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Use of Metal-Rich Slag as a Source of Grit and Its Effects on Pigeon Health and Fitness

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ABSTRACT

Uptake, utilization and physiological effects of slag as a source of grit were studied in laboratory pigeons provided with an *ad libitum* supply of a) industrial metal-rich slag b) pristine gravel or c) a combination of the above, over a one-year period. Although retention times undoubtedly differed, both grit types appeared to remain within the gizzard until near-completely abraded, thus resulting in near-maximal exposure of the bird to intrinsic metal loads present. Mortalities were confined to the slag-treated group (62%) and birds given choice (11%) with no deaths among gravel-treated controls. Surviving slag-treated birds indicated marked weight increases in both liver (2-fold) and kidney (~ 46%) whereas body fat scores and breast muscle mass were substantially reduced. Slag ingestion likewise affected marked hypertrophy of the parathyroid glands. Tissue metal assays indicated a striking accumulation of Fe (7-8 fold) in liver, and to a lesser extent in bone (3.2-fold) and kidney (1.5-fold) of slag-treated birds. Histologically, hepatocytes and invading clusters of macrophages appeared stuffed with numerous pigmented cytoplasmic granules – histochemically identified as predominantly stored Fe. These findings point to the development of an acute toxic Fe storage condition, not unlike avian hemosiderosis (iron storage disease), as the prime cause of morbidity and eventually death of these slag-treated birds. Long bones of slag-treated birds were substantially more pliable, of reduced strength, and characterized by significantly reduced Ca levels compared to control birds. Histological examination of keel and tibiotarsal bones revealed moderate to severe mineral resorption, with thinning of cortical layers in lamellar bone and reduced cross-strut formation in cancellous bone. Evidence of active woven bone formation at these foci was likewise confirmed, thus corroborating the marked hypertrophy of parathyroids and implied changes in parathyroid function. Given free choice, birds were basically non-selective, rather than exclusive, in their uptake of gravel versus slag. Given that slag was not avoided, and in light of its profound adverse physiological effects, concern for the ecological implications of the vast quantities of exposed slag within our environment and its potential health risks to wild bird populations is warranted.

1. Introduction

The longstanding mining and smelting of metal-rich ores has resulted in the production of vast amounts of slag typically stock piled in the vicinity of production or transported and deposited in environments further afield where it has been used in a variety of applications. Included among the latter are its extensive use in road construction, formation of railway beds, bridge building, construction of retaining buttresses, breakwalls, causeways, and dikes, surface stabilization of inclines and ravines, production of cement and concrete aggregates and occasional use as base substrate for parking areas and airport runways [1,2]. The extensive displacement and varied uses of these metal-rich slags have resulted in the introduction and dispersal of metals into otherwise pristine environments where they can pose a potential risk to various biota including resident avian and mammalian wildlife populations.

A case at hand is the historical mining/smelting activities of the INCO Ltd operations based at Sudbury, Ontario where mining for Ni, Cu and a large suite of other lesser-abundant metals has continued over the past 130 years [3]. While widespread pollution of both terrestrial and aquatic environments through metal-particulate fallout from the Sudbury-area smelter emissions has been well documented [4,3], few studies have been focused on the environmental impact of slag translocated to pristine sites outside the Sudbury Industrial Basin. Historically, huge quantities of slag derived from the Sudbury-based smelters were distributed throughout eastern Central Ontario where they have been incorporated into the building of roads and railway beds most of which remain to this day.

Most birds, and granivorous species in particular [5], depend on a continuous uptake of pebble-sized grit to assist in the grinding functions of the gizzard, and are known to routinely obtain such pebbles from available sources within their home range environment. When sparse or unavailable locally, certain species may travel as much as several miles in order to acquire suitable grit [6]. Sites of exposed rock outcropping, gravel pits, and roadsides are among the most frequented sites for the purpose of procuring such grit [7,5]. The possibility that uncovered slag used in the formation of road ways, railway beds, or various other anthropogenic structures within eastern Central Ontario may likewise be used as a source of grit by birds resident in the area raises concern because of the substantial concentrations of various metals known to characterize the Sudbury-ore slag. No studies investigating the direct uptake of slag from such anthropogenic sources (nor from terrestrial stock piles and tailing sites) by avian wildlife species and its potential effects on their health were found during our literature searches.

The present laboratory study examines the use of metal-rich slag and pristine gravel as a grit source for pigeons maintained under controlled conditions over a one-year period. The objectives were to: a) determine the extent to which the two grit materials, provided separately or in combination, were utilized by birds maintained on a regular seed grain diet; b) document the pattern of grit accumulation, retention and subsequent breakdown within the avian gastrointestinal tract; c) determine the extent to which various metal contaminants present within the slag grit provided, namely arsenic (As), calcium (Ca), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), nickel (Ni), lead (Pb) and selenium (Se), were reflected in metal burdens of the liver, kidney and bone of the bird; and d) determine the extent to which the health and fitness of the bird were compromised as a result of the metal exposures and tissue loadings incurred.

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Paramount to assessing the toxicity of dietary grit taken in by birds are two fundamental challenges. The first involves determining the duration of the exposure period during which grit is retained within the bird (i.e. grit turnover time) and hence the period over which metals are available for absorption. Secondly and perhaps more importantly, is understanding the extent to which such grit is reduced and degraded during grinding processes within the gizzard.

In the absence of clear and definitive answers on these two issues, two lines of speculative thought have been put forth. One postulates that grit is retained and used for relatively brief periods of time (few days) during which the very sharpest and most-jagged of the surface edges are smoothed and the largely-unaltered particle then allowed to pass. Such rapid turnover implies that much of the exposure results from contact with surface-bound metals present on the grit particles. Such metals are exchangeable under the acidic conditions present within the avian digestive tract and constitute the 'bioaccessible' or 'extractable' fraction of metals available for uptake from the grit. The second scenario purports a much more extensive and aggressive use of the ingested grit. In this case, it is believed that grit is retained for extended periods (several weeks/months) and vigorously engaged in the mastication of food to the extent that its erosion and disintegration are near complete prior to elimination. Under such conditions, the majority of all metals present within the grit would become available for uptake by the bird: hence, in this context, the more relevant term describing grit toxicity would be 'total' metal burden.

Given the uncertainty as to which of the above two scenarios more accurately reflects the dynamics of grit abrasion within the avian digestive tract, we have quantified both the 'extractable' and 'total' levels of selected metals present in the grit used in the present study. In addition, an attempt was made to not only quantify the volume of grit consumed by each treatment group of birds but to also assess the extent of erosion incurred by comparing the size profiles of grit collected from the gizzard and from feces against that fed within the diet.

2. Experimental Methods

2.1 Field Collection and Preparation of Grit

Both gravel and slag substrates used to prepare dietary grit for the birds in this study were collected directly from the field, thus representing materials exposed to the long-standing, natural-weathering effects prevailing within their respective environments. A composite stock supply of slag was prepared by collecting and pooling surface materials from 4 relatively isolated, slag-covered, travel right-of-ways within eastern Central Ontario. The sites were spaced > 2 km apart and located in areas supporting only infrequent vehicular traffic. Collections involved surface material (< 10 cm in depth) only and were taken from the central portion of the travel way.

The stock supply of gravel was obtained from the millings of a limestone quarry situated west of Meldrum Bay on Manitoulin Island, Ontario. There are no major sources (past or present) of industrial activity or atmospheric fallout within 100 km of this collection site.

In both cases, stock materials were sieved through a No. 5 screen (U.S.A. Standard Testing Sieve) to remove over-sized particles followed by a No. 10 screen to eliminate finer particulates. The result was a mix of grit pebbles ranging between 2-4 mm in diameter. Combining and homogenizing of subsamples was carried out using an aggregate sample splitter. To prevent mold formation during storage, the prepared grit supplies were autoclaved at 165 °C.

2.2 Treatment and Husbandry of Birds

The experiment was performed in accordance with an animal care protocol approved by the University of Guelph Animal Care Committee on behalf of the Canadian Council on Animal Care. Pigeons (*Columbia livia domesticus*) (4–9 weeks of age; n=61) obtained from a local commercial meat-stock breeder were used in the study. To minimize potential confounding effects of reproductive activities, an attempt was made to sex the young squabs and provide only males. Given the difficulty of reliably sexing live squabs at this age, genders were subsequently confirmed by gonadal inspection following death or sacrifice of the birds at the end of the study.

Birds were randomly assigned to one of three treatments groups: those receiving a) gravel grit (controls; n=17); b) slag grit (experimentals; n=26); or c) a combination of the gravel and slag grits provided separately (free choice group; n=18). Birds in each treatment group were provided a continuous *ad libitum* supply of grit throughout the treatment period, which lasted one year.

Birds were individually identified by numbered leg bands with colour coding according to treatment group. Each treatment group was maintained in a flight cage of proportionate size (6.3' wide x 10.2' high x 12'-24' long) so that bird densities compared favourably at 1.7-2.3 birds/100 ft^3. Cages were equipped with roosting perches as well as individual nest boxes, and were bedded with a combination of straw and wood shavings. The room in which the flight cages were housed was maintained at 20 ± 1 °C with light intensity of 60 lux (floor level) and a daily photoperiod which was adjusted monthly in accordance with environmental lighting conditions in the local area.

Prior to being incorporated into the study, birds were visually inspected for ectoparasite burdens, treated for endoparasites using ivomectin (2 mg/L) in their drinking water for 5 days and subsequently checked for clearance by fecal egg counts 1 week later. Throughout the study, all birds were maintained on an *ad libitum* diet of whole grain seeds (corn, wheat, peas, and safflower) obtained commercially, and provided drinking water from a deep well source with non-metallic plumbing. Grit, provided in free-standing floor trays, was replenished at 2-3 week intervals; in order to estimate intake, any contaminating bedding materials were removed and the residual grit volumes measured before replenishment.

To monitor physical condition and weight changes, the birds were caught, inspected and weighed monthly. Individuals showing a 10% reduction from their initial body weight were subsequently monitored on a weekly basis. If, and when, such declines reached 20%, the bird was immediately euthanized accordingly to the established Animal Use Protocol. Deceased and euthanized specimens were forwarded to the Animal Health Laboratory, Department of Pathobiology, U of Guelph for post-mortem examination and histopathological assessments.

Rather than removing any inadvertently-included female at first signs of egg laying, it was deemed less traumatic to allow both members of the mated pair (pigeons typically pair for life) to remain in the study and introduce measures to curtail the extent of the reproductive activity initiated. Accordingly, any eggs produced were allowed to remain in the nest box for approximately one week and then removed, thereby disrupting the egg laying/incubating cycle.

2.3 Collection of Body Organ/Tissue Samples Following Exposure

At the end of the experimental period, live body weight was recorded and a heparinized blood sample (1-1.5 mL) drawn from the basilic wing vein prior to euthanizing the bird with an 1.15 mL injection of sodium pentobarbital (340 mg/mL) administered intraperitoneally. Three microcapillary tubes of blood were centrifuged and values averaged for determination of hematocrit. The remainder of the blood sample was forwarded to the Animal Health Laboratory, Department of Pathobiology, U of Guelph for determination of the following hematological parameters: white blood cell count (WBC), hemoglobin content (Hb), hematocrit by refractometry (HCT), mean corpuscular hemoglobin concentration (MCHC), protein total solids (Protein TS), and heterophil, lymphocyte, monocyte, eosinophil, basophil and azurophil counts.

