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Determination of the Effect of Bone Meal as an Ameliorant on Lead Contaminated Soils

[1]Edmond Gatimu, [2]Mwinyikione Mwinyihija and [1]Ken Killham
[1]School of Biological Sciences, Plant and Soil Sciences, University of Aberdeen, St. Machar Drive, Cruickshank Building, Aberdeen, AB24 3UU, UK
[2]Veterinary Laboratories Kabete, Private Bag 00625, Nairobi, Kenya

Abstract: Bone meal is a by-product of the livestock and fish industry whose main constituent is calcium phosphate. Therefore the aim was to investigate the effect of bone meal on leaching of lead from an agricultural soil spiked with different lead sources. The results successfully demonstrated the effectiveness of bone meal as an ameliorant, when a reduction of specific lead compounds was observed. However, the source of bone meal and its method of processing could influence the final results through contamination of the leachate.

Key words: Bone meal, toxicity, ameliorant, lead contamination

INTRODUCTION

Bone meal is a by-product of the livestock and fish industry. The main constituent of bone meal is calcium phosphate (Valsami-Jones, 2000). In previous studies by Hodson *et al*. (2001) using Scanning Electron Microscopy (SEM), it was revealed that metal splinters containing variable amounts of Iron (Fe), Zn, Cu, Ni and Pb were present as particles mostly derived from the crushing process involved in bone meal manufacture. A variety of phosphate amendments, including soluble phosphate (K_2HPO_4) and rock phosphate (apatite) have been investigated but are reportedly too fast or too slow, respectively, when compared to bone meal as a source of phosphate (Hodson *et al*., 2000).

The importance of bone meal has been reported (The Columbia Encyclopaedia, Sixth Edition, 2001) as an organic fertilizer source due to its readily available phosphate and nitrogen (about 23-30% available phosphate and 2-4% nitrogen) content. In addition, bone meal has been used as a feed supplement to farm animals to provide crucial mineral food constituents (i.e., calcium and phosphorus). However health considerations associated with bone meal have become a matter of increasing concern (e.g., Creutzfeldt-Jakob Disease, which is the human form of Mad Cow Disease, Salmonella and Bovine Spongiform Encephalopathy (BSE)) and therefore necessary, initial precautionary principles need to be observed.

Valsami- Jones *et al*. (1998) noted similarities between some synthetic apatites and bone meal. They suggested that poorly crystalline apatites such as those found in crush bone (bone meal) could therefore provide a cost-effective, natural-phosphate source for remediation of soils contaminated with certain metals. Hodson *et al*. (2000) went on to report that the preliminary experiments incorporating bone meal were encouraging. However, Walworth *et al*. (2003) reported that the use of bone meal supplies additional organic compounds that exert an O_2 demand on the system relative to that experienced with DAP (diammonium phosphate). Therefore, they observed that any bioremediation design utilizing bone meal must account for the additional O_2 demand through provision of adequate aeration.

Corresponding Author: Dr. Mwinyikione Mwinyihija, Veterinary Laboratories Kabete, Private Bag 00625, Nairobi, Kenya Tel: 254 0722 429807

In most of the experiments related to remediation of metal contaminated soils, application of bone meal was found to be a suitable source of phosphate for such remediation (Laperche *et al*., 1998). For example metal immobilization was found to be due to both the pH rise associated with bone meal dissolution and almost certainly, formation of metal phosphates (Hodson *et al*., 2000). In recent years, researchers have also taken advantage of the high content of phosphorus in bone meal, to use it as an ameliorant of soils contaminated with heavy metals, based on the fact that most of metal phosphates have very low solubility, rendering toxic metal unavailable (Ma *et al*., 1993; Cotter-Howells and Caporn, 1996). Heavy metal contaminated soils may cause risks to entire ecosystems and humans. Furthermore, metal contaminated sites are widespread through the world and the risk associated with such sites depends on soil characteristics (e.g., content of clay, sesquioxides, organic matter and pH), climate (e.g., precipitation, wind) and the chemical characteristics of the contaminants present (Friesl *et al*., 2004). Therefore the need to use, efficient and cost effective sources of ameliorants for remediation is pertinent (e.g., bone meal which is widely available as a by-product of the animal and fish industry).

