ppm level in soybean oil on bacon slices, prepared with and without incorporation of sodium erythorbate (550 ppm) in the normal pickle (150 ppm of nitrite), gave 80–90% reduction of nitrosamine formation in the cook-out fat.

(4) Under household frying conditions, the C_{16} ascorbyl acetal treated bacons give vanishingly small amounts (<1 ppb) of nitrosamines.

(5) The mode of application of the acetal is not critical. Thus, the ascorbyl C_{16} acetal, when sprinkled as a solid or added as a solution in soybean oil to the frying pan in which the bacon slices are subsequently fried, gave the same excellent (>90%) reductions in nitrosamine content as when applied directly to the bacon slices in soybean oil at the same level (1000 ppm).

(6) Unlike AP, the C_{16} acetals of both ascorbic and erythorbic acids retain their activities (>90% inhibition of nitrosamines) for at least 35 days at +3 °C when applied to bacon at the 1000-ppm level; the reduction is more pronounced with NPyr than with NDMA. The residual nitrosamine contents in the rashers and cook-out fats were 0–3 and 1–4 ppb, respectively, despite the fact that optimized frying conditions producing maximum amounts of nitrosamines were used.

(7) The smoking operation during the processing of bacon has little, if any, effect on the nitrosamine levels of cooked bacon, suggesting that C-nitrosophenols are not implicated in nitrosamine formation, e.g., via transnitrosation.

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Metal Uptake by Crops Grown over Entrenched Sewage Sludge

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Oats (*Avena sativa* L.), wheat (*Triticum aestivum* L. em. Thell), and chard (*Beta vulgaris* var. Cicla) were grown on plots containing entrenched digested and limed raw sewage sludge to determine the metal uptake by the crop and the effect of surface pH adjustment on this uptake. The digested and control plots were split with half receiving surface applications of lime to determine the effects of liming the surface soil on metal uptake. The limed raw sludge plot was not split because its surface soil was already pH 6.7. Metal uptake by the crops reflected metal content of the sludge and the pH of the sludge and subsoil more than the pH of the surface soil. Chard accumulated higher metal levels than either wheat or oats which had similar metal accumulations. Crops grown in limed raw sludge plots had metal levels equal to or less than those of crops grown on control plots with limed surface soil. Although liming the surface soil reduced metal uptake by crops grown on digested sludge plots, metal uptake was still significantly greater than that of crops grown on limed raw sludge or control plots. These results suggested that land containing entrenched limed sludges (raw or digested) may be used to grow agricultural crops if the sludge is relatively low in heavy metals.

The principal method currently used for the disposal of limed raw sewage sludge produced at the Blue Plains Wastewater Treatment plant in Washington, DC is entrenchment. The regional, 309 million gallon per day facility produces about 200 wet tons of digested sludge per day which is land spread on cooperator farms and about 700 wet tons of limed raw sludge, of which one-half is composted and the other half entrenched at selected sites in Montgomery and Prince Georges counties, Maryland. Entrenchment or "trenching", which has been conducted since 1974, is expected eventually to be replaced by sewage sludge composting because of the resource recovery benefits of the sludge compost product (Hornick et al., 1979).

The method for sludge entrenchment involves placing sludge in trenches which are 60 cm wide, 75–135 cm deep, and 60–90 cm apart. The disposal rate for sewage sludge filter cake of about 20% solids is 2900 metric tons/ha, which is significantly higher than that allowed for surface application and one of the major benefits of the method. When sludge is surface applied, it is recommended that the rate equal the nitrogen fertilizer requirements of the crop which for most crops equals an application rate in the range of 5–40 metric tons ha, dry weight basis (CAST, 1976). Because entrenched sludge is buried relatively near the soil surface, agricultural benefits from such sludge were foreseen and demonstrated in a greenhouse experiment (Taylor et al., 1978).