Carcasses were frozen over-night and then transported in coolers with dry ice to the Department of Biology, Laurentian University (Sudbury, Ontario) where they remained frozen (-25 °C) until dissected. Upon thawing, the thoraco-abdominal cavity was exposed and the following organs removed, and their weights recorded (nearest 0.01 g): liver, both kidneys, gizzard (contents removed) and both tibiotarsal bones. Subcutaneous and visceral fat stores were collectively evaluated using a subjective scoring scale of 1-4, with 4 indicating greatest abundance. Profile of the breast musculature with respect to the underlying keel bone was used to subjectively assess the degree of muscle atrophy, with a) well-rounded convex profile designated as no atrophy with good to adequate muscle mass present b) flat none-curved profile implying moderate atrophy with moderate mass remaining and c) concave profile indicating substantial atrophy with marked reduction in mass. Bone strength was likewise subjectively evaluated according to the degree of resistance provided during cutting of the keel and leg bones with paper-cutting scissors: the categories were as follows, a) crisp grating cut with very considerable resistance indicative of good strength b) less abrasive with reduced resistance indicating moderate strength and c) soft low-friction cut with very little resistance implying markedly reduced strength.

2.4 Recovery and Characterization of Gizzard and Fecal Grit

Gizzard contents were examined under a dissecting scope (6.3X mag) and all grit pellets removed and identified as gravel or slag in nature. In each case, pellets were sequentially sifted through a series of sieves (No.10, No.12, No.18, and No.35; U.S.A. Standard Testing Sieves, West Chester, PA.) allowing for separation into size groupings of > 2.0 mm, 1.7-2.0 mm, 1.0-1.7 mm, and 0.5-1.0 mm in diameter. (Particles < 0.5 mm were rare and excluded in the survey due to difficulties in accurately

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characterizing such fine material). In each size cohort, numbers of pellets present and their accumulative weight were recorded.

Fecal samples, obtained from each nest box by systematically sub-sampling at 3 month intervals, were ashed at 450 °C to remove organic content. The resulting dry ash was then passed through the above series of sieves to recover grit particles of the same size groupings as fore-mentioned. Gravel and slag pellets were separated under a dissecting microscope (6.3X mag) and their numbers and total weights recorded as above.

2.5 Necropsies and Histopathological Examinations

Necropsies, consisting of a detailed visual inspection of each major organ system, were conducted on all birds. Histopathological studies were undertaken on birds that died during the study (n=2) or were euthanized due to > 20% weight loss (n=16), along with two sub-groups of 4 control birds and 4 from the slag plus gravel treatment group, both of which had survived to completion of the study and served for comparative purposes. Tissues showing signs of abnormality along with corresponding tissues from the reference controls were sampled, fixed, embedded in paraffin and stained (hematoxylin-eosin) for histopathological assessment by a professional avian pathologist (Dr. M. Brash, Department of Pathobiology, U of Guelph).

2.6 Preparation and Digestion of Feed, Grit and Body Tissue Samples

Feed, grit and body tissue samples were analyzed for ‘total’ burdens of the following metals: arsenic (As), calcium (Ca), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), nickel (Ni), lead (Pb) and selenium (Se). Grit samples were subjected to two additional extraction procedures in an attempt to quantify pH-dependent extractable fractions of each element.

Feed samples were hand sorted and each seed type pulverized using a food grinder. A 0.5 g sample was digested in 8 mL of HNO_3 and 2 mL of 30% H_2O_2 while subjected to pulsatile heating in a microwave oven (Milestone Ethos 1600 URM, HPR 1000/10 system, Burlington, ON). The digested sample was cooled and brought to volume (25 mL) with deionized water.

Extractable metal levels in grit were determined from a 1.0 g sample following sequential acid extractions; the first extraction was conducted in 40 mL of 1 M acetic acid (Fisher Scientific trace metal grade) held at 40 °C for 24 h (with sample agitation @ 136 rpm). The leachate was centrifuged for 20 min at 2500 rpm and the supernatant collected for determination of the metal fraction designated as BAA-extractable (extracted by acetic acid). Residuals of the acid treated sample were then incubated with 40 mL of 0.5 M $NH_2OH.HCl$ (hydroxamine hydrochloride) with pH adjusted to 1.5 using 2 M HNO_3 and the sample held at 40 °C for 16 h. Following centrifugation for 20 min at 2500 rpm, the supernatant was collected for determination of the BHY-extracted metal fraction (extractable By HYdroxamine hydrochlorine). The above protocols for BAA- and BHY-extracted metal fractions are modifications of that established by Tessier et al., [8] for the speciation of particulate trace metals in sediments, and correspond to fractions 2 and 3 respectively as described therein.

To determine ‘total’ metal levels, grit samples were finely pulverized using a ball mill (SPEX Industries, Inc., NJ, U.S.A.). A 0.25 g sample was weighed into a 100 mL Savillex Teflon digestion bomb (ATS Scientific Inc., Eden Prairie, MN) into which 6 mL of concentrated HNO3 and 2 mL of 30% H_2O_2 were added. The mixture was heated in a sand block at 170-190 °C for one h. Upon cooling, a 2 mL aliquot of concentrated HF (ACS grade) was added and the sample re-heated to 170-190 °C (in the sand block) for 90 min. The vials remained in the block for an additional 30 min with the caps removed. Cooled samples were treated with 0.2 g of boric acid and brought to volume (25 mL) with deionized water.

In preparation for digestion, all body tissue samples were freeze-dried. Soft tissues (liver and kidney) were then hand-ground and sieved to remove non-degraded vascular components while bone samples were fragmented into small pieces and major marrow fat inclusions removed. Samples weighing 0.2 g (soft tissue) and 0.5 g (bone) were digested in 6.4 mL of HNO_3 and 1.6 mL of H_2O_2 (30%) using pulsatile microwave heating, and brought to volume (25 mL) with deionized water.

2.7 Metal Assays

Digested samples were analyzed for Ca, Cu, Fe and Ni content using a Perkin Elmer Flame Atomic Absorption Spectrophotometer (Model 703, Shelton, CT.), with the aid of graphite furnace for Ni levels in soft tissues and Cr levels in grit. As, Cd, Co, Cr, Pb and Se concentrations were obtained by Inductive Coupling Plasma Mass Spectrophotometry (ICP-MS) (Perkin Elmer Elan Model 6000, Shelton, C.T.), with the exception of As and Se levels in grit which were determined by atomic fluorescence spectrophotometry following hydride formation (PS Analytical - Millenium System; Kent, England). Procedural blanks were included in each sample run to control for metals introduced by the digestion and filtering processes. Quality control was ensured through the regular use of duplicate sample extractions and matrix spiking. Data validation was undertaken through the use of Certified Reference Materials (DOLT-#2 Dogfish liver; DOLT-3 Dogfish liver; TORT-2 Lobster hepatopancreas; DORM-2 Dogfish muscle; and PACS-2 Marine sediment) obtained from NRC, Canada. Metal concentrations are expressed as μg/g calculated on a dry weight basis.

2.8 Statistical Analyses

Central tendencies (genders combined) have been reported as mean values ± standard error of the mean. Group data sets were analyzed by ANOVA with the Tukey *post hoc* tests used to distinguish significantly different subsets. In cases where the data failed to meet the homogeneity of variance requirement, the differences were verified by Kruskal-Wallis testing for nonparametric data. Nominal fat scores were assessed by Chi-square. All text statements indicating significant difference are the result of statistical testing at the $p< 0.05$ level unless otherwise specified.

3. Results

3.1 Mortality

Forty-three of the 61 birds in the study survived till the end of the treatment period. Of the 18 (29.5%) that succumbed, 16 showed rapid weight losses (> 20%) prior to being euthanized while 2 others died suddenly without apparent signs of deterioration. Mortalities occurred primarily in the slag-treated group where 16 (61.5%) of the initial 26 birds succumbed. The remaining 2 deaths occurred among the 18 birds provided with both gravel and slag. No deaths were observed among the 17 controls that received gravel only.

Deaths were not observed until after the first 4.5 months of treatment. Thereafter, deaths occurred at a rate of 1-4 per month, with the last one occurring two weeks prior to the termination of the study.

A total of 22 females had been inadvertently included in the study, with 8 blindly assigned to the slag treatment group, 11 to the gravel plus slag treatment group and 3 to the control group. Because of the disproportionate gender representation within and among treatment groups, it has not been possible to evaluate the effects of treatment on female versus male birds in a statistical manner. However, mortalities in the slag treatment group (where 89% of deaths occurred) involved 4 (25%) females and 12 (75%) males, values unlikely to differ markedly from the proportions in which the two genders were represented within the group [n=8 (31%) females and n=18 (69%) males]. Thus, in the absence of further data, it would appear unlikely that a definitive gender bias existed in the mortalities noted among the slag-treated birds of this study.

3.2 Grit Ingestion Rates

Mean volume of grit ingested, calculated as cc/bird/week, did not differ significantly between birds given slag and those receiving gravel, with mean values of 5.60 (± 0.55) and 5.68 (± 0.32) respectively. Birds provided with a choice of grit types ingested a significantly higher mean volume of grit (8.13 ± 1.69 cc/bird/week) than either of their counterpart groups ($p < 0.05$). Within the group afforded a choice as to grit type, ingestion rates were more variable but, on average, resulted in twice as much gravel (66%) being selected relative to slag (34%).

3.3 Metal Levels in Feed

Wheat and corn constituted the most prevalent grain types present within the feed (38 and 29% by wt respectively), with safflower seeds (20%) and green peas (13%) making up the remainder of the mix (Table 1).

Mean metal concentrations within the individual grain types, along with that computed for the composite feed mix as a whole (latter calculated as product of mean concentration within the seed type x relative abundance of latter within the mix), are likewise reported in Table 1. With the exception of Pb, measureable levels of the assayed metals were present in all four grain types examined.

In general concentrations computed for feed as a whole were low, with only four elements showing mean values > 0.5 μg/g. The exceptions included Ca, Fe, Cu, and Ni for which concentrations averaged 93.8, 37.5, 3.4 and 1.8 μg/g respectively. In the case of Ca and Cu, wheat and peas contributed disproportionately relative to other seed types, and collectively accounted for > 90% of the total amount of Ca in feed and

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approximately 69% of the Cu present. Wheat was also a noteworthy contributor to Fe levels, accounting for approximately 45% of the total Fe content of the mix.