During this study, a series of experimental pots were designed for leachate collection (Fig. 1) to investigate the potential of bone meal as an ameliorant. The application of bone meal was carried out in the context of measuring its remediation potential for soils contaminated with various forms of lead (PbS, $PbNO_3$, $PbCO_3$ and $PbNO_3$) contaminated soils.

There has been very little work on the application of bone meal as an ameliorant to agricultural (Craibstone estate in Scotland) contaminated soils and, therefore work was carried out in this study to observe how soils spiked with different lead compounds PbS, $PbSO_4$, $PbNO_3$ and $PbCO_3$ would respond to bone meal amelioration during a prolonged period of leaching (230 days).

The aim of this study was to investigate the effect of bone meal on leaching of lead from an agricultural soil spiked with different lead sources.

METHODS AND MATERIALS

Sampling Collection and Experimental Set-Up

The topsoil used in these experiments was collected from the top 25 cm of an agricultural field on the Craibstone estate, which is approximately 9 km North West of Aberdeen, at an elevation of 100 m. The pots had a diameter of 150 mm and a surface area of 17,600 mm^2. A Whatman 42 filter paper was placed on the base of each pot to prevent coarse material from passing through. Leaching pots were arranged on a leaching bench with holes wide enough to hold them. Funnels with aligned filter paper (Whatman 42) inside were placed under each pot placed on the leaching bench to collect leachate in a conical flask placed on the bottom of the shelf as shown in Fig. 1.

Preparation of Experimental Pots

Thirty pots were packed with either a mixture of soil, lead compounds and Bone meal, or soil with lead compound only.

Control: 3 pots of soil only Soil
3 pots of soil + Bone meal
Samples: 3 pots of soil + Pb compound
3 pots of soil + Pb compound + Bone meal

Since 4 Pb compounds were considered (PBS, $PbSO_4$, $PbNO_3$, $PbCO_3$), the total number of samples was 24. This translates to thirty pots together with 6 pots of control.

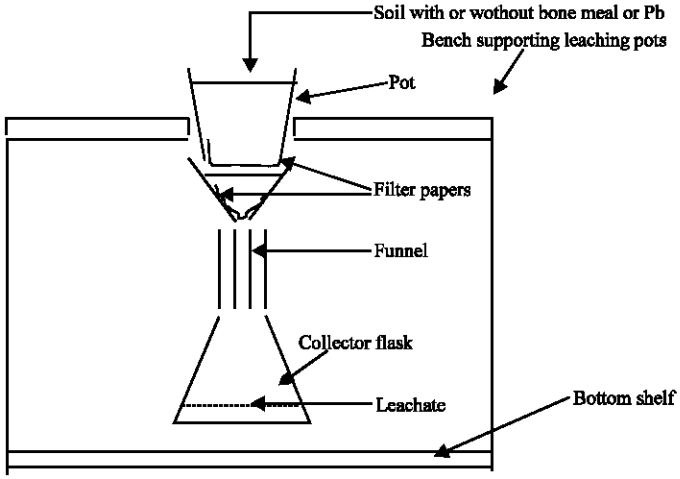


Fig. 1: Diagram of experiment set-up

The bone meal was added to the soil in the proportion of 1 g of bone meal to 50 g of soil. Since 1 kg of soil was packed into each leaching pot, 20 g of bone meal were added to each pot. Bone meal, lead compounds and soil were mixed together in plastic bags and shaken for 2 min to make sure that bone meal and lead (Pb) were evenly distributed throughout the soil. In addition, control samples without lead compounds, with and without bone meal, were prepared.

The area where the bottles were placed was protected with black, plastic material to minimise the effect of light on leachate chemical properties. All the experimental treatments were carried out in triplicate. The controls used are soils spiked with lead compounds but not treated with bone meal.