The methods used for sludge entrenchment vary with the amount of sludge to be buried on a daily basis (Sikora and Colacicco, 1979). After a site has been used for sludge entrenchment, the recommended practice is to level the mounds which result when the sludge-filled trenches are

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backfilled, add lime and fertilizer, and establish a grass such as Kentucky-31 tall fescue (*Festuca arundinacea* Schreb.) to stabilize the site (Walker et al., 1978). Maryland State Public Health officials have ruled that the sludge entrenchment sites may not be utilized for growing food chain crops because research has not completely verified that all crops so grown are safe to enter the food chain. A food chain crop was mistakenly planted at a municipal entrenchment site on one occasion by a neighboring farmer, and the resulting crop of field corn was permitted to grow to maturity. After heavy metal analyses of the grain and comparison of these levels with those in control corn grown on adjacent fields containing no sludge trenches and with levels of metals in corn grain produced across the United States the corn from the sludge entrenchment site was determined to be safe for entrance into the food chain (Sikora and Colacicco, 1980). The entrenched sludge was limed raw sludge from the Blue Plains Wastewater Treatment plant, considered, because of its low metal content, a "domestic" sludge (Chaney et al., 1976).

In 1972, research field plots containing trenches filled with sludges of different physical and chemical characteristics were established at the Beltsville Agricultural Research Center. The objectives of that research were to determine the effect of sludge type, trench size, and soil type on nitrogen and metal transformations in the sludge, on pathogen survival, on dewatering rate of the sludge, and on characteristics of the leachate from the sludges. Data from these investigations have been reported previously (Sikora et al., 1978a, 1979, 1980; Walker et al., 1975).

Land used for the entrenchment of sludge is normally leased or purchased by the municipality or sanitary commission and held for approximately 5 years after trenching has ceased, which is the estimated length of time required for the stabilization of the buried sludge (Sikora et al., 1979). After this time, the land can be sold or the lease terminated but there are no specific requirements for its future utilization. One possibility is to use the land for agricultural purposes, but thus far the effects of entrenched sludges on the quality of many agronomic crops have not been reported. The possibility does exist for agricultural use of sludge entrenchment land, as numerous studies have demonstrated the low-risk use of many sludges for crop growth (CAST, 1976). However, the quality of the sludge, the soil type, and the pH of both sludge and soil all affect the yield and quality of the resulting crops.

For determination of the effect of entrenched sludges on the quality of small grains and leafy vegetables, a field crop study was initiated in which three crops were grown on plots containing two types of sludge. Because heavy metal uptake by food chain crops is of primary concern (CAST, 1976), levels of zinc, copper, and cadmium in the crops were the determining quality factors. For determination of the effect of elevated surface soil pH on metal uptake, one-half of some plots were limed.

MATERIALS AND METHODS

Plots established in 1972 containing entrenched digested sludge or limed raw sludge were used for the crop study. The raw sludge was obtained from the Lower Potomac Wastewater Treatment plant in Lorton, VA, and the digested sludge was obtained from the Blue Plains Wastewater Treatment plant. Both sludges had relatively low heavy metal contents (Table I). The dimensions of the trenches were 60 cm wide by 60 cm deep and they were placed 60 cm apart with each plot containing at least four adjacent trenches. Three months after the trenches were established in 1972 the mounds over the trenches were leveled, lime and fertilizer were applied, and Kentucky-31 tall fescue, *F. arundinacea* Schreb., was planted. The fescue was planted to stabilize the surface of the soil and utilize a portion of the nutrients in the entrenched sludge. The entrenched sludges were considered stabilized after 4 years because N mineralization in the sludges was negligible (Sikora et al., 1979, 1980). Single plots for the crop study were staked out in each field plot containing trenches established in 1972. The digested and raw sludge plots were 12 by 12 m and the control plot was 7.5 by 15 m. The control plot was in an area where trenches were dug and backfilled without a sludge addition. The soil series was Galestown-Evesboro loamy sand. For the present study, each plot was disked, which turned the grass cover under, and then rototilled. A tractor-pulled grader rake was used to remove the grass clumps and level the plots. Hand raking was used as the final plot preparation at which time the entrenched sludges were estimated to be 20–25 cm below the soil surface.

Soil Sampling. The surface soil of each plot was sampled by taking 10 samples with a 1.25-cm soil probe to a depth of 15 cm and making a composite for metal analysis by the methods of Chaney et al. (1976).