Table 1 Components of feed, their respective metal concentrations and computed metal levels in composite feed mix provided. Values reported as mean (±S.E.); n=6 for feed components; n=5 for element assays. Within elements, mean values sharing a common superscript are not significantly different ($p \leq 0.01$) as indicated by ANOVA with Tukey post hoc and confirmed by Kruskal Wallis test

Component	Corn	Wheat	Peas	Safflower	Composite feed mix
% of total diet (by weight)	28.84 (±1.55)	37.99 (±2.24)	13.37 (±0.91)	19.81 (±0.69)	100
Metal levels (µg/g)					
As	.000^{a}	.004^{ab} (±.002)	.008^{b} (±.002)	.010^{b} (±.000)	0.005
Ca	11.40^{a} (±.926)	133.74^{b} (±2.550)	254.14^{c} (±8.262)	28.88^{a} (±.704)	93.795
Cd	.010^{ab} (±.004)	.056^{c} (±.006)	.023^{b} (±.001)	.006^{a} (±.000)	0.029
Co	.008^{a} (±.003)	.010^{a} (±.000)	.058^{b} (±.002)	.010^{a} (±.000)	0.016
Cr	.348^{a} (±.053)	.626^{b} (±.038)	.374^{a} (±.008)	.488^{ab} (±.053)	0.485
Cu	1.686^{a} (±.100)	4.032^{c} (±.032)	6.390^{d} (±.136)	2.960^{b} (±.095)	3.459
Fe	23.008^{a} (±1.025)	44.122^{c} (±1.197)	48.056^{c} (±1.449)	38.964^{b} (±.890)	37.541
Ni	1.456^{a} (±.151)	1.300^{a} (±.295)	3.282^{b} (±.348)	2.364^{ab} (±.465)	1.821
Pb	ND	ND	ND	ND	ND
Se	.124^{a} (±.005)	.096^{a} (±.007)	.328^{c} (±.014)	.214^{b} (±.010)	0.159

Table 2 Comparison of metal burdens (total and two acid-extractable fractions) in gravel and slag used as grit sources. Values are means (µg/g dry wt) followed by standard error in parenthesis; n=5. Equalities assessed by Student t-test and confirmed by Kruskal-Wallis test with $p \leq 0.01$

Element	Fraction	Grit Type	
		Gravel	Slag
Arsenic	BAA	ND	ND
	BHY	ND	ND
	TOTAL	1.67 (±0.23)	< 123.07 (±7.36)
Calcium	BAA	122,407 (±22,328)	> 4 (±1.41)
	BHY	7,434 (±1058)	> 17 (±2.30)
	TOTAL	132,326 (±17,287)	> 15,573 (±1430)
Cadmium	BAA	ND	< 0.03 (±0.002)
	BHY	ND	< 0.01 (±0.004)
	TOTAL	0.12 (±0.11)	< 4.36 (±0.45)
Cobalt	BAA	0.25 (±0.05)	< 1.41 (±0.09)
	BHY	0.06 (±0.02)	< 5.40 (±0.45)
	TOTAL	26.83 (±1.66)	< 916.66 (±27.46)
Chromium	BAA	0.38 (±0.08)	> ND
	BHY	0.10 (±0.01)	< 0.31 (±0.02)
	TOTAL	2.97 (±0.64)	< 424.70 (±8.24)
Copper	BAA	1.79 (±0.22)	< 3.85 (±0.77)
	BHY	0.23 (±0.05)	< 4.02 (±0.58)
	TOTAL	5.58 (±0.64)	< 1522.26 (±102.06)
Iron	BAA	118.14 (±15.30)	< 267.06 (±22.75)
	BHY	23.32 (0.58)	< 2424.20 (±165.11)
	TOTAL	751.90 (±55.83)	< 347,798 (±5910.20)
Nickel	BAA	3.78 (±0.79)	< 7.70 (±0.13)
	BHY	0.48 (±0.14)	< 5.75 (±0.69)
	TOTAL	8.18 (±1.51)	< 1813.34 (±65.29)
Lead	BAA	0.17 (±0.03)	< 3.02 (±0.66)
	BHY	0.05 (±0.01)	< 1.99 (±0.52)
	TOTAL	3.35 (±0.68)	< 627.68 (±65.07)
Selenium	BAA	ND	ND
	BHY	ND	ND
	TOTAL	0.07 (±0.02)	< 5.03 (±0.17)

Table 3 Ratio of 'total' metal burdens in slag relative to gravel, provided to birds as grit source (n=5)

Element:	As	Ca	Cd	Co	Cr	Cu	Fe	Ni	Pb	Se
Ratio	73.7	0.12	36.3	34.2	143.0	272.8	462.6	221.7	187.4	71.9

3.4 Metal Levels in Grit

Metal concentrations in gravel and slag grits are presented in Table 2, showing mean values for both 'extractable' (BAA- and BHY-) fractions and for the 'total' amount present. As anticipated, 'total' metal levels (with the exception of Ca) were consistently and markedly higher in slag than in gravel; for individual elements, differences ranged from 34-fold to as much as 463-fold higher (Table 3). Among those elements showing the greatest elevations in slag relative to gravel were Fe (463-fold), Cu (273-fold), Ni (222-fold), Pb (187-fold) and Cr (143-fold). Elevations in 'total' As, Se, Cd and Co were lesser in magnitude, ranging from 34- to 74-fold higher than respective levels within gravel.

'Total' Ca levels, on the other hand, showed a reverse pattern, in that mean concentration in gravel (132,326 µg/g) was 8.5-fold higher than seen in slag (15,573 µg/g) (Tables 2 and 3).

For most elements, the extractable fractions constituted relatively minor proportions of the 'total' metal burden present in the grit. This was particularly true in the case of slag, where BAA- and BHY-extracted fractions of each element, whether considered singly or in combination, were consistently less than 1% of the 'total' content (Table 4). Likewise in gravel, 4 of the 10 elements (As, Cd, Co and Se) showed a similar trend in that extractable fractions were either non-detectable or represented not more than 1% of the 'total' burden present. The remaining 6 elements assayed in gravel (Pb, Cr, Fe, Cu, Ni and Ca) showed noticeably higher proportions of extractable metals present; among these six elements, BHY-extracted fractions comprised 1.5 to 5.9% of the 'total' burden present while BAA fractions in these same samples ranged from 5.1 to 92.5% of the 'total' burden (Table 4). Certain elements in this latter group, namely Cu, Ni and Ca showed atypically high proportions of the metal burden to be present in extractable form; considered jointly, BAA- and BHY-fractions for Cu, Ni and Ca comprised more than 36, 52 and 98% respectively of the 'total' burden present in gravel.

Table 4 Proportion of BAA- and BHY- extractable fraction(s) relative to total metal burdens in gravel and slag provided to birds as grit source. Values are percentages calculated from mean concentrations reported in Table 2; n=5

	Gravel			Slag		
Element	BAA: Total	BHY: Total	BAA + BHY: Total	BAA: Total	BHY: Total	BAA + BHY: Total
Arsenic	ND	ND	ND	ND	ND	ND
Calcium	92.50	5.62	98.12	0.03	0.11	0.14
Cadmium	ND	ND	ND	0.60	0.23	0.83
Cobalt	0.93	0.22	1.15	0.15	0.59	0.74
Chromium	12.80	3.37	16.17	ND	0.07	0.07
Copper	32.08	4.12	36.20	0.25	0.26	0.51
Iron	15.71	3.10	18.81	0.08	0.70	0.78
Nickel	46.21	5.87	52.08	0.43	0.32	0.75
Lead	5.08	1.49	6.57	0.48	0.32	0.80
Selenium	ND	ND	ND	ND	ND	ND

3.5 Profile of Grit in Gizzard

Postmortem examinations revealed that grit was present in the gizzard of virtually all birds (34/35 = 97%). In only one bird, a male from the gravel-only treatment group, was the gizzard devoid of grit inclusions.

The majority of birds presented gizzards with relatively large grit pellets; 97.1% of birds (all treatment groups combined) yielded pellets > 2 mm in diameter, 91.4% had pellets from 1.7 - 2.0 mm, and 85.7% with pellets of 1.0 - 1.7 mm (Table 5). In contrast, pellets of the finest size category (0.5 - 1.0 mm) occurred in only 51.4% of all birds. This trend towards a reduced prevalence of gizzards with the finest of grit particles present was a clear and consistent pattern seen across all treatment groups regardless of whether grit types (gravel and slag) were considered individually or in combination (Table 5), and is interpreted as a reduced retention of grit within the gizzard as particle size progressively diminished.

A total of 2403 grit pellets removed from the 34 gizzards were classified as to size. Most were relatively large, with more than 46% exceeding 2 mm in diameter, 17.6% between 1.7 – 2.0 mm and 31.5% from 1.0 – 1.7 mm (Table 5). By comparison, those belonging to the smallest size class (0.5 – 1.0 mm) constituted only 4.6% of the total number. This general trend in which relatively few small-sized grit inclusions were dispersed among greater numbers of substantially larger grit was a clear and consistent pattern noted across all three treatment groups regardless of whether grit types (gravel and slag) were considered individually or in combination (Table 5). The reduced abundance of relatively fine grit within the gizzard is interpreted as additional evidence that grit, upon substantial erosion within the gizzard, was then expelled from the organ.

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Birds ingesting slag as the only grit source retained noticeably more pellets within the gizzard than did those consuming gravel. The former group (10 birds) accounted for a total of 1301 pellets (average = 130.1 pellets/bird) whereas the 13 control birds receiving gravel yielded 240 pellets (average = 18.5 pellets/bird) (Table 5). Birds provided with a choice of grit types showed an intermediate mean number of pellets present in the gizzards (862 = 71.8 pellets/bird). The distribution of slag relative to gravel pellets in the gizzards of these latter birds followed the pattern noted above in that slag pellets occurred at a higher mean rate (42.9 pellets/bird) than did gravel (28.9 pellets/bird).

Average weights for pellets within the four size classes are reported in Table 5. Mean values ranged from a high of 32.5 x 10^{-3} g for pellets of the largest size class (> 2 mm; gravel and slag combined) to a low of 1.1 x 10^{-3} g for those of the finest size class (0.5 – 1.0 mm; gravel and slag combined) – a difference of approximately 30-fold. This latter value is interpreted as evidence of very substantial erosion in the size/weight of most grit particles while in the gizzard.

Table 5 Prevalence, abundance and size-weight profiles of gizzard pellets in the three treatment groups

Treatment Group	Pellet size (mm)	Proportion of gizzards with pellets present		Number and percentage of pellets present		Average weight of pellets
Grit type:		n	%	n	%	(g x 10^{-3})
Controls						
Gravel	> 2	12/13	92.3	69	28.8	32.1
	1.7 - 2.0	11/13	84.6	41	17.1	10.3
	1.0 - 1.7	10/13	76.9	119	49.6	4.9
	0.5 - 1.0	4/13	30.8	11	4.6	2.3
	All sizes	12/13	92.3	240	100	13.5
Experimentals						
Slag	> 2	10/10	100	656	50.4	33.8
	1.7 - 2.0	10/10	100	240	18.5	15.5
	1.0 - 1.7	10/10	100	352	27.1	9.2
	0.5 - 1.0	6/10	60.0	53	4.1	0.7
	All sizes	10/10	100	1301	100	22.4
Group given choice						
Gravel	> 2	12/12	100	144	41.5	29.5
	1.7 - 2.0	10/12	83.3	55	15.9	19.6
	1.0 - 1.7	10/12	83.3	123	35.5	5.4
	0.5 - 1.0	5/12	41.7	25	7.2	1.1
	All sizes	12/12	100	347	100	17.4
Slag	> 2	10/12	83.3	243	47.2	31.0
	1.7 - 2.0	8/12	66.7	87	16.9	14.3
	1.0 - 1.7	8/12	66.7	163	31.7	6.9
	0.5 - 1.0	5/12	41.7	22	4.3	1.4
	All sizes	11/12	91.7	515	100	19.3
Gravel and slag combined						
	> 2	12/12	100	387	44.9	30.5
	1.7 - 2.0	11/12	91.7	142	16.5	16.4
	1.0 - 1.7	10/12	83.3	286	33.2	6.3
	0.5 - 1.0	8/12	66.7	47	5.5	1.2
	All sizes	12/12	100	862	100	18.5
All treatment groups combined						
Gravel and slag combined						
	> 2	34/35	97.1	1112	46.3	32.5
	1.7 - 2.0	32/35	91.4	423	17.6	15.3
	1.0 - 1.7	30/35	85.7	757	31.5	7.4
	0.5 - 1.0	18/35	51.4	111	4.6	1.1
	All sizes	34/35	97.1	2403	100	20.1

3.6 Profile of Grit in Feces

Grit was present in all 61 fecal samples examined. The majority of samples contained pellets that were small; 100% (all treatment groups combined) contained pellets of the smallest size class (0.5 – 1.0 mm) and 77% contained pellets of the second smallest size (1.0 – 1.7 mm) (Table 6). In comparison, only 28 – 31% of all fecal samples contained pellets in the two larger size classes. This pattern of fewer samples with large grit while the majority of samples contained small grit was repeated for fecal samples obtained from birds on all three treatments. This pattern was likewise apparent whether slag and gravel were considered separately or in combination.