Bioassay

Lux-marked bacterial biosensors were used during the study and the preparation of the biosensor and luminometer measurements were carried out as described. One hundred miceoliter of the resuscitated biosensor suspension was added to the samples at 15 sec intervals, accurately timed for measurement in the Bio Orbit 1253 luminometer (Labtech International, Uckfield, UK). Each sample was exposed to the sensor for exactly the same time. Samples were incubated for 15 min before light output measurements were carried out at 15 sec intervals. This ensured the same exposure time to the potentially toxic elements for cells in each of the cuvettes.

Chemical Analysis

Stock Solution Preparation

1.599 g of lead nitrate, Pb $(NO_3)_2$ (analytical grade) was carefully weighed and dissolved in deionized distilled water. When dissolution was complete, it was acidified with 1 mL of 1M HNO_3 and diluted to 1 L with deionized water.

Preparation of Standard Solutions and Calibration

Standard Lead Solution was prepared by diluting the stock (lead) solution. Concentration ranges starting from 0.1, 0.5, 100, 200, 400, to a maximum of 800 g L^{-1}) were used as calibration standards. All standard and sample soil solutions were prepared to approximately 0.1 mol L^{-1} in HNO_3. Care was taken to use specially purified water (deionized water) when diluting samples to final volume for quality control purposes.

Deionized water was also used during the final rinsing of all the plastic and glassware. This was after rinsing them first, in solution (with diluted nitric acid) in order to remove any possible traces of lead on them. During the determination of concentration two replicate determinations of absorbance were made for each sample. A blank of dionized water was used to zero the instrument.

A 10 μL sample was injected very carefully with the help of an auto sampler into the cold graphite furnace and, by means of an automatic temperature programmer, dried at 120°C for 35 sec and at 140°C for another 35 sec, then heated to 200°C and allowed to cool for 15 sec. These steps were performed, automatically, to remove solvent and any removable volatile matrix. Actual atomization of the sample followed and was performed at 1800°C, very rapidly, for 5 sec. During this time the signal from the chamber (absorbance) was recorded and displayed on the screen as a function of time. Finally the furnace was heated for 5 sec at 2600°C. The purpose was to remove any residues and prepare the instrument for next sampling phase. During the atomization step, the absorbance was monitored at 283.3 nm, using a slit width of 0.7 nm, set at low level. Purging with argon was interrupted automatically during the absorbance scan. Background correction was provided by means of the deuterium background corrector, which automatically compensated for broadband absorption interferences.

Data Analysis

Two-way analyses of (ANOVA-Analysis of Variance) (except for biosensor experimental data which is One-way ANOVA) were carried out using the statistical package Minitab for windows, release 12.1 (State College, PA, USA). Mean differences were determined using t-test (paired two samples for means) and Pearson Correlations using Excel program (Microsoft™ Office 2000). Significant differences between treatments were elucidated using Least Significance Difference (LSD) values. Graphs were generated using SigmaPlot for Windows version 9.0 (Jandel Corporation, CA and USA).

RESULTS

Chemical Analysis

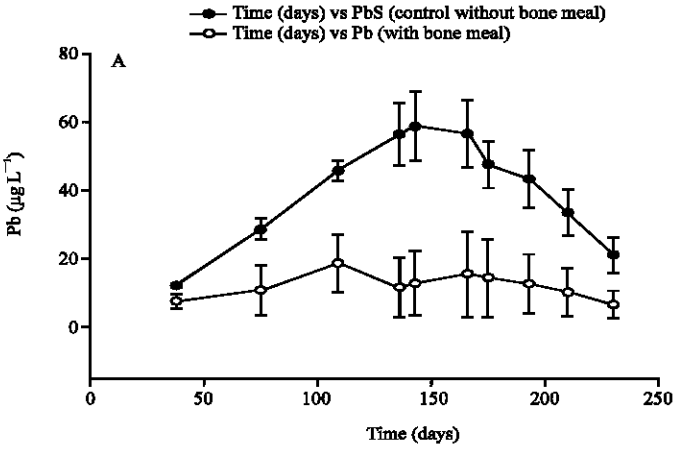
Effect of Bone meal on Lead Concentration of Leachate

