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RESEARCH ARTICLE

Comparison of analytical procedures for measuring phosphorus content of animal manures in China

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Abstract The concentration and components of manure phosphorus (P) are key factors determining potential P bioavailability and runoff. The distribution of P forms in swine, poultry and cattle manures collected from intensive and extensive production systems in several areas of China was investigated with sequential fractionation and a simplified two-step ($NaHCO_3$-NaOH/EDTA) procedures. The mean total P concentration, determined by the sequential fractionation procedure of intensive swine, poultry and cattle manure, expressed as $g \cdot kg^{-1}$, was 14.9, 13.4 and 5.8 $g \cdot kg^{-1}$, respectively, and 4.4 $g \cdot kg^{-1}$ in extensive cattle manure. In intensive swine, poultry and cattle manure about 73%, 74% and 79% of total P, respectively, was bioavailable (i.e., P extracted by H_2O and $NaHCO_3$) and 78% in extensive cattle manure. The results indicated the relative environmental risk, from high to low, of swine, poultry and cattle manure. There is considerable regional variation in animal manure P across China, which needs to be considered when developing manure management strategies.

Keywords diet phosphorus, manure phosphorus, sequential P fractionation

1 Introduction

Animal manure can be a valuable source of phosphorus (P) for improving soil fertility. However, in areas with intensive livestock production, long-term field application of animal manure without considering the P forms and bioavailability cannot only improve soil fertility, but can also lead to soil P accumulation and an acceleration of P losses in surface runoff[1–4]. Given that mineral phosphate resources are being depleting, developing best management practices to optimize recycling of manure P, minimizing reliance on rock phosphate and reducing adverse environmental effects of animal manure application to crop land are important objectives for future sustainability of agriculture.

Potential manure P losses in animal production and potential bioavailability of manure P to plants after field application may not only be related to how much manure P is applied to fields, but also directly related to the proportion of manure inorganic P and how easily the manure P is dissolved in water[5,6]. The concentration of total P and the proportion of inorganic P (P_i) and organic P (P_o) fractions in animal manure varies considerably, mainly as a result of the animal species and production systems[7–9]. Animal manure P is composed of P_i and P_o of varying degrees solubility. The P_o fractions may vary from 10% to 80% of total P[10,11]. When animal manure is applied to the field, some manure P forms, such as orthophosphates and low-molecular-weight P_o (myo-inositol P), may be more soluble and bioavailable than others. Thus, the identification and quantification of the P forms in animal manure is necessary to understand manure P dynamics in soil and evaluate the potential bioavailability of manure P. The existing information on manure P is normally based on a sequential fractionation procedure developed by Hedley et al.[12], a procedure originally used for soil P characterization. The Hedley procedure differentiates manure P fractions based on operational definitions of bioavailability, including deionized water-extractable P (H_2O-P), 0.5 $mol \cdot L^{-1}$ $NaHCO_3$-extractable P ($NaHCO_3$-P), 0.1 $mol \cdot L^{-1}$ NaOH-extractable P (NaOH-P), 1.0 $mol \cdot L^{-1}$ HCl-extractable P (HCl-P) and concentrated sulfuric acid extractable P (residual-P)[12–15]. Manure P can be sequentially classified into readily soluble and stable fractions on the basis of solubility by the Hedley procedure. Deionized water and $NaHCO_3$ extract readily soluble manure P fractions, including orthophosphates, phospholipids, DNA and simple phosphate monoesters. These manure P fractions are normally

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bioavailable in soil. Whereas, NaOH and HCl extract the relative stable P fractions, such as phytic acid, which are poorly soluble in soil. This procedure has been used successfully in many studies. For example, Ajiboye et al.[16] found that the majority of P forms in fresh swine manure was in the labile P fractions extracted by $NaHCO_3$ and NaOH. He et al.[17] reported that deionized water, 0.5 mol·L^{-1} $NaHCO_3$, 0.1 mol·L^{-1} NaOH, 1.0 mol·L^{-1} HCl and concentrated H_2SO_4-HNO_3 extracted 48%, 19%, 18%, 11% and 3% of the total P in the swine manure, respectively. The sequential fractionation procedure can supply the comprehensive evaluation of manure P composition which can be used to evaluate the bioavailability and the environmental risk of animal manure after field application. However, this procedure involves many experimental steps that are long and complex. Consequently, a simplified two-step fractionation procedure was developed by Turner and Leytem[15], which involved extraction of readily soluble manure P fractions in 0.5 mol·L^{-1} $NaHCO_3$ followed by extraction of stable manure P fractions in a solution containing 0.5 mol·L^{-1} NaOH and 50 mmol·L^{-1} ethylenediaminetetraacetic acid (EDTA). Compared to the Hedley procedure, this convenient two-step fractionation procedure not only simplifies the experimental steps, but also separates structurally-defined manure P fractions with environmental relevance by considering the nature of P compounds in manures[15].

Over recent decades, the consumption of animal products in China has increased substantially due to the growth in the number of animals per farm. For example, the mean milk consumption per capita has increased in China from 2.9 kg·yr^{-1} in 1961 to 31 kg·yr^{-1} in 2007. The mode of animal production has shifted gradually from family-based farms to intensive industrial-scale feedlot systems, with more input of protein and feed additives in animal feeds, such as dicalcium phosphate, sodium bicarbonate and salt[11,18]. This intensification has come with higher dependence on purchased and concentrated feeds to meet at least the minimum nutritional requirements, with less attention being given to excessive feeding. Consequently, the concentration of dietary P commonly exceeds the actual demands of animals in China. This high-P feeding will inevitably lead to an increase of P concentration in animal manure. It has been reported that as total P in animal manure increases through increased dietary P, so does the proportion of P_i[6]. For instance, as dietary P of dairy cattle exceeds actual P requirements by 25%–40%, a significant fraction (about 80%) of P consumed was passed in the manure[19]. Consequently, P losses from agricultural land intensively amended with animal manures has become one of the greatest contributors to nonpoint pollution in China.