A total of 1820 grit pellets were recovered from the 61 fecal samples, with the majority characteristically small; 71.6% fell within the smallest size group and 23.6% within the second smallest size class whereas the two larger size classes comprised only 2.3 and 2.4% of the pellets retrieved (Table 6). This preponderance of finer grit particles within fecal samples prevailed throughout each of the treatment groups and was likewise apparent whether the two grit materials (gravel and slag) were considered individually or in combination.

In general, fecal samples were characterized by an abundance of fine, intensely-eroded grains of grit material with only the occasional inclusion of a few larger, less-modified grit pellets – a pattern consistent for all three treatment groups (Fig. 1).

Average weights for fecal pellets within the four size classes are reported in Table 6. For all gravel and slag pellets combined, mean values ranged from a high of 19.4 x 10^{-3} g for the largest size class (> 2 mm) to a low of 0.6 x 10^{-3} g for those of the smallest size class (0.5 – 1.0 mm) – a difference of 32.3-fold.

Visual inspection of the weight data (Tables 5 and 6) indicated that the average weight of fecal pellets within the smallest size class (0.5 – 1.0 mm; all treatment groups combined) constituted approximately half that of gizzard pellets in this same size category (0.6 vs 1.1 x 10^{-3} g), thus implying that among fecal samples a greater proportion of pellets had been reduced to the lower limits of this size category.

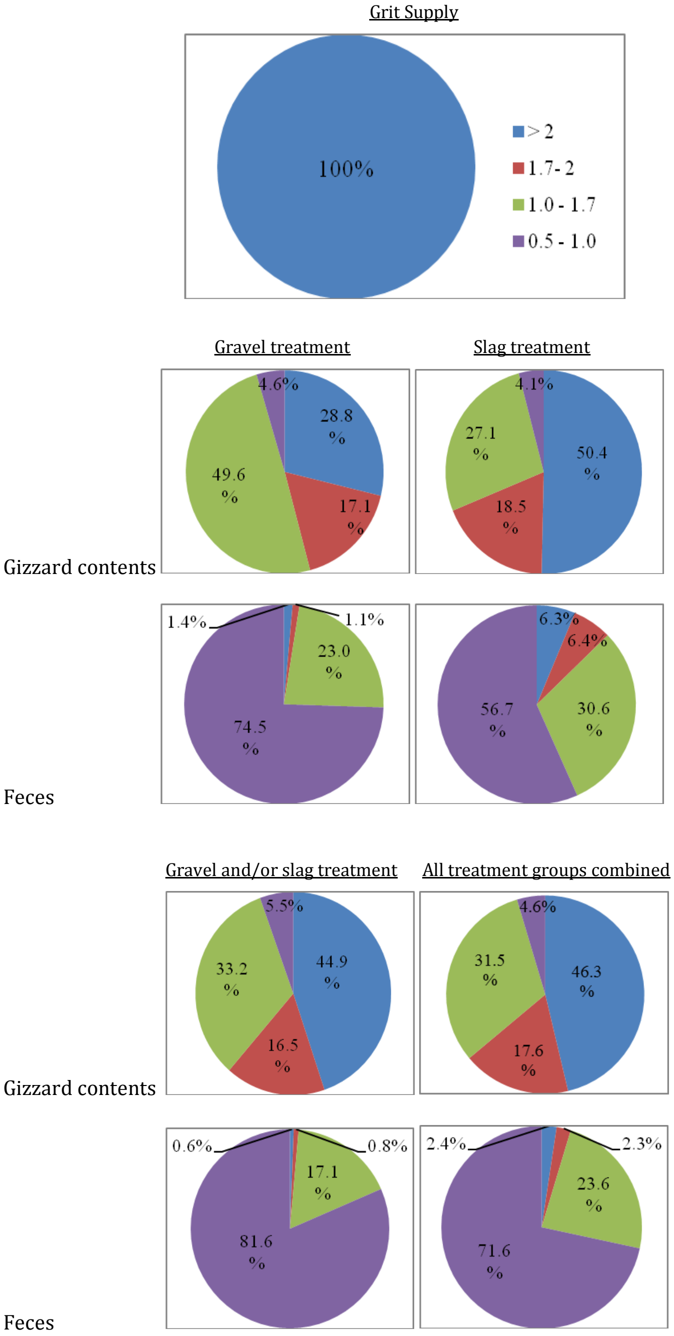

Fig. 1 Pie charts showing relative proportions of the various size classes of grit in grit supply, gizzard contents and fecal samples of treatment groups maintained on gravel, slag or a combination (choice) of the two grit types

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Table 6 Prevalence, abundance and size-weight profiles of fecal pellets in the three treatment groups

Treatment Group	Pellet size (mm)	Proportion of fecal samples with pellets present		Number* and percentage of pellets present		Average weight* of pellets
Grit type:		n	%	n	%	(g x 10^{-3})
Controls						
Gravel	> 2	2/18	11.1	13.7	1.4	11.9
	1.7 - 2.0	5/18	27.8	10.4	1.1	9.5
	1.0 - 1.7	10/18	55.6	226.3	23.0	3.4
	0.5 - 1.0	18/18	100	732.1	74.5	0.6
	All sizes	18/18	100	982.5	100	1.5
Experimentals						
Slag	> 2	13/26	50.0	28.3	6.3	22.4
	1.7 - 2.0	12/26	46.2	28.6	6.4	10.4
	1.0 - 1.7	22/26	84.6	137.2	30.6	5.5
	0.5 - 1.0	26/26	100	254.4	56.7	0.7
	All sizes	26/26	100	448.5	100	4.1
Group given choice						
Gravel	> 2	0/17	0	0	0	-
	1.7 - 2.0	0/17	0	0	0	-
	1.0 - 1.7	15/17	88.2	54.9	16.4	3.0
	0.5 - 1.0	17/17	100	280.6	83.6	0.6
	All sizes	17/17	100	335.5	100	1.0
Slag	> 2	2/17	11.8	2.2	4.1	27.6
	1.7 - 2.0	2/17	11.8	3.1	5.8	12.2
	1.0 - 1.7	8/17	47.1	11.5	21.4	4.2
	0.5 - 1.0	12/17	70.6	37.0	68.7	0.6
	All sizes	13/17	76.5	53.8	100	3.1
Gravel and slag combined						
	> 2	2/17	11.8	2.2	0.6	27.6
	1.7 - 2.0	2/17	11.8	3.1	0.8	12.2
	1.0 - 1.7	15/17	88.2	66.4	17.1	3.2
	0.5 - 1.0	17/17	100	317.6	81.6	0.6
	All sizes	17/17	100	389.3	100	1.3
All treatment groups combined						
Gravel and slag combined						
	> 2	17/61	27.9	44.2	2.4	19.4
	1.7 - 2.0	19/61	31.1	42.1	2.3	10.3
	1.0 - 1.7	47/61	77	429.9	23.6	4.0
	0.5 - 1.0	61/61	100	1304.1	71.6	0.6
	All sizes	61/61	100	1820.3	100	2.1

**values standardized to 0.5 g sample of ashed feces*

3.7 Morphometrics and Hematological Parameters

Data on body and organ weights, fat scores and various hematological parameters for the three treatment groups are summarized in Table 7.

Mean body weights at the beginning of the exposure period did not differ significantly among the treatment groups (x̄ = 614.4 ± 9.2 g; n=61). Likewise, for those birds surviving at the end of treatment, mean body weights did not differ by treatment (x̄ = 618.1 ± 12.9 g; n=43).

Liver weights in slag-treated birds averaged 29.61 ± 3.53 g, and were significantly higher than mean weights seen in either control birds or those provided with both slag and gravel. When expressed as a percentage of total body weight, livers of the slag-treatment group averaged 5.06%, and represented a 2-fold increase over average values seen in either of the other treatment groups.

Kidney weights followed a similar trend in that values for the slag-treatment group averaged 4.65 (± 0.25) g which was significantly higher than mean values in either of the other two groups. Expressed as a function of body weight, mean kidney weights for the slag-treated group exceeded those for the other two treatments by ~ 46%.

Gizzard weights averaged 9.58 (± 0.52) g in the control birds, 10.51 (± 0.63) g in the slag-treated birds and 10.85 (± 0.34) g in birds provided with a choice. These mean values did not differ significantly from each other.

Mean weights of the left tibiotarsal bone ranged from a low of 2.05 (± 0.11) g in the slag treatment group to a high of 2.39 (± 0.06) g in the free choice group. While these means were significantly different from each other, neither differed significantly from the intermediate value seen in the control group (x̄ = 2.25 ± 0.07 g).

Fat scores (based on subcutaneous and visceral stores collectively) of the slag-treatment group averaged 1.10 with 9 of the 10 birds scored in the leanest category (1) and the remaining bird in the next-to-leanest class (2). Mean scores for the control group and the group receiving gravel plus slag were 2.38 and 2.25 respectively. Whereas these latter means did not differ significantly from each other, both were significantly higher than that of the slag group (Chi-square value = 8.543; p=0.036), with 21 of the 25 birds scored in the 2 (moderate fat) through 4 (very fat) categories.

Hematocrit levels within the blood were generally high with almost identical mean values noted among control birds [53.9 (± 0.65) %] and those receiving gravel plus slag [54.2 (± 1.09) %]. Birds maintained on slag, on the other hand, indicated a significantly lower mean value at 49.2 (± 1.19) %. This suppression in hematocrit levels of slag-treated birds relative to controls was confirmed in HCT values obtained by refractometry (Table 7).