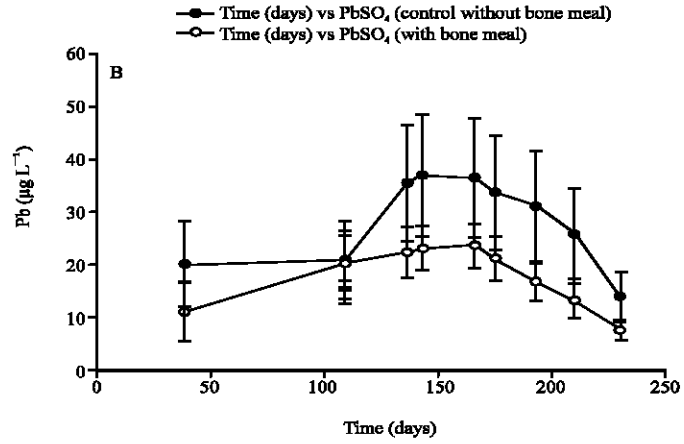
The effect of bone meal on soils (treated/untreated with bone meal) contaminated (spiked) with PbS, $PbSO_4$, $PbNO_3$ and $PbCO_3$ over a period of 230 days was evaluated as shown in Fig. 2(A-E). When bone meal was used as an ameliorant, different effects on individual lead compounds were observed. Generally, bone meal as an ameliorant had an effect on all soils contaminated with PbS and $PbCO_3$ ($p<0.001$), $PbSO_4$ and $PbNO_3$ ($p<0.01$) irrespective of the length of exposure. However, when length of time (days) was considered, the lowest Pb leachate concentration in the medium term was demonstrated for $PbCO_3$ at 143 days (bone meal treated value of 20 μg L^{-1} compared to the untreated value >60 μg L^{-1}). However a bone meal treatment effect over time (days) was clearly observed in PbS ($p<0.01$) and $PbNO_3$ ($p<0.05$). For the full term duration of experiment (230 days), the bone meal treatment effect was clearly observed in PbS ($p<0.01$) and $PbNO_3$ ($p<0.05$). Table 1

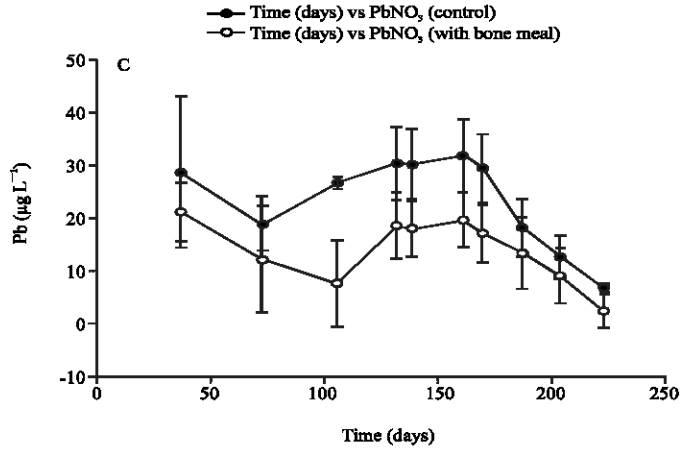
Table 1: Comparison of significance of the effect of bone meal treatment and time

(Source of Pb)	Treatment (Bone meal)	Time factor
PbS	***	*
$PbSO_4$	**	ns
$PbNO_3$	**	*
$PbCO_3$	ns	ns

*** $p<0.001$; ** $p<0.01$; * $p<0.05$; ns = not significant


Time (days) vs PbS (control without bone meal)
Time (days) vs Pb (with bone meal)
A
Pb (μg L⁻¹)
Time (days)


Time (days) vs PbSO4 (control without bone meal)
Time (days) vs PbSO4 (with bone meal)
B
Pb (μg L⁻¹)
Time (days)


Time (days) vs PbNO3 (control)
Time (days) vs PbNO3 (with bone meal)
C
Pb (μg L⁻¹)
Time (days)