Liming and Fertilization. The designated plots were split into portions containing limed and unlimed surface soils, with the exception of the plot located over the limed raw sludge which already had a surface soil pH of 6.7. The lime requirement to raise the pH to 6.5 was estimated by using the buffer method of Adams and Evans (1962). The liming rates as determined by the buffer method were 2500 and 3400 kg of hydrated lime/ha for the digested and control plots, respectively. NPK fertilizer was added at a rate equal to 56 kg of N, 112 kg of P, and 112 kg of K per ha.

Planting. On October 9, 1977, "Potomac" winter wheat (*Triticum aestivum* L.) was planted in rows 137 cm apart and perpendicular to the sludge trenches. The following spring all the plots received an additional 84 kg/ha of N, P, and K, and "Clintford" oats (*Avena sativa* L.) and "Fordhook Giant Swiss" chard (*Beta vulgaris* var. Cicla) were planted on March 30, 1978, and April 6, 1978, respectively. These two crops were planted between the wheat so that the final spacing was 46 cm between all rows. The rows of crops were alternated on each plot, with each row representing a replication for a total of three replications. The final configuration consisted of three replicates of three crops grown in single plots split to accommodate limed and unlimed surface soils (except the plot containing the limed raw sludge).

Harvesting. Diagnostic samples of both whole oat and wheat plants were sampled at the boot stage on May 5, 1978. The diagnostic leaf (youngest fully expanded) of swiss chard plant was harvested on June 30, 1978, and the mature oat and wheat plants were harvested on July 7, 1978. Twenty-five plants were randomly sampled from each treatment.

Plant Preparation. All plant samples were washed by repeatedly being dipped in distilled water, followed by 0.1% sodium lauryl sulfate solution, and followed by distilled water again. All plant samples were dried in a forced air oven at 60 °C and later ground through a 20-mesh screen by the use of a stainless steel Wiley Mill. (Trade names are presented for the convenience of the reader and do not constitute any preferential endorsement by the USDA over similar, available products.) The oat and wheat boot stage and swiss chard diagnostic leaf samples were ground completely while the mature oats and wheat were separated into grain and stover samples before

Table I. Chemical Composition of Sludges Prior to Entrenchment in 1972

chemical analysis	sludge[a] limed raw	digested
total Kjeldahl N	34800	29400
ammonium N	2000	1600
nitrate N	0	0
total Zn	400	2050
extractable Zn	59	–[b]
total Cu	200	627
extractable Cu	13	-
total Cd	2	18
extractable Cd	0.5	-
% solids	19	22
pH	11.2	6.9

[a] Millgrams per kilogram, oven dry weight. [b] See text for explanation.

grinding. After all the samples were ground, they were prepared for total metal analysis by the methods in Chaney et al. (1976).

Statistical Analysis. Duncan's Multiple Range Test was used to determine the significant difference in heavy metal levels as a result of treatment for each plant material.

RESULTS AND DISCUSSION

The entrenched sludge plots used for the crop study were sampled periodically from 1972 to 1976. The sampling involved cross sectioning a trench and sampling the sludge and the surrounding soil with depth. The samples were analyzed for organic and inorganic nitrogen (N), chlorides, percent solids, volatile solids, and extractable and total metals (Sikora et al., 1979, 1980). Data indicated that the majority of the heavy metals did not leach from the entrenched sludge and therefore could possibly be absorbed by crops grown over these trenches. In the digested sludge trenches, zinc movement from the sludge into the soil below the trench was detected through soil profile analysis but the amount of zinc found in the soil below the trench was only a small fraction of the total zinc in the entrenched sludge (Sikora et al., 1980).

The metal levels were different in the limed raw and digested sludges placed in the trenches in 1972 (Table I), and the extractable metals in the entrenched sludge from the last cross section in 1976 exhibited the same differences in concentration (Table II). Table II also contains extractable metal levels in soil sampled at the same depth from the surface as the sludge samples but between trenches. These soil metal levels are indicative of those that might be found in the control plots, which were not sampled with depth. Because our experience has shown that leachate from the trenches does not move laterally in the sandy soils, samples taken with depth between the trenches (30 cm from either trench) indicate background levels of metals in the soil. The level of extractable metals was lower in the entrenched limed raw sludge than in the entrenched digested sludge after 4 years. The extractable metal data on the original digested sludge were not available, but data from the samples taken over 4 years indicated that the percentage of the total metals in the entrenched digested sludge which were extractable by DTPA–TEA did not change with time (Sikora et al., 1980).