Table 7 Comparison of body/organ weights, fat scores and hematological parameters among the three treatment groups. Values given as mean with ± standard error in parenthesis and n value underscored. For each parameter, means sharing a common superscript are not significantly different (p ≤ 0.05) as indicated by ANOVA with Tukey post hoc and confirmed by Kruskal-Wallis test, or in the case of fat scores alternatively by Chi-square

Parameter	Treatment Group		
	Gravel	Choice	Slag
Initial wt. (g)	613.71^a	593.89^a	629.04^a
	17 (18.08)	18 (16.56)	26 (13.78)
Final wt. (g)	612.29^a	650.44^a	576.40^a
	17 (20.91)	16 (19.80)	10 (24.65)
Liver wt. (g)	13.80^a	16.92^a	29.61^b
	13 (.74)	12 (.85)	10 (3.53)
Liver wt. (% body wt.)	2.26^a	2.60^a	5.06^b
	13 (.10)	12 (.17)	10 (.46)
Kidney wt. (g)	3.19^a	3.70^a	4.65^b
	13 (.17)	12 (.10)	10 (.25)
Kidney wt. (% body wt.)	0.53^a	0.57^a	0.81^b
	13 (.02)	12 (.02)	10 (.04)
Gizzard wt. (g)	9.58^a	10.85^a	10.51^a
	13 (.52)	12 (.34)	10 (.63)
Bone wt. (g)	2.25^{ab}	2.39^b	2.05^a
	13 (.07)	12 (.06)	10 (.11)
Fat Scores	2.38^b	2.25^b	1.10^a
1→4 (fatest)	13 (.21)	12 (.28)	10 (.10)
Hematocrit (%)	53.88^b	54.24^b	49.19^a
	17 (.65)	15 (1.09)	10 (1.19)
HCT Refractometry (L/L)	0.49^b	0.49^{ab}	0.45^a
	17 (.01)	16 (.01)	10 (.01)
Hemoglobin (g/L)	156.65^b	155.75^b	140.90^a
	17 (2.25)	16 (3.62)	10 (3.52)
Mean corpuscular hemoglobin	318.50^a	320.06^a	310.80^a
concentration (g/L)	16 (7.26)	16 (2.38)	10 (2.21)
Protein (total solids)	42.88^a	44.81^a	44.00^a
(g/L)	17 (1.89)	16 (2.05)	10 (2.13)
White blood cells (x	10.32^a	7.88^a	10.96^a
10^9/L)	17 (1.38)	16 (1.05)	10 (1.86)
Heterophils (x 10^9/L)	3.88^a	4.53^a	8.17^b
	17 (.40)	16 (.72)	10 (1.63)
Lymphocytes (x 10^9/L)	5.50^b	2.82^{ab}	2.23^a
	17 (1.04)	16 (.37)	10 (.51)
Monocytes (x 10^9/L)	0.39^a	0.32^a	0.34^a
	17 (.11)	16 (.08)	10 (.14)
Eosinophils (x 10^9/L)	0.28^b	0.04^a	0.03^a
	17 (.16)	16 (.02)	10 (.02)
Basophils (x 10^9/L)	0.28^a	0.17^a	0.19^a
	17 (.07)	16 (.06)	10 (.05)
Azurophils (x 10^9/L)	0	0	0
	16	15	10

Hemoglobin values paralleled the trends noted for hematocrit, in that slag-treated birds indicated a significant (10%) reduction from mean values determined for the control group and likewise for the group receiving gravel plus slag.

Among blood cell types examined, treatment effects were observed only in the case of heterophils, lymphocytes and eosinophils. On average, heterophil numbers in slag-treated birds were significantly elevated by ~ 2-fold and lymphocyte numbers suppressed to ~ 50% of the respective values seen in control birds. Eosinophil counts were severely reduced (by ~ 90%) in both the slag-treatment group and in birds receiving gravel plus slag compared to mean values seen in the controls.

No significant treatment effects were noted in numbers of monocytes, basophils or total white blood cells. Azurophils were not observed to be present in any of the blood samples examined.

Blood levels of MCHC (mean corpuscular hemoglobin concentration) and Protein TS (protein - total solids) did not differ significantly among treatment groups.

3.8 Metals in Body Tissues

3.8.1 Liver

Metal concentrations in liver are summarized and compared among the three treatment groups in Table 8. Of the 10 elements examined, the most pronounced treatment effects were noted for Fe and Ca. Mean Fe values ranged from a low of 3060 (± 229) µg/g in controls to a high of 23,624 (± 1082) µg/g in the slag-treated group – a highly significant 7.7-fold increase. An intermediate mean hepatic Fe concentration of 8271 (± 817) µg/g was noted in birds given both gravel and slag.

By comparison, Ca levels within the liver as well as group differences attributable to treatment effects were less striking; mean concentration in the slag-treatment group (438 ± 99 µg/g) was 2.3-fold higher than in controls (163 ± 13 µg/g). Mean concentration in birds provided with both grit types (259 ± 32 µg/g) was intermediate in value, although not significantly different from either of the former values.

In contrast to the above two elements, mean concentrations of As, Cd, Cr, and Cu were surprisingly low (< 1 µg/g, with the exception of Cu) and indicated an unexpected reverse trend; mean values in slag-treated birds were significantly lower (up to 50%) compared to controls and in the case of Cd and Cr, likewise significantly lower than in birds with choice.

Although likewise generally low (< 3 µg/g), mean concentrations of Co, Pb and Se indicated statistically significant accumulations of these elements within the liver of slag-treated birds when compared to either of the other treatment groups.

Hepatic nickel concentrations were low (< 2 µg/g). Birds with a choice as to grit types indicated a significantly lower mean value than did control subjects, while slag-treated birds showed a mean value not significantly different from either of the aforementioned groups.

Table 8 Comparison of metal concentrations in liver among the three treatment groups receiving gravel, slag or a combination (choice) of the two as grit source. Values are reported as means (µg/g dry wt.) with standard error in parenthesis. Within elements, mean values sharing a common superscript are not significantly different (p ≤ 0.05) as indicated by ANOVA with Tukey post hoc and confirmed by Kruskal Wallis test

Element	Gravel (n=17)	Choice (n=18)	Slag (n=26)
Arsenic	0.057^{b} (.004)	0.039^{a} (.004)	0.040^{a} (.005)
Calcium	162.958^{a} (12.825)	259.083^{ab} (31.515)	437.881^{b} (99.344)
Cadmium	0.239^{b} (.032)	0.255^{b} (.039)	0.097^{a} (.008)
Cobalt	0.052^{a} (.005)	0.169^{a} (.041)	0.400^{b} (.062)
Chromium	1.014^{c} (.085)	0.804^{b} (.018)	0.594^{a} (.027)
Copper	9.786^{b} (1.476)	7.210^{ab} (.565)	4.962^{a} (.285)
Iron	3060.200^{a} (228.7)	8270.833^{b} (816.67)	23,623.885^{c} (1081.73)
Nickel	1.508^{b} (.341)	0.362^{a} (.129)	1.315^{ab} (.318)
Lead	0.065^{a} (.025)	0.051^{a} (.018)	0.632^{b} (.094)
Selenium	2.546^{a} (.186)	2.374^{a} (.078)	3.170^{b} (.157)

Table 9 Comparison of metal concentrations in kidney among the three treatment groups receiving gravel, slag or a combination (choice) of the two as grit source. Values are reported as means (µg/g dry wt.) with standard error in parenthesis. Within elements, mean values sharing a common superscript are not significantly different (p ≤ 0.05) as indicated by ANOVA with Tukey post hoc and confirmed by Kruskal Wallis test

Element	Gravel (n=17)	Choice (n=18)	Slag (n=26)
Arsenic	N.D.^{a}	0.031^{b} (.003)	0.052^{c} (.004)
Calcium	224.518^{a} (9.490)	230.100^{a} (15.826)	235.369^{a} (29.422)
Cadmium	0.795^{a} (.089)	1.400^{b} (.165)	0.709^{a} (.054)
Cobalt	0.090^{a} (.007)	0.368^{a} (.061)	1.206^{b} (.121)
Chromium	1.242^{c} (.129)	0.872^{b} (.044)	0.614^{a} (.037)
Copper	9.017^{a} (.865)	7.908^{a} (.402)	9.936^{a} (.517)
Iron	875.788^{a} (81.884)	1071.483^{a} (87.513)	1046.250^{a} (109.259)
Nickel	1.603^{a} (.449)	0.809^{a} (.188)	2.300^{a} (.430)
Lead	N.D^{a}	0.111^{b} (.018)	0.199^{c} (0.28)
Selenium	4.895^{a} (.245)	4.679^{a} (.170)	4.958^{a} (.175)

3.8.2 Kidney

Metal levels in the kidney are summarized and compared among the three treatment groups in Table 9. Of the various elements, Ca and Fe were present at comparatively high concentrations ($\bar{x}$ > 200 µg/g and $\bar{x}$ > 800 µg/g, respectively). However, treatment effects were not indicated for either element in that mean concentrations reported for the three treatment groups did not differ significantly from each other. Cu, Ni and Sn, all present at substantially lower levels ($\bar{x}$ < 10 µg/g, $\bar{x}$ < 3 µg/g and $\bar{x}$ < 5 µg/g, respectively), likewise failed to show any significant treatment effects. In contrast, As, Co, and Pb, typically present in the control birds at levels ranging from non-detectable to < 0.1 µg/g, indicated significant accumulations within slag-treated birds and in the case of As and Pb also within birds given choice. Mean Cd concentrations indicated a significant accumulation (2-fold) in birds provided with a choice of grits but not in those maintained on slag alone. Unlike other elements, mean Cr concentrations were highest in the control birds (1.2 ± 0.13 µg/g), significantly lower in birds of choice (0.87 ± 0.04 µg/g) and significantly further reduced in the slag-treated subjects (0.61 ± 0.04 µg/g).

3.8.3 Bone

Five elements, namely Co, Fe, Ni, Pb and Sn, showed significantly higher mean concentrations in the bones of slag-treated birds than in those of the control group, thus signifying significant accumulations (Table 10). Mean levels in birds given a choice of grit types were consistently intermediate between the respective values seen in controls and those indicated in the slag-treatment group, although not all of the latter group comparisons showed differences that reached statistical proportions. For example, Fe levels ranged from a low mean value of 77 (± 9) µg/g in controls to a high of 243 (± 60) µg/g in slag-treated birds – a significant 3.2-fold increase. Mean Fe concentration in birds provided with both grit types (143 ± 27 µg/g) was intermediate in value, but not significantly different from mean values for either the controls or the slag-treated birds.

Ca, the most abundant of the elements, indicated an anomalous reverse pattern. Compared to mean concentrations in control birds (79,906 ± 2896 µg/g) and among birds provided with both grit types ($\bar{x}$ = 86,240 ± 4177 µg/g), Ca levels in the bones of slag-treated birds were substantially reduced ($\bar{x}$ = 66,288 ± 1862 µg/g). Mean values for control birds and birds with choice did not differ significantly.

Cu and As showed a somewhat similar reverse pattern. Mean concentrations ranged from a high value in controls (3.13 ± 0.12 µg/g and 0.03 ± 0.002 µg/g respectively) to a significantly lower level in the slag-treatment group (2.48 ± 0.15 µg/g and 0.02 ± 0.002 µg/g respectively). Birds that received a choice of grit types averaged intermediate values that failed to differ significantly from their respective mean values seen in either controls or the slag-treated group.

No statistically significant treatment effects were indicated for Cd or Cr levels in bone.