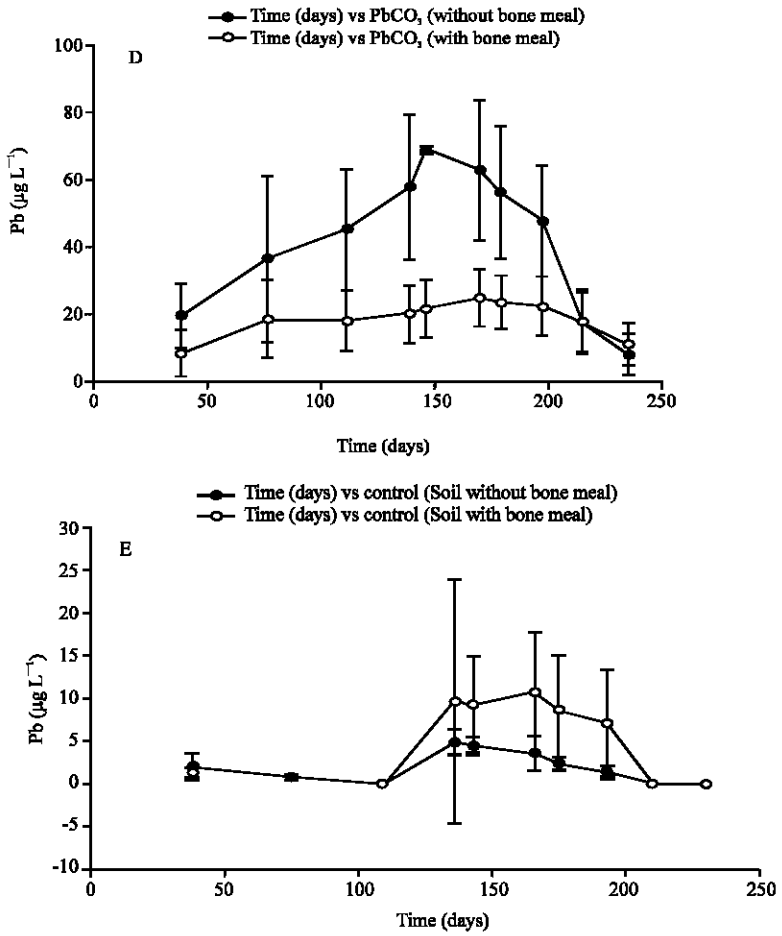


Fig. 2: Lead concentration (µg L^{-1}) in leachate from soil contaminated with (i) PbS, ii) $PbSO_4$, (iii) $PbNO_3$ and (iv) $PbCO_3$ (treated/untreated with bone meal) over a period of 230 days

showed the significance of the time factor when lead compounds were treated with bone meal. All the treatments were affected by time as a factor except for $PbSO_4$. The time factor for contaminated soils treated with bone meal is critical for design of mitigation strategies so as to address emerging constraints from the contaminants. Comparison between the PbS and $PbSO_4$ ($r = 0.8$), PbS and $PbCO_3$ ($r = 0.8$) treatment means showed a significant difference ($p<0.001$), while demonstrating an amelioration with bone meal. An increase in Pb leached from various control (i.e., no bone meal) samples over time after the equilibration phase was not associated with an increase in leachate from samples treated with bone meal. The difference between the blanks (control with/without) was not significant.

Effect of Bone meal Treatment on pH Values of Leachate from Samples Spiked With Lead Compounds

The treatment of the lead contaminated soil samples with bone meal as an ameliorant significantly ($p<0.05$) affected the pH values (Fig. 3). For example control soil samples (i.e., without the addition of Pb and Bone meal) showed a pH value of 6.05±0.16 but when bone meal was added to the soil