Metal analysis of surface soil sampled after crop harvesting was performed to determine whether the plot preparation and sampling over 4 years had brought sludge to the surface (Table III). Compared to the control plots, metal levels were much higher in the digested sludge plots and slightly higher in the limed raw sludge plots. These results indicated that some mixing of the buried sludge and surface soil had occurred through sampling or plot preparation, but the levels in the surface soil (Table III) and in the entrenched sludge (Table II) were sufficiently different so that uptake by plant roots in the sludge and in the soil should result in detectably different metal levels.

Metal analysis data for the crops are presented in Table IV. No attempt was made to measure yields or to choose plants which were growing over the trenches vs. over the space between trenches. Twenty-five plants were randomly sampled along a row for each replicate sample.

The crops selected for the study differed in relative metal uptake and rooting patterns. Chard is generally considered to be a metal accumulator crop, is shallow rooted, and takes up significantly more Zn, Cd, Mn, and somewhat more Cu in acid soils than in neutral or alkaline soils. Accordingly, chard had the highest levels of all metals of the crops grown (Table IV). Zinc and cadmium levels in chard were highest from the digested sludge plots, followed by the control and limed raw sludge plots. Liming the surface soil had little effect on chard metal concentrations. The effect entrenched limed raw sludge had on the soil characteristics, including pH, resulted in a significantly lower metal uptake by chard. Kirkham (1980) reported a soil column study in which irradiated raw sludge was placed in 2-cm layers at different depths below the soil surface and on the soil surface. The composition of wheat grown on the different columns was compared, and wheat grown on the soil column containing sludge at 18–20 cm depth had less absorption of heavy metals than that of wheat grown on the soil column with sludge on the surface. Although the metal levels in chard were increased, the levels were not as great as those reported for chard grown on long-term sludge utilization farms (surface soil incorporation of high metal sludges) where Cd in chard reached as high as 150 mg/kg dry weight and Zn, 1800 mg/kg dry weight (Chaney and Hornick, 1978).

Oat and wheat samples taken at boot stage had comparable metal concentrations for all treatments with one exception. Oats had markedly higher Zn and Cd levels from the digested plots without lime additions to the surface soil. As with chard, the lower metal concentrations and higher pH of the sludge zone in the limed raw sludge trench plots led to lower Zn and Cd levels in both oats and wheat than in the plants grown on the control plots.

Stover samples from oats and wheat had similar metal concentrations except for Zn and Cd levels in oats from the digested sludge plots with the unlimed soil surface

Table II. DTPA-TEA-Extractable Metal Concentrations [Mean (Range)] in Entrenched Sludge and Soil Taken at 30–40 cm below the Soil Surface Plots in 1976

sample	pH	extractable metal concn[a] Zn	Cu	Cd
digested sludge	5.5	403 (361-479)	156 (145-171)	2.96 (1.97-3.45)
soil between digested sludge trenches	4.7	0.40 (0.23-0.70)	0.30 (0.28-0.53)	0.04 (0.02-0.06)
limed raw sludge	7.4	17.0 (4.6-36.3)	12.4 (5.0-24.8)	0.22 (0.12-0.40)
soil between limed raw sludge trenches	5.2	0.22 (0.08-0.38)	0.15 (0.10-0.20)	0.02 (0.01-0.02)

[a] Milligrams per kilogram, oven dry weight.

Table III. DTPA-TEA-Extractable Metal Concentrations [Mean (Standard Error)] in Surface Soil (0-15 cm) of Plots Containing Entrenched Sludge after Harvesting Crops[a]

plot	metal concn, mg/kg Zn	Cu	Cd
digested	10.2 (0.13)	6.3 (0.15)	0.17 (0.01)
digested[b]	14.5 (0.133)	7.4 (0.78)	0.23 (0.01)
limed raw	4.2 (0.03)	3.4 (0.06)	0.09 (0.01)
control	3.7 (0.09)	3.1 (0.19)	0.07 (0.01)
control[b]	3.4 (0)	1.9 (0.07)	0.05 (0.01)

[a] Samples from limed and unlimed split plots made up the composite samples from the digested and control plots. [b] Surface-limed plots.