Table 10 Comparison of metal concentrations in bone among the three treatment groups receiving gravel, slag or a combination (choice) of the two as grit source. Values are reported as means (µg/g dry wt.) with standard error in parenthesis. Within elements, mean values sharing a common superscript are not significantly different (p ≤ 0.05) as indicated by ANOVA with Tukey post hoc and confirmed by Kruskal Wallis test

Element	Gravel (n=13)	Choice (n=12)	Slag (n=10)
Arsenic	0.027^{b} (0.002)	0.024^{ab} (0.002)	0.019^{a} (0.002)
Calcium	79,905.85^{b} (2895.98)	86,240.00^{b} (4177.09)	66,288.25^{a} (1862.00)
Cadmium	0.005^{a} (0.002)	0.003^{a} (0.001)	0.002^{a} (0.001)
Cobalt	0.178^{a} (0.011)	0.237^{a} (0.023)	0.316^{b} (0.022)
Chromium	0.295^{a} (0.027)	0.264^{a} (0.039)	0.308^{a} (0.019)
Copper	3.131^{b} (0.122)	2.958^{ab} (0.183)	2.476^{a} (0.151)
Iron	76.577^{a} (8.931)	142.804^{ab} (27.296)	242.765^{b} (60.196)
Nickel	3.355^{a} (0.184)	3.828^{ab} (0.373)	4.602^{b} (0.270)
Lead	0.231^{a} (0.188)	0.236^{a} (0.079)	0.833^{b} (0.179)
Selenium	0.172^{a} (0.021)	0.234^{ab} (0.033)	0.317^{b} (0.022)

3.8.4 Overview

In summary, pronounced treatment effects were noted for two elements. Fe levels in slag-treated birds increased over baseline values seen in the controls, with accumulations occurring preferentially in the liver (7.7-fold), followed by bone (3.2-fold) and finally the kidney (1.5-fold). Concurrently, tissue Ca levels were altered in these slag-treated birds, resulting in a 2.7-fold increase in hepatic concentrations, no net change in renal levels and a 17% reduction in the Ca content of bone.

3.9 Summary Observations during Post-Mortems

3.9.1 Gravel-Treated (Control) Birds

All 17 birds examined showed adequate breast muscle mass. Fourteen of these birds showed good subcutaneous/visceral fat stores, while 2 (both males) showed moderate subcutaneous and internal fat deposits and one bird (a male) had only minimal amounts of fat stores present. All 17 control birds had good bone strength. Very little variation was noted among the spleens and livers.

3.9.2 Slag-Treated Birds

Of the 25 birds examined, 23 had moderate atrophy of breast muscle mass, while one (a male) had adequate mass and one (a male) showed a marked reduction in breast musculature. The majority of these birds showed either minimal subcutaneous/visceral fat stores (14 of 25) or noticeably reduced deposits (11 of 25). With respect to bone strength, 16 (10 males, 6 females) showed marked reduction in overall bone strength, including keel bone and long bones, while 4 (2 males, 2 females) indicated mild reduction in overall bone strength and 3 (all males) had good bone strength. Twelve of 13 spleens examined were classified as small while 1 was considered slightly enlarged. Most livers were noticeably enlarged and discoloured (tan/brown).

3.9.3 Birds Given Gravel/Slag Choice

Of the 18 birds examined, 6 (1 male and 5 females) showed mild to moderate decrease in breast musculature while 12 birds had good breast muscle mass. In 13 birds (6 males, 7 females), subcutaneous/visceral fat scores were judged to be good while those in 3 others (1 male, 2 females) were considered to be minimal and those in 2 others (both females) were moderately reduced. Bone strength was good in 13 birds (7 males, 6 females) and moderately reduced in 5 others (all females). In 3 birds the spleen was considered to be small, and in several birds the liver was noted to be slightly enlarged and mildly discoloured (tan/brown).

3.10 Summary of Histopathological Abnormalities Observed

Most frequently observed histological abnormalities occurred within the parathyroid glands, thyroids, liver, spleen, various digestive organs and bone (tibiotarsus and keel).

3.10.1 Parathyroid Glands

One (or occasionally both) glands were successfully located and examined histologically, in 10 of the slag-treated birds examined. In all cases, the glands were grossly enlarged, typically exceeding 2.5 – 3.0 mm in diameter and occasionally reaching as much as 4 – 5.5 mm (Fig. 2).

Fig. 2 Photograph showing grossly enlarged parathyroid gland (5.5 mm) in dissected slag-treated bird (#18)

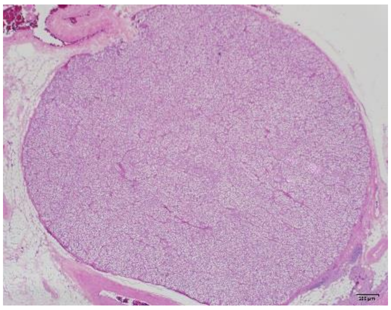

Fig. 3 Microphotograph of sectioned parathyroid gland in slag-treated bird (#14), stained with H & E

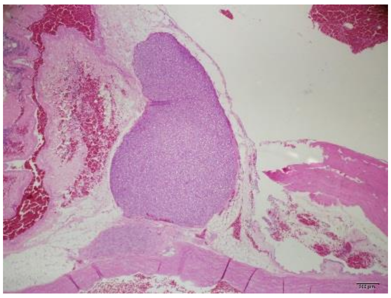

Fig. 4 Microphotograph of sectioned parathyroid gland with accessory structures in gravel-treated (control) bird (#3), stained with H & E

In 9 of the 10 cases, the parathyroid glands of this slag-treatment group showed hypertrophied chief cells with increased amounts of cytoplasm bearing discrete lipid vacuoles that were arranged in ribbons (Fig. 3). Similar observations were noted in the one bird successfully examined from the group receiving slag plus gravel. Neither glandular hypertrophy nor the extensive cytoplasmic vacuolation of chief cells were identified in the parathyroids of the 3 control birds examined (Fig. 4); parathyroid gland dimensions in controls ranged from 0.5 – 2 mm.

3.10.2 Thyroid

Few treatment-related effects were noted in thyroid histology. Thyroid glands were not grossly enlarged and so were difficult to capture; as a result, not all birds from all groups had the thyroids examined histologically. In 3 of the 5 birds from the slag treatment group, the thyroid gland follicles were lined by hypertrophied epithelium and were either devoid of colloid or contained pale eosinophilic colloid (Fig. 5). Two of these birds also had a few small interstitial lymphocytic foci. The remaining 2 pigeons had thyroid glands with relatively normal appearing colloid-laden follicles similar to the thyroid glands seen in the control and gravel plus slag treatment groups (Fig. 6).

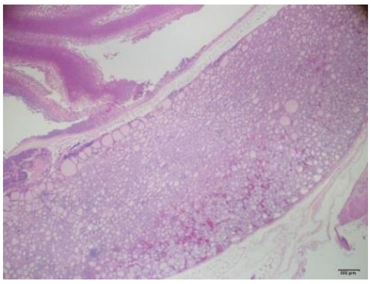

Fig. 5 Microphotograph of sectioned thyroid gland from slag-treated bird (#16). Note the hypertrophied epithelium of the follicles and reduced amount of pale eosinophilic colloid present (H & E stained section)

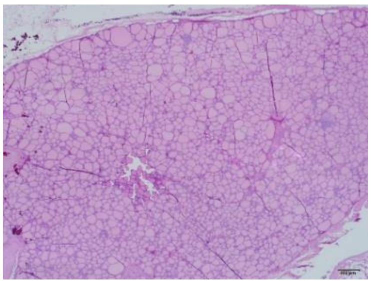

Fig. 6 Microphotograph of sectioned thyroid gland from gravel-treated (control) bird (#3), showing normal follicles lined by more normal flattened epithelium and filled with substantial amounts of eosinophilic colloid. (H & E stained section)

3.10.3 Liver

Histological examination revealed remarkably consistent alterations within the liver of virtually all slag-treated birds. Numerous variably sized clusters, ranging from small to coalescing aggregates, of macrophages with fewer mature and immature heterophils, plasma cells and lymphocytes were noted throughout the parenchyma (Fig. 7). In several birds, similar small to moderately sized macrophage clusters were aggregated around the portal triads of the tissue. Irrespective of location, macrophages were typically swollen and characterized by an abundance of densely-packed cytoplasmic granules which varied in colour from waxy blue/grey to yellow/green to deep gold/brown (Fig. 8). Hepatocytes, as well as Kupffer cells where occasionally noted, were likewise swollen and the cytoplasm appeared stuffed and bulging with numerous large gold/brown granules (Figs. 7 and 8). Mild biliary epithelial proliferation was apparent in the majority (7/12) of these slag-treated birds. Typical distribution and appearance of hepatocytes in control birds are provided in Fig. 9.

Among birds maintained on slag plus gravel grit, liver sections indicated mild histiocytosis, with small numbers of macrophages and occasionally lymphoid cells commonly located near portal blood vessels. These macrophages as well as the hepatocytes generally had relatively low numbers of small brown cytoplasmic granules.

To determine the nature and content of the pigment-laden granules accumulated in the hepatocytes and macrophages of these two treatment groups, several formalin-fixed, paraffin-embedded hepatic sections were subjected to routine histochemical stains for copper, iron and lead. Iron was identified as the dominant metallic constituent present in the pigmented cytoplasmic granules (Fig. 10).

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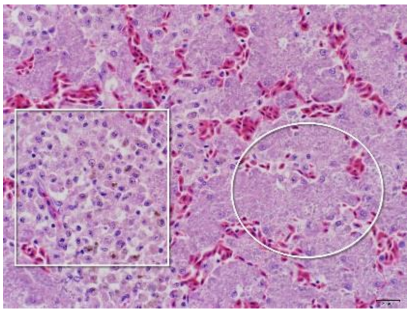

Fig. 7 Microphotograph of liver section from slag-treated bird (#19), showing an aggregate of macrophages (square) and area dominated by hepatocytes with limited numbers of individual macrophages scattered throughout (circle). Hepatocytes are enlarged and contain a large amount of pigmented granules in their cytoplasm. Section stained with H & E

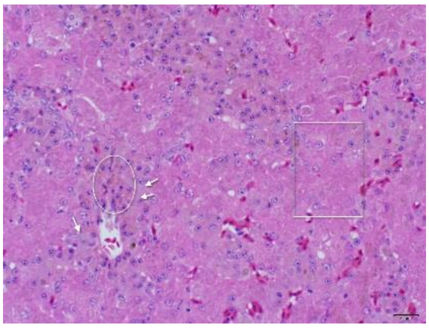

Fig. 8 Microphotograph of liver section from slag-treated bird (#19), showing a cluster of swollen macrophages (circle) with an abundance of densely-packed cytoplasmic granules (arrows) and swollen hepatocytes (rectangle) containing numerous densely-packed granules. Section stained with H & E

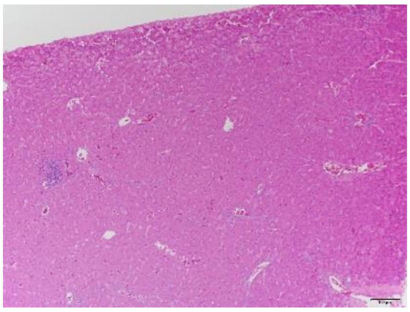

Fig. 9 Microphotograph of liver section from gravel-treated (control) bird (#2), with uniform distribution of normal hepatocytes. H & E stained

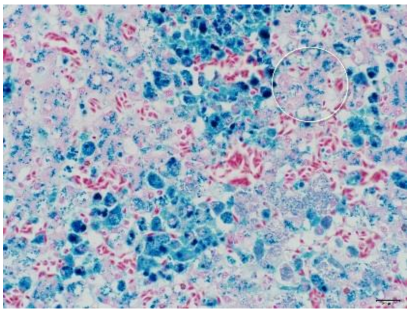

Fig. 10 Microphotograph of liver section from slag-treated bird (#16), stained with Perl's stain to show abundance of iron-containing granules (blue pigment) in cytoplasm of hepatocytes (circled)

3.10.4 Spleen

Increased numbers of macrophages bearing heavily pigmented granules, and commonly situated near blood vessels in the spleen, were noted in the majority (11/12) of slag-treated birds. Several of the birds maintained on the slag plus gravel treatment condition showed a similar pattern, but generally involved fewer macrophages with lower levels of cytoplasmic granulation.