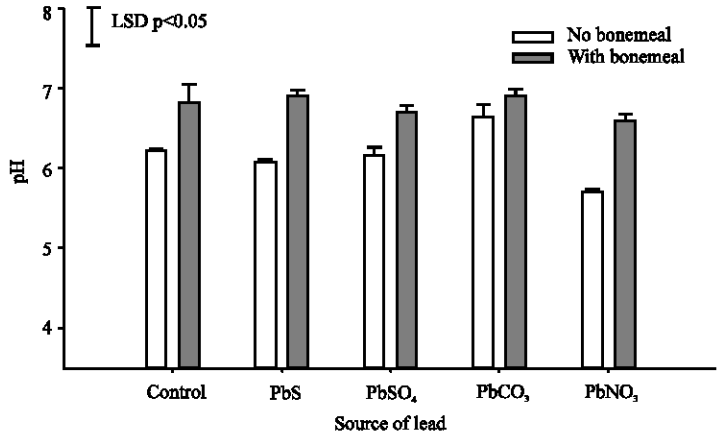


Fig. 3: Effect of Bone meal treatment on pH of soil samples spiked with different lead compounds

samples the pH immediately decreased to 5.70±0.14. Similarly when Pb compounds (PbS, $PbSO_4$, $PbNO_3$ and $PbCO_3$) were added (in the absence of bone meal) to the soil samples the pH values also decreased with the highest decrease noted with PbS (pH 5.5±0.003). This result suggested that the decrease in pH values in most of the samples were attributed to both the presence of bone meal and Pb compounds. However, when the samples previously spiked with Pb compounds were treated with bone meal, lower pH values were maintained except for $PbNO_3$ (6.09±0.20) which was insignificantly higher than the control and also higher than $PbNO_3$ samples not treated with bone meal (5.62±0.003). Most probably the increase of pH observed after initially spiking the samples with $PbNO_3$ was through a synergistic or additive effect caused by the treatment of bone meal.

Biosensor Based Toxicity Test of the Leachate

Effect of Filtration of Leachate on Biosensor Response

The leachates were subjected to a toxicity test, to measure the bioavailable portion of Pb immediately after the determination of its concentration was carried out. Leachate collected from different soil samples of various lead compounds treated with bone meal, filtered/unfiltered were exposed to a *lux*-marked *E. coli* HB101pUCD607 biosensor to measure the percentage bioluminescence. The analysis of luminescence demonstrated the different levels of toxicity of the leachate samples as shown in Table 2. High toxicity is represented by a low percentage luminescence relative to the control.

Overall application of bone meal as an ameliorant had a significant ($p<0.05$) effect on lead spiked soils as demonstrated with the % bioluminescence results (Table 2). However initial analysis of the data on filtered samples before treatment with bone meal indicated that all the samples were significantly different ($p<0.05$) except for $PbCO_3$ (Table 2). Further analysis of the results on filtered samples indicated that the effect (Table 2) of bone meal was negligible between treated samples and their controls. However, the effect of filtration on samples treated with bone meal ($p<0.05$) was demonstrated only on soils spiked with PbS and $PbSO_4$. In comparison to filtration, the unfiltered samples treated with bone meal (Table 2) were measured for luminescence, indicating a difference in toxicity levels between $PbNO_3$ and its control ($p<0.05$).

Moreover when the effect of filtration was measured for samples not treated with bone meal, differences in luminescence were observed only with PbS and $PbSO_4$ when compared to $PbCO_3$, $PbNO_3$ and No Pb samples. The effect of filtration was demonstrated when the samples were treated

Table 2: Analysis (ANOVA and LSD) of luminescence-based toxicity data for leachates from samples treated with/without Bone meal

	Without bone meal		With bone meal	
Samples	Mean filtered	Mean unfiltered	Mean filtered	Mean unfiltered
NoPb	70.42(1.63)	66.62(0.45)	75.32(5.87)	74.90(4.18)
PbS	80.22(3.65)	79.05(2.07)	81.55(12.35)	75.04(2.85)
$PbSO_4$	84.10(7.12)	80.10(0.47)	79.39(3.37)	80.84(3.95)
$PbCO_3$	68.30(0.79)	68.30(0.79)	72.14(0.29)	71.15(0.77)
$PbNO_3$	62.31(1.34)	70.82(9.66)	70.41(3.01)	83.82(4.35)
LSD (5%)	6.77	8.06	11.72	6.34
p-value	ns	ns	ns	ns