However, the data on P concentration in animal manure reported in China in the 1990s (i.e, 8.5–9.5, 8.7–9.9 and 4.1–4.5 g·kg^{-1} in swine, poultry, and dairy manure, respectively) may not reflect the composition of manure P in current rearing systems. The results of Li et al.[11] indicated a significant increase of total P in animal manures collected from a range of animal farms. To better understand manure P dynamics and further enhance the capacity to managing manure to reduce P losses to the environment, it is important and necessary to evaluate the P composition in animal manure under current rearing systems, particularly the P_i fractions. Therefore, the objective of this study is to comprehensively assess manure P composition and concentration in intensive swine, poultry and cattle production and in non-intensive cattle production using the Hedley procedure and the simplified two-step fractionation procedure. The data obtained can provide much-needed information for farmers in China to make better management decisions in relation to manure P.

2 Materials and methods

Fresh and undisturbed swine, cattle and poultry manures were collected from different animal farms located in several areas of China.

With the grassland grazing system mainly concentrated in Inner Mongolia, three intensive cattle farms (> 200 head of cattle) and four extensive cattle farms (< 10 head of cattle) were selected for collection of manure (from calves and cows) and feed samples in this area. An intensive dairy farm (> 200 head of cattle) and two extensive dairy farms were selected to collect manure and feed samples in Tai'an, Shandong. Manure and feed samples from intensive swine farms were collected from Beijing (> 5000 swine), Quzhou, Hebei (> 100 swine) and Jining, Shandong (> 100 swine). Manure and feed samples in the intensive poultry farms were collected from Quzhou, Hebei (> 10000 chickens) and Jining, Shandong (> 10000 chickens). The detailed information about the farms is provided in Table 1.

At least five samples of each type of animal manure were collected from different locations in the facility, and then combined and subsampled. Manure and feed samples were stored in a portable refrigerator below 4°C and analyzed within a week.

Manure samples were oven-dried at 65°C, and then ground to 2 mm for the sequential extraction procedure. The sequential fractionation procedure of Hedley and the simplified two-step procedure involving the extraction of different manure P fractions by 0.5 mol·L^{-1} $NaHCO_3$ and a solution containing 0.5 mol·L^{-1} NaOH and 50 mmol·L^{-1} EDTA were described in Li et al.[11] and Turner and Leytem[15], respectively. The feed samples were digested with concentrated H_2SO_4-HNO_3 for 75 min at 350°C (APHA 1995) and the concentration of total P in feeds was determined using the phosphomolybdate blue method

Table 1 Detailed information of the animal farms

Area	Site	Types of farms	Number of animals (age)		
			Calf	Adult cattle	Milk cow
Inner Mongolia	Hohhot-farm 1	Intensive	200 (10–12 months)	0	80 (2 years)
	Hohhot-farm 2		200 (10–12 months)	0	400 (2 years)
	Hohhot-farm 3		200 (10–12 months)	0	50 (2 years)
	Hohhot-farm 4	Extensive	0	7 (18–20 months)	0
	Hohhot-farm 5		0	8 (18–20 months)	0
	Hohhot-farm 6		0	5 (18–20 months)	0
	Hohhot-farm 7		0	7 (18–20 months)	0
Shandong	Tai'an-farm 1	Intensive	150 (18–20 months)	0	60 (2 years)
	Tai'an-farm 2	Extensive	0	6 (18–20 months)	0
	Tai'an-farm 3		0	7 (18–20 months)	0
			Piglet	Porker	Sow
Beijing	Shunyi District	Intensive	2000 (1 months)	4000 (6 months)	2500 (1–2 years)
Hebei	Quzhou County	Intensive	70 (2 months)	0	35 (1–2 years)
Shandong	Jining-farm 1	Intensive	50 (2 months)	50 (6 months)	16 (1–2 years)
	Jining-farm 2		50 (3 months)	30 (8 months)	21 (2 years)
	Jining-farm 3		65 (3 months)	0	43 (2 years)
			Chicken	Chicken	Chicken
Hebei	Quzhou County	Intensive	6000 (110 days)	0	5500 (500 days)
Shandong	Jining-farm4	Intensive	4000 (40 days)	3700 (130 days)	3500 (500 days)
	Jining-farm5		5500 (70 days)	0	5000 (450 days)

(APHA 1995)[20].

Analysis of variance was conducted using the SAS statistical software (SAS 2001, SAS Institute Inc., Cary, NC, USA). Significant difference between means was assessed by LSD at the 0.05 probability level.

3 Results

3.1 Comparison of animal dietary P

There was substantial variation of animal dietary P concentration collected from different farms (Fig. 1). Total dietary P concentrations were 2.6–7.3 $g \cdot kg^{-1}$ ($\overline{x}$ = 5.0 $g \cdot kg^{-1}$) for intensive swine, 3.8–5.0 $g \cdot kg^{-1}$ ($\overline{x}$ = 4.5 $g \cdot kg^{-1}$) for intensive poultry, 2.2–4.3 $g \cdot kg^{-1}$ ($\overline{x}$ = 3