3.10.5 Digestive Organs

Few treatment-related effects were noted in the histology of the digestive tract. Small clusters of macrophages bearing yellow/gold cytoplasmic granules, and frequently occurring in association with heterophils, were dispersed throughout tissues of the digestive tract in slag-treated birds. These cell clusters were concentrated primarily within the mucosa, and were most prevalent throughout the gizzard, duodenum and terminal portions of the small intestine.

3.10.6 Bone

Most birds examined from the slag treatment group (8/10) had moderate to severe resorption of bone tissue within the tibiotarsus and the keel. Cortical layers in these bones were thin and typically marked by discontinuous borders, particularly along the periosteal and endosteal surfaces, where resorptive cavities lined with increased numbers of osteoclasts prevailed (Fig. 11). Areas of cancellous bone showed reduced cross-strut formation with irregularly scalloped borders and similarly increased numbers of osteoclasts present, compared to the normal profile observed in gravel-treated control birds (Fig. 12). Invariably, within regions undergoing such resorptive changes, foci of woven bone formation were likewise identified (Fig. 11). In addition to the presence of spicules of osseous tissue, these areas were typically marked by increased numbers of osteoblasts and fibroplasia.

In general, comparable observations indicative of resorption of lamellar bone and active formation of woven bone were observed in the bone samples obtained from the single subject examined from the slag plus gravel treatment group. Occasionally among slag-treated birds, areas of acute myonecrosis were noted at the base of the keel bone – pectoral muscle border. Such sites were marked by substantial resorptive changes in the periosteal surface of the keel bone and involved the adjoining fibers, predominantly those of the deeper layers of the muscle. Increased numbers of macrophages and heterophils were noted among the necrotic fibres.

Visual inspection of long bone marrow (all treatments combined) yielded estimates of cellularity mostly between 15 – 30%, but with 2 birds (one from the slag group and the other from the slag plus gravel group) reaching an upper value of 80%. Estimates of myeloid:erythroid ratio in the marrow were generally 1:1 with two birds (both from the slag-treated group) showing a higher ratio of 2:1 and one bird (from the slag plus gravel group) reaching a ratio of 3:1. Both cellularity values and myeloid:erythroid ratios were highly variable and no clear or consistent treatment-related patterns were apparent apart from the observation that the few occasional anomalies noted here all occurred among birds receiving slag as their sole or partial grit source.

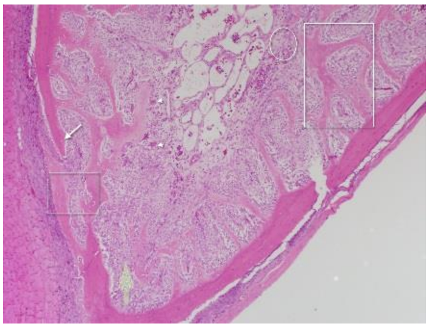

Fig. 11 Microphotograph of keel bone of slag-treated bird (#14), undergoing resorption of trabecular bone by osteoclasts (arrow heads). Note the thin cortical layer showing discontinuity (arrow) with replacement by woven bone (square). Larger region of active woven bone formation (rectangle) and fibroplasia (circle) are also identified. H & E staining

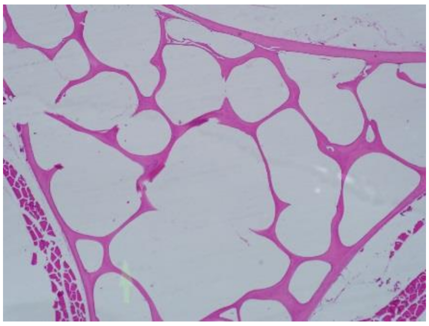

Fig. 12 Microphotograph of keel bone of gravel-treated (control) bird (#3), showing normal cross-strut formation and solid thick layers comprising the cortex and trabeculae. H & E staining

4. Discussion

The most imminent and pronounced of treatment effects noted in this study was the early onset of morbidity and mortality eventually leading to loss of 61.5% of birds maintained on the slag-grit treatment. The health and survival of all control birds, along with all but two subjects maintained on the free choice grit regime (gravel and slag), further substantiate slag and its ingestion as the principle causative factor leading to death.

Rates of grit ingestion, while similar for slag- and gravel-treated birds, were estimated to be moderately higher (44%) among birds provided with a choice. The reason for this apparent disparity among treatment groups presently remains unclear. Gender differences, particularly those relating to a perceived need for greater calcium intake by egg-laying females, are an unlikely explanation as measures taken to curtail reproductive cycles resulted in relatively few eggs being produced. It is possible, however, that this higher value obtained for the free choice group is attributable to behavioural differences in feeding and wastage patterns as this latter group had been provided with two separate grit feeders containing a total volume of grit twice that provided to the other treatment groups in the study. Certainly any increased spillage or wastage by birds accustomed to a surplus would falsely inflate the estimate of grit usage rather than provide a true measure of the amount actually consumed.

Of fundamental importance to assessing the level of exposure and potential health risk from metal-laden slag, is an understanding of the extent to which grit, once ingested, is eroded and worn down during digestive processes within the gizzard. Such activity can be expected to directly reflect the release and availability of metals for absorption. In the present study, virtually every gizzard contained grit with all size classes represented, thus confirming the view that grit was not only routinely ingested by the bird but was systematically degraded during the mastication process. The pronounced shift from predominantly larger-sized grit in the gizzard (overall 95.4% of gizzard pellets exceeded 1.0 mm; Table 5; Fig. 1) to the much greater dominance by very fine grains of grit recovered in fecal samples (overall 71.6% of fecal pellets < 1.0 mm; Table 6; Fig. 1) further substantiates the intense erosion that had occurred in these birds. From a theoretical perspective, assuming grit particles to be spherical in shape, and acknowledging that volume = $4/3\ \pi r^3$, it is possible to quantitatively estimate the extent of this reduction in mass. Given that ingested pellets ranged from 2 – 4 mm in diameter and that the majority of these were diminished to 0.5 – 1.0 mm prior to elimination in the feces, it is estimated that most pellets underwent an average reduction in mass amounting to ~ 95% (range of 87.5 – 99.8%) of their original volume. During this process, it is reasonable to assume that the bulk of the metal burden present in these eroding pellets would have been released and made available for potential absorptive uptake by the bird.

The above findings are clearly at variance with the perception that raw grit, once ingested, remains within the gizzard for only a brief interval during which time only the roughest and most-jagged edges of the particle are worn away before being eliminated. The latter scenario, if true, would imply only limited release and exposure to surface-bound metals on the grit particle, thus resulting in relatively little risk of metal toxicity to the bird. Clearly the experimental birds of the present study had been subjected to the bulk of the metal burdens present in slag and, as a result, many developed manifold morbidity changes that frequently led to death.

The above results are also clearly at variance with the observations and conclusions derived from a simulated bird digestion study purported to examine the liberation and availability of metals from ingested slag as described by researchers at Environment Canada and reported by SAIC Canada [9]. In the aforementioned simulation study, samples of 1 – 3.35 mm slag likewise collected from abandoned travel corridors within eastern Central Ontario were subjected to conditions believed to prevail within the avian gizzard (i.e. pH of 2.1, 40 °C temperature environment) while mechanically agitated on a rolling mill (while fluid suspended) for approximately 20 hours. Following the tumbling period, the supernatant of the extraction fluid was analyzed for 26 metals (including 9 of the 10 elements assayed in the present study). While metal concentrations detected were higher than values obtained from TCLP tests (Toxicity Characteristic Leaching Procedures based on Ontario Ministry of Environment's waste guidelines) and likewise SPLP tests (Synthetic Precipitation Leaching Procedures using a procedure that simulates acid rain conditions), there were "no particularly elevated levels of metals liberated under the acidic conditions of the test procedure" used to mimic the avian digestive system. In assessing the potential risk to birds from consumption of slag as grit, the cited authors [9] considered Cr, Cd and Pb to be the primary elements of concern. However, based on the fact that the concentrations of these metals fell within an order of magnitude of the Canadian drinking water quality guideline values and were below the MOE Leachate Criteria, these authors concluded that it was unlikely that concentrations of metals measured in the leachate would pose a risk to birds consuming slag material as grit.

In contrast to the findings and conclusions drawn from the above-described simulation study, we observed and report substantial morbidity and mortality among live birds maintained on slag as a grit source. Given that total inorganic metal concentrations in the slag sources used in the two studies were generally of the same order of magnitude, it would appear that the blatant contrast in findings can be attributed to a flawed experimental design, namely the failure of the adopted *in vitro* liberation and extraction procedures to satisfactorily replicate the true grinding actions and physiological conditions prevailing within the avian gizzard. As a further subsidiary experiment to examine the effect of increased abrasion of slag on the availability of metals, the above-cited authors subjected a single slag sample (size class 3.35 – 9.0 mm) to agitation in a ball mill with 5 ceramic balls (2.5 cm in diameter) added to further enhance abrasive action. Following the 24-hour tumbling period, all slag material < 3.35 mm was collected and acid-extractable metals quantified. Concentrations of metals rendered available again proved minimal. The most significant of observations noted here, however, was the fact that after 24 hours of action only 4% of the slag material had abraded – clearly a meager fraction of the 88 – 99% disintegration of slag pellets demonstrated to occur between ingestion and fecal elimination in the birds of the present study. As a result, it is our opinion that the data and conclusions reported in the simulated bird digestion tests of the SAIC study [9] provide little useful information and, in fact, would appear to constitute a misleading literature in the quest toward understanding the health risks of slag ingestion in birds.

Grit turn-over times, the period during which pellets are retained in the gizzard before passage and elimination, were not determined in the present study. The importance of retention times in assessing metal exposure rates has been emphasized [10]. Gionfriddo and Best [11] reported that retention times could vary from days to as long as a year depending on a variety of factors including physical characteristics of the grit such as size, shape, solubility and hardness. The consistently higher numbers of pellets observed within the dissected gizzards of slag-treated birds compared to gravel-fed birds suggest that slag may have been retained for longer periods of time. This contention is further supported by the greater prevalence of slag noted in the gizzards of birds given free choice despite the fact that gravel constituted a greater proportion of the total grit ingested by these birds. Reasons for the assumed higher retention time for slag are unclear but may relate to the basic structure and physical properties of the material. Hardness testing of the two materials using the Rockwell hardness methodology [12] yielded an average index of 81.2 (n = 3) for slag and 35.4 (n = 4) for gravel. This marked difference was substantiated by a parallel test, the Brinell hardness test [13]; slag = 634 (n = 1) and gravel = 327 (n = 1). Irrespective of methodology, the indices for slag were approximately 2 times higher than those for gravel, thus indicating a greater hardness and durability, factors which are likely to have contributed to the assumed slower rate of erosion and greater retention time characterizing slag grit. Additionally, from a purely chemical perspective, gravel grit characterized by its substantial Ca reserves would be expected to more readily disintegrate under the acidic conditions prevailing within the avian digestion tract. Nonetheless, despite unknown rates of erosion and retention times involved, both gravel and slag appeared to remain within the gizzard of the bird until most pellets had been near-fully degraded, thus resulting in near-maximal exposure to intrinsic metal loads present.