Figures in parenthesis show standard errors of the mean * $p<0.05$, n = 9; ns = not significant

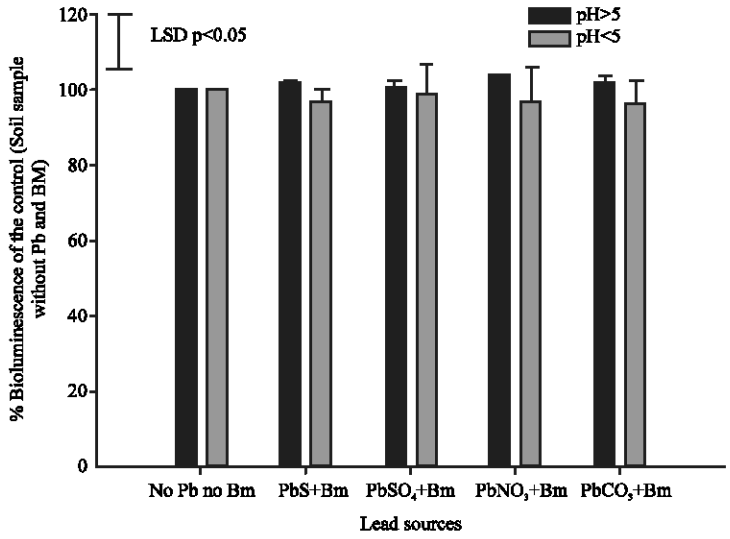


Fig. 4: Effect of pH adjusted below and above 5 on Pb bioavailability in samples amended with Bone meal

either with or without the bone meal thus suggesting colloidal nature of the contaminant. The overall effect of applying bone meal on filtered and unfiltered samples (Table 2) was evaluated and the results demonstrated that the effect of bone meal was not dependent on filtration. However, when individual samples were compared between each other, the greatest effect on samples that were filtered was observed for $PbNO_3$ + Bone meal (BM) (83.8%±4.3) and $PbSO_4$ (80.8±4.0). In addition, when the samples were filtered, PbSO4 (84.1±7.1) and PbS (80.2±3.7) indicated the highest bioluminescence values compared to the other compounds.

Further comparison of samples and their specific controls showed a difference ($p<0.05$) in $PbCO_3$ with the unfiltered samples and, when individually compared, they demonstrated a strong negative correlation (r = -0.81). This also applied to $PbNO_3$ but indicated a weaker negative correlation (r = -0.3) in comparison to $PbCO_3$.

Effect of Bone Meal on pH (Adjusted Above and Below 5) of Leachate to Biosensor Response

The adjustment of pH had no significant effect (p = 0.08) on all the Pb compounds (PbS, $PbSO_4$, $PbNO_3$ and $PbCO_3$) analysed (Fig. 4). Consequently no interaction effect (p = 0.09) was observed between the effect of adjusting the pH (above and below 5.0) with the difference in Pb compounds.

DISCUSSION

The stabilisation of metals as phosphates in metal contaminated soil is increasingly recognised as a potentially cost effective *in situ* remediation technology (Cotter-Howells, 1996). The current method of treating metal-contaminated soils essentially focuses on isolating the soil from the ecosystem by capping or removal of the soil and dumping it elsewhere (Wood, 1997). Therefore, the application of bone meal in this study offered a possible alternative as a potentially viable source for *in situ* remediation of contaminated sites without disruption to the ecosystem profile.

The use of bone meal generates highly insoluble metal phosphates (Nriagu, 1984) and it has been suggested that their low solubility renders such metals non-bioavailable (Ma *et al*., 1993; Cotter-Howells and Caporn, 1996). Hodson and Valsami (1999) also reported that bone meal appeared to reduce metal release from heavily contaminated soils with pH ranging from 2.7 to 7.1. To be able to evaluate this phenomenon of generating non-bioavalable metal phosphates, a *lux*-marked biosensor (to indicate the bioavailable fraction of the contaminant) was successfully applied to complement the chemical analysis (which provides the total concentration of the contaminant). The results from this study generally demonstrated that the application of bone meal as an ameliorant (providing a stable and slow release source of phosphate) had an intercepting effect both on total Pb released from soil spiked with several Pb compounds (PbS, $PbSO_4$, $PbNO_3$, $PbCO_3$). This observation is in agreement with reports from Hodson and Valsami-jones (1999) and Hodson *et al.* (2000, 2001) who reported that bone meal additions reduce both metal released from metal-contaminated soils and metal availability, as determined by chemical extractions and subsequent metal analysis. They also closely linked the immobilisation of metals to the dissolution of bone meal in this study.