Table IV. Zinc, Copper, and Cadmium Concentrations (mg/kg Dry Weight) in Crop Tissues Grown on Plots Containing Entrenched Sewage Sludges

tissue	treatment	soil pH	metal concn Zn	Cu	Cd
whole chard	control	5.0[a]	142 ab[b]	14.0 a	1.03 ab
	control	6.5	158 ab	19.2 a	0.97 a
	raw	6.7	53 a	23.0 ab	0.25 a
	digested	5.4	322 c	27.2 b	1.54 ab
	digested	6.7	254 bc	25.8 b	1.47 ab
oat boot	control	5.0	50.4 ab	5.3 b	0.27 c
	control	6.5	29.6 a	5.0 a	0.15 b
	raw	6.7	17.8 a	4.4 a	0.08 a
	digested	5.4	111.2 c	6.0 b	0.82 e
	digested	6.7	61.1 b	5.4 b	0.43 d
oat stover	control	5.0	35.3 ab	2.6 a	0.42 b
	control	6.5	20.5 a	3.1 a	0.39 b
	raw	6.7	5.3 a	2.7 a	0.10 a
	digested	5.4	171.2 d	3.5 ab	1.48 c
	digested	6.7	71.4 c	3.3 a	0.57 b
oat grain	control	5.0	57.3 b	4.6 ab	0.18 ab
	control	6.5	52.1 b	4.3 a	0.15 a
	raw	6.7	27.6 a	3.7 a	0.08 a
	digested	5.4	84.5 c	5.4 c	0.72 d
	digested	6.7	65.1 b	4.6 ab	0.35 c
wheat boot	control	5.0	38.7 b	4.5 b	0.14 a
	control	6.5	28.0 b	4.2 a	0.15 a
	raw	6.7	17.1 a	4.6 b	0.10 a
	digested	5.4	62.8 c	4.8 c	0.47 b
	digested	6.7	48.0 bc	5.1 d	0.38 b
wheat stover	control	5.0	42.8 b	2.1 a	0.29 a
	control	6.5	42.2 b	2.1 a	0.28 a
	raw	6.7	5.3 a	2.4 a	0.18 a
	digested	5.4	76.4 c	3.2 ab	0.61 bc
	digested	6.7	69.7 c	2.8 ab	0.50 ab
wheat grain	control	5.0	44.4 b	3.8 a	0.12 a
	control	6.5	44.8 b	4.2 a	0.13 a
	raw	6.7	27.2 a	4.1 a	0.08 a
	digested	5.4	48.4 b	4.8 ab	0.20 b
	digested	6.7	49.2 b	4.8 ab	0.23 b

[a] Control and digested sludge plots were split with one-half being limed, and these plots are indicated by the higher pH. [b] Within results for one metal and one crop tissue, values followed by the same letter are not significantly different at the 5% level.

which were higher. These data indicate that oats accumulate higher levels of Zn and Cd than wheat when grown in acidic, metal-enriched soils.

Metal concentrations in grain samples were of particular interest because, unlike corn, oats and wheat translocate substantial amounts of metals to their grain (Bingham et al., 1975). With the exception of Cu in wheat grain, plants from the limed raw sludge plots had the lowest Zn, Cu, and Cd levels followed by the control and the digested sludge plots. Oat grain had significantly lower metal levels in plants from plots with limed surface soils. Although liming the surface soils resulted in lowering the metal concentrations in oat and wheat tissue, this treatment had much less effect on metal uptake than the lime added as limed raw sludge in the entrenched sludge zone.

Copper is absorbed and retained mainly in the roots of plants, and foliar Cu levels are seldom influenced by moderate Cu additions (CAST, 1976). In this study, copper levels in oat grain and oat and wheat tissue in the boot stage were influenced by treatment but in all cases treatment effects were minor.