The total extent of metal exposure incurred in slag-fed birds was a combination of the exposures derived from slag and those resulting from feed sources. The relative contributions of these two sources cannot be determined with accuracy. However, the amount of Fe, for example, available from the average weekly intake of slag (5.6 cc/bird) would be expected to approximate 1.95 g, assuming total disintegration in the gizzard. The equivalent amount of Fe available though feed sources would require that the bird consume ~ 52 kg (115 lbs) of the feed mix per week. As pigeons are typically able to maintain themselves on a daily seed ration of 40-45 g (0.66 lb/week), clearly the major source of metal exposures incurred in the slag-stressed birds of the present study was via grit with feed sources contributing only a very minor fraction.

The accumulation of Fe in the liver (and to a lesser extent bone tissue) of slag-treated birds was more pronounced than treatment effects seen for any of the other metals examined. In slag-treated birds, hepatic Fe concentrations averaged 23,624 μg/g, with values for individual birds reaching as much as 40,502 μg/g (while in bone average and individual values approximated 243 μg/g and 532 μg/g respectively). These hepatic concentrations exceeded those of the control group by more than 7-fold and likewise exceeded levels typically reported for birds manifesting iron storage disease or hemosiderosis - defined as "histopathologically

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quantified evidence of 'more than expected' deposition of hemosiderin (storage form of Fe), without accompanying clinical disease" [14]. For avian species known to be particularly susceptible to excessive Fe storage, mean hepatic concentrations of 5500 μg/g, that is approximately one fourth of the concentrations noted here, have been reported [14-16]. The latter values were documented in autopsied samples from captive zoo-caged specimens that had been maintained on diets suspected of Fe elevations or that had received known levels of Fe supplementation for an extended period prior to death. In line with the view that diet constitutes a prime etiological factor contributing to the condition [17], the slag material used as a grit source in the present study was characterized as having excessive levels of Fe present ($\bar{x}$ = 347,798 ± 5910.20 μg/g vs $\bar{x}$ = 751.90 ± 55.83 μg/g in gravel ingested by controls).

The abundant proliferation and widespread macrophagic infiltration of liver, spleen and gastrointestinal mucosa in slag-treated birds constitutes a toxicological response to excessive metal exposure and uptake. Staining confirmed that the abundant macrophage population, in addition to hepatocytes and Kupffer cells, had sequestered substantial quantities of Fe stored as densely packed cytoplasmic granules – a form that would remove the contaminant metal from circulation and thereby limit further systemic toxicity. This finding is consistent with those of previous studies demonstrating increased numbers of metal-binding macrophages in response to a variety of Fe overload conditions in birds [18] and humans [19]. The above observations lead us to believe that the slag-treated birds of the present study had developed a chronic Fe storage condition not unlike that of avian hemosiderosis as reported in the literature [20-22]. Furthermore, this condition which can reach toxic proportions, particularly at the excessive tissue Fe levels reported here, is likely to have been one of the prime causes leading progressively to a state of acute iron toxicity, bird morbidity and eventually to the high numbers of bird deaths observed.

Calcium uptake and retention were likewise markedly altered in the slag-treated birds of the present study as indicated by the 2-3 fold higher levels present in the liver and the lowered concentrations in bone tissue relative to other treatment groups. Although markedly lower than seen in gravel grit, calcium levels in the slag were substantial (> 15,000 μg/g) and, assumed to be largely bioavailable, should have been more than adequate to meet the essential daily Ca requirements of the bird, a contention supported by the elevated hepatic Ca stores noted in these slag-treated birds. Nevertheless, the gross hyperplasia of their parathyroid glands, a typical response to chronic Ca disruptions in avian and mammalian systems [23], implies a marked reduction of blood Ca reserves accompanied by the mobilization of stored Ca from bone tissues in an attempt to maintain systemic Ca homeostasis. The reduction in bone calcium levels noted among the slag-treated birds corroborates well with the observation that during dissections virtually all members of this treatment group presented tibiotarsal and keel bones that were unusually pliable and of noticeably reduced strength. The basis for these chemical and physical changes (calcium loss and reduction in bone strength) were verified histologically with direct evidence indicating that osseous tissue had been actively undergoing resorption in both cortical and trabecular regions of the bone, resulting in thinning of cortical layers and loss of cross-struts respectively, with the formation of woven bone occurring concomitantly.

The metabolism of individual elements can rarely be considered in isolation as it is well known that various elements, including both toxic and essential metals, can mutually interfere, especially at high concentrations, in the physiological processes of elemental absorption, accumulation and/or retention [24]. Although bone Ca levels were not assayed directly, Kim and co-workers recently demonstrated a negative association between bone mineral density and toxic Fe levels in human subjects [25]. Further evidence that iron-overloading results in trabecular and cortical thinning of bone accompanied by increased resorption of bone minerals has been reported in a murine model [26]. Given the substantial concentrations of calcium and iron within slag and the magnitude and direction of changes in tissue burdens of these two elements following its ingestion, it would seem improbable that these two elements exerted independent influences on metabolic processes in the bird. Accordingly, it seemed prudent to examine the inverse relations of these two elements and their potential interactive effects in compromising the mechanical properties of bone. Further studies in this area, employing x-ray absorption and mass spectrometry techniques, are currently in progress with publication pending.

In addition to cortical layers and trabecular regions, the marrow-containing medullary portions of bone appeared to be likewise adversely effected by toxicity levels in the slag-treated birds. Although patterns were highly variable, marrow cellularity and myeloid:erythroid ratios in several birds showed striking irregularities suggestive of suppressed cell formation activity in marrow tissue. More specifically, erythropoiesis, a major physiological function of red bone marrow [27], is likely to have been adversely affected by prevailing toxic conditions, thus accounting for the lowered hematocrit and hemoglobin levels observed among slag-treated birds of the study. Evidence that excess iron can damage bone marrow stromal cells and exert a negative impact on hematopoiesis in mice has been recently reported [28].

The substantial atrophy of breast muscle accompanied by near depletion of body fat stores denoted an ongoing negative energy balance among slag-treated birds and suggests that such birds had been metabolically stressed for some time prior to their death or sacrifice. Such factors, although unlikely the direct cause of mortality, were undoubtedly prime contributors to a state of reduced fitness and the development of certain behavioural and endpoint morbidities (e.g. reduced and lethargic activity patterns) frequently noted in this metal-stressed group. The striking hypertrophies noted in liver and kidneys of surviving birds within the slag-treatment group reflect the critical role of these two organs in detoxification processes. Bothwell [29] reported that severe cases of hemochromatosis, a congenital condition characterized by abnormally high hepatic Fe accumulation, frequently resulted in hepatomegaly with symptoms of liver dysfunction. Enlargement of the liver with accompanying hepatic tissue damage were likewise reported in several avian species subjected to a deadly outbreak of iron storage disease [22]. Accordingly, the pronounced hepatomegaly, and possibly the renal hypertrophy noted here as well, may be viewed as morphophysiological indicators of reduced fitness in the metal-stressed bird. Other fitness parameters, including assessment of the status and performance of the immunological system, as discussed by Porto and De Sousa [30] and Walker and Walker [31], were not examined but may represent a worthwhile direction for future studies. In addition to being less invasive, such approaches would be expected to provide greater sensitivity and earlier detection of subtle changes in the health and fitness of the metal-stressed bird.

Gender differences in the response of birds maintained on slag grit were not addressed in this study. It is speculated that such differences, if any, would be minimal in birds of non-reproductive status. Under conditions of continued laying the metabolic activities and dietary needs of the laying female can be expected to be enhanced, particularly as relates to Ca metabolism and its essential role in eggshell formation. Our efforts to suppress laying activity by interrupting female hormonal cycles through the removal of newly-laid eggs from the nest box were not totally successful; the occasional production of an egg(s) at a later date and the presence of developing eggs within the oviduct of certain autopsied specimens indicated that at least a few females had been in a reproductively active laying condition - either sporadically or for a brief portion of the treatment period.

Numbers and identities of laying birds could not be ascertained with certainty, nor could the extent of their involvement in egg laying activities be determined. Given these uncertainties and the overall low and uneven representation of females within the treatment groups, the present study is unable to shed new light on the effects of slag ingestion on egg laying and the metabolic status of the laying hen nor on the differential effects of slag on female versus male birds. Notwithstanding, as reasoned earlier (see Results section), mortalities did not appear to be gender biased.

The present study was designed as a laboratory study carried out under specific and, for the most part, carefully controlled conditions for the purpose of providing a fundamental understanding of the use of slag as a grit source and assessing its physiological effects on bird health and fitness. As such, it has not been our intention to extrapolate our findings to wild populations or conditions of environmental contamination existent in the field. Behavioural feeding patterns among birds remained largely non-examined except for inclusion of the one treatment group to which both types of grit were offered concurrently. Based on measured volumes provided, members of this latter cohort indicated a 2-fold greater consumption of gravel relative to slag, thus tentatively suggesting a selective preference for the former. Nevertheless, the number of gravel pellets present in the gizzard of these birds was consistently fewer than slag pellets present. The explanation for this incongruity, however, may well lie in the faster turnover rate proposed for gravel as discussed earlier. At any rate, it must be acknowledged that all birds in this cohort relied on the uptake of grit to aid in digestion as indicated by its pervasive presence in their gizzards and furthermore that, with but one exception, all members had taken up a combination of both slag and gravel, rather than discriminating to the exclusion of either material. This latter observation strongly supports the view that birds may not be discriminatory in their selection of grit material(s) under environmental conditions where industrial slag and more natural gravel-based options are both available within their habitat. Interestingly, the presence of black volcanic lava particles have been observed among the gizzard contents of wild free-ranging swans and geese [32], suggesting that these species may likewise be relatively non-selective in their grit intake. The ecological implications of residual slag deposits within the environment and an assessment of their risk to resident avian species will need to be addressed directly through carefully designed field studies; such investigations will need to take into account the substantial potential for mortality and severe pathophysiological impacts identified in this laboratory-based study.

5. Conclusion

Industrial slag as a source of grit had multiple deleterious effects, frequently resulting in death, in laboratory pigeons, due to its substantial intrinsic metal residues. As with gravel, slag grit remained in the gizzard until near-completely abraded, thus accounting for widespread uptake and accumulation of injurious metal loads in various vital organs. Hemosiderosis (Fe storage disease) was probably the primary causative factor leading to death. Slag ingestion evoked parathyroid hypertrophy with accompanying mineral resorption in long bones leading to reduced hardness and structural strength. Other subsidiary changes, including enlarged liver and kidneys, marked atrophy of breast musculature and body fat reserves as well as alterations in various hematological parameters may be useful morphophysiological indicators of reduced fitness and declining health in the metal-stressed bird. Given choice, birds were not highly selective in their uptake of grit types available to them, suggesting concern for the ecological implications of the vast quantities of exposed slag within our environment and its potential health risks to wild bird populations is warranted.

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