Lead concentration data for the leachates from the current study (chemical analysis) indicated a reduction in the leaching of Pb compounds irrespective of an application of an ameliorant (i.e., both control and treated samples) after 230 days period, suggesting that over time, metal pollution from leached soil matrices may attenuate to some degree. However, time is a crucial factor in developing the viability of a remediation process. Therefore, in that context, the highest reduction in lead leaching due to bone meal amelioration was observed for $PbCO_3$ in mid term (i.e., 136-175 days) when bone meal was applied as an ameliorant. This behaviour of $PbCO_3$, which had the highest Pb total concentration before the application of bone meal as the ameliorant, is in agreement with an observation by Bataillard *et al.* (2003) who reported that initial speciation of metal (i.e., lead) was influenced by solubility: oxide = carbonate>sulphate>sulphide (on a decreasing order of solubility). They also showed that when lead was added as sulphate, between 10 and 20% of lead particles dissolved, regardless of the soil type with lead sulphide progressively oxidising over time (as observed in table 1 where time as a factor was not significant on $PbSO_4$). This is a crucial consideration in relation to similar observations made in this study where determination of centrifuged samples to obtain total concentration of Pb showed much narrower differences between bone meal treated and untreated $PbSO_4$ and PbS leachate samples. This was in contrast to $PbCO_3$ and $PbNO_3$, which as reported earlier, have a higher solubility and are thus more likely to release lead into the interstitial pore water of the soil matrix.

The bioassay results generally showed that the effect of filtration was attributable to physically held (e.g., $PbSO_3$ and PbS which are less soluble in aqueous media) but not chemically bound Pb compounds (e.g., $PbNO_3$ and $PbCO_3$ are more soluble in aqueous media) in the soil matrices. This observation was also demonstrated as shown in Table 2 where it was clear that the higher the solubility of lead compound, the lower the effect of filtration. In relation to bone meal as an ameliorant, this suggests that leachate from $PbNO_3$ and $PbCO_3$ contaminated sites would not require filtration as an initial pre-treatment requirement for remediation. Previous studies using bioassays (e.g., *Colpoda stinii*, *Pseudomonas fluorescens* and *Escherichia coli*) to measure toxicity of the leachate, showed lead in the leachates to be toxic to all the test organisms (Hodson *et al*., 2001). Determination of toxicity during

the current study was based on the bioluminescence response of the *lux*-modified biosensor, *E. coli* HB101 pUCD607, which had previously been marked with the *lux* CDABE genes (isolated from *Vibrio fischeri)* using the multi-copy plasmid pUCD607 (Amin-Hanjani *et al*., 1993). The environmental relevancy and wide pH range (3-10) of the biosensor (Palmer *et al*., 1998) provided reliable results which indicated a reduction in toxicity on application of bone meal, suggesting a reduction in the free metal concentrations in concurrence with the chemical analysis data.

CONCLUSION

The study demonstrated that the use of bone meal as an ameliorant was efficient and effective in addressing lead mobility issues related to soils contaminated with lead compounds. An effect of application of bone meal was observed for all the lead compounds tested with the highest reduction observed for $PbCO_3$ based on chemical analysis and bioassay using biosensor techniques. However, the source of bone meal and its method of processing could influence the final results through contamination of the leachate. In addition the health and safety issues in sourcing of the bone meal require strict control and care (i.e., should be sourced from disease free zones) and during its application require precautionary principles (especially when used as fine, ground particles) to be applied due to risks through inhalation.

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