Several factors indicate that the crops were absorbing metals from the entrenched sludges. In field studies performed on long-term sludge utilization farms, oat Zn and Cd levels were substantially reduced by liming the surface soil (Chaney and Hornick, 1978). At a site amended with sludge from city no. 13, oat grain Cd was reduced from 3.38 to 0.54 mg/kg when soil pH was raised by liming from 5.6 to 6.6; at a site amended with sludge from city no. 9, oat grain Cd was reduced from 2.12 to 0.38 mg/kg when soil pH was raised from 4.8 to 6.6. In the present study, oat grain Cd decreased from 0.72 to 0.35 mg/kg and wheat grain Cd did not change significantly when the surface soil on the digested sludge plots was adjusted from pH 5.4 to 6.7. Therefore, metal uptake was influenced more by the metals in the subsoil and, although liming decreased metal uptake in most instances, significant metal uptake from the entrenched digested sludge was recorded.

Oats and wheat usually absorb much less Zn and Cd than chard when grown in acid soils amended with sludge. This occurrence is partially due to inherent differences in Zn and Cd absorption by crops (Bingham et al., 1975) and partially due to different rooting patterns. Although grain crops have the majority of their root mass in the top 20 cm of soil, they are considered to be deeper rooted than the short season leafy vegetables; other variables governing rooting depth are plant water stress and impeding soil layers (Robertson et al., 1980; Ward et al., 1978). The Zn data indicated that both chard and oat plants absorbed metals from the entrenched digested sludge.

Williams and David (1977) reported on the effect P fertilizer additions on uptake of Cd by clover. They found that Cd added with P fertilizer to a range of soils resulted in greater plant uptake of Cd, especially in P-deficient acid soils. Other researchers have reported that plant roots proliferate in P-rich fertilizer band areas (Barber, 1977), and municipal sewage sludges contain high levels of P which should result in similar root proliferations. Thus, crop absorption of heavy metals from entrenched sludges that are acidic is expected. Crops grown on digested sludge which had the higher extractable metal content and the lower final pH of the two entrenched sludges showed the greatest metal accumulation. Surface pH adjustment with lime resulted in a lowering in metal uptake primarily in oats, with little change recorded in chard or wheat.

Cadmium concentration of food chain crops grown in sludge-amended soils is of primary concern (Chaney and Hornick, 1978). In this study where sludge was buried below the plow layer but at rates up to 10 times that recommended for surface application (CAST, 1976), there is concern over Cd concentrations in crops. Although Cd levels in chard and in oat and wheat grain increased on digested sludge trench plots, these levels were much lower than that found in crops grown on soils where surface soils contained similar Cd concentrations. Baker et al. (1979) found wheat grain Cd concentrations of 1.4 mg/kg on plots with pH 6.7 and 10.0 mg/kg total Cd level. LaConde et al. (1978) found a Cd concentration in wheat grain of 1.3 mg/kg on plots with pH 6.7 and 7.7 mg/kg DTPA–TEA-extractable Cd. Thus, the levels of Cd in crops grown over entrenched digested sludge increased, but the levels were not excessively high.

The data showed that crops grown over sludge trenches take up metals from the sludge in a manner which reflects the type of crop planted and sludge buried. The metal levels in the crops were relatively low probably because the entrenched sludges had low metal contents. Therefore, it appears possible that agricultural use of low metal sludge entrenchment sites is possible. However, caution must be exercised in the interpretation of these data since they are from 1 year's study. Study of crop quality at actual entrenchment sites is necessary to confirm these data and accumulate data on other possible crops.

The data showing the lower metal uptake from limed, entrenched sludge when compared to limed control plots suggest that the impact of heavy metals in entrenched sludges can be minimized, e.g., by use of high levels of lime to condition sludges before dewatering and entrenchment. This practice has been shown to reduce the migration of metals from entrenched high-metal sludges (Sikora et al., 1978b). Also, after the sludges have been entrenched, the surface soils should be kept at pH levels commensurate with good agricultural practice (i.e., pH 6.5).

CONCLUSIONS

Crops that differed in rooting patterns and their absorption of heavy metals were grown on plots containing entrenched sewage sludge buried for 5 years. Metal uptake by these crops reflected the metal content of the sludge and the pH of the sludge and subsoil more than the pH of the surface soil. Chard accumulated higher levels of metals than either wheat or oats which had similar metal accumulations. Crops grown in plots containing entrenched, limed raw sludge had metal levels equal to or less than levels in crops grown in the limed control plots, which contained no sludge. The results indicate that lime stabilization of sludges prior to entrenchment reduces metal uptake.

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