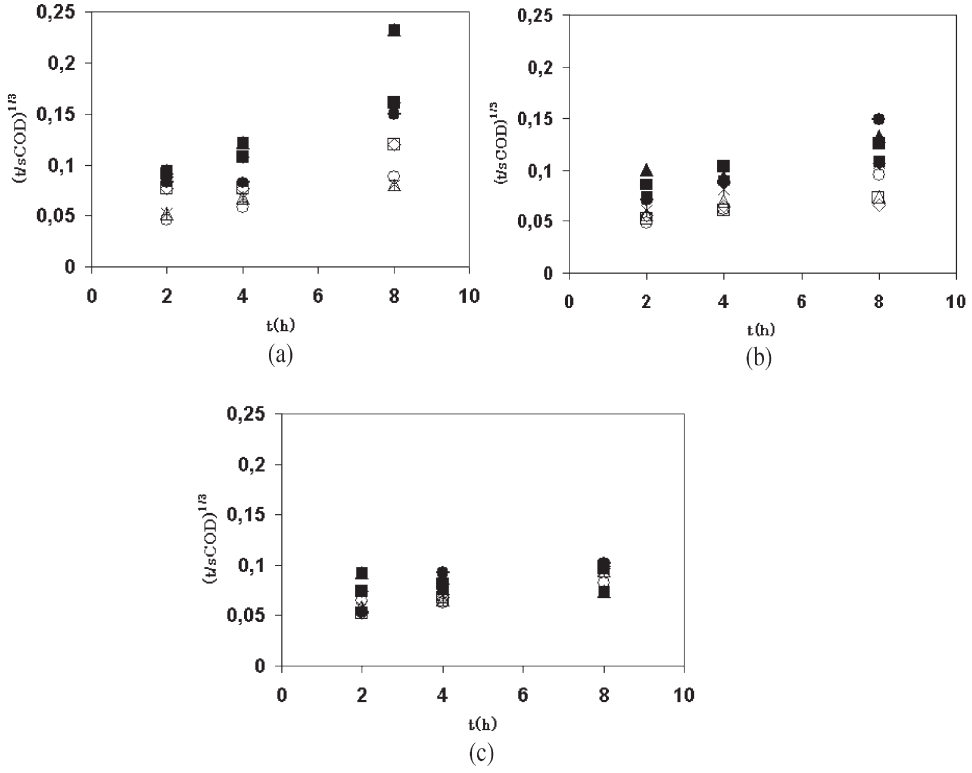


***Figure 8.*** *The rate of hydrolysis kinetics for sludge. (a) 70°C, (b) 100°C, (c) 120°C, (◆) pH 2, (■) pH 3, (▲) pH 4, (■) pH 5, (✱) pH 6, (–) pH 7, (□) pH 8, (◇) pH 9, (△) pH 10, (×) pH 11, (○) pH 12.*

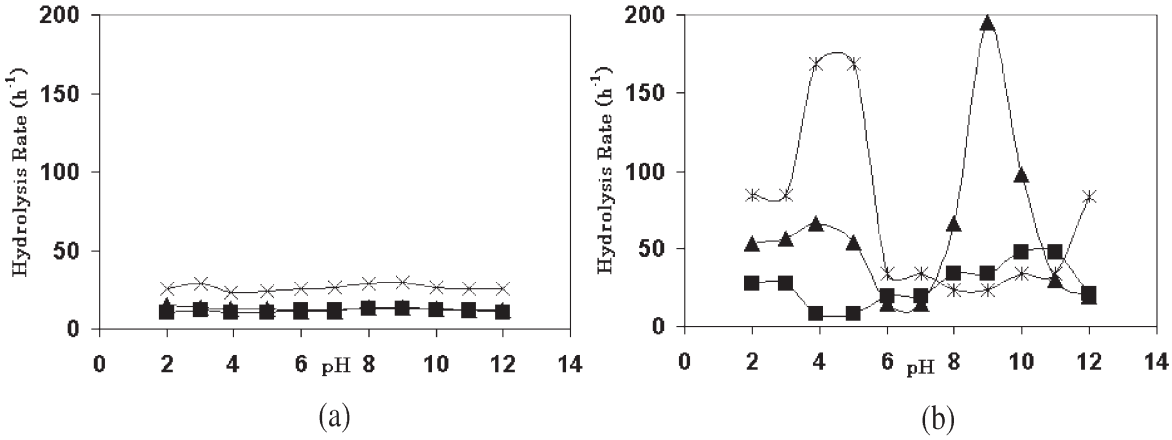


***Figure 9.*** *Hydrolysis rate for yeast (a) and activated sludge (b) as a function of pH and temperature at (■) 70°C, (▲) 100°C, (×) 120°C.*

- The hydrolysis kinetics of the sludge and yeast are examined at constant temperature and pH. The results are fitted to the first order reaction kinetics.
- The hydrolysis rate of waste activated sludge is found 194.8 $h^{-1}$ at 100°C. When it is compared with the hydrolysis rate constants of yeast and waste activated sludge, it is seen that the hydrolysis of activated sludge occurs more rapidly than that of yeast.
- The sCOD increases at extreme pHs both on acidic and alkaline side. The solubility of yeast has increased up to 70–80% above pH11. At 5 hours reaction time and 120°C the extent of hydrolysis reaches to 80%.
- At 120°C, after 5 hours reaction time, it is seen that the hydrolysis of yeast was continued.
- sTKN concentration increases as the temperature, pH, and reaction time increase.
- Almost half of total nitrogen has been solubilized at 5 hours reaction time and 120°C temperature.
- One fifth to one tenth of total soluble nitrogen is in the form of ammonia nitrogen and the rest is in the form of organic nitrogen.
- Highest solubility rate constant ($h^{-1}$) at 120°C are higher then the values at temperature of 70 and 100°C.
- The activation energy of sludge is lower than that of yeast. This indicates hydrolysis rate of sludge is higher than hydrolysis rate of yeast.
- The usage of hydrolysis production in anaerobic treatment is important. Anaerobic treatment should be applied after these results.

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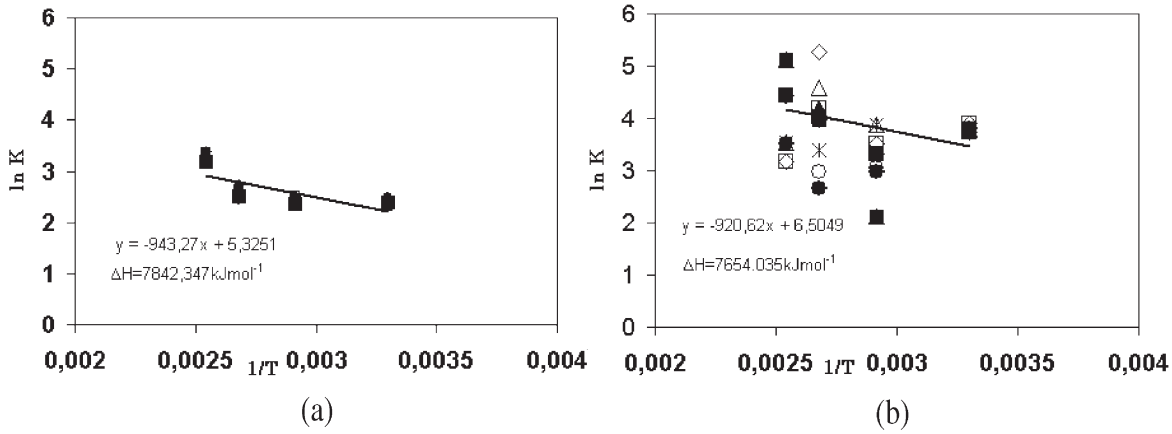


***Figure 10.*** *Activation energy for (a) yeast and (b) activated sludge as a function of pH and temperature at (◆) pH 2, (■) pH 3, (▲) pH 4, (■) pH 5, (✱) pH 6, (–) pH 7, (□) pH 8, (◇) pH 9, (△) pH 10, (×) pH 11, (○) pH 12.*

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*Table 5. Comparison of state-of-the-art matrix resins with VPSP/BMI copolymers.*

| Resin System | Core Temp. (DSC peak) | $T_E$ | Char Yield, % |
|---|---|---|---|
| Epoxy (MY720) | 235 | 250 | 30 |
| Bismaleimide (H795) | 282 | >400 | 48 |
| VPSP/Bismaleimide copolymer | | | |
| C379: H795 = 1.9 | 245 | >400 | 50 |
| C379: H795 = 1.4 | 285 | >400 | 53 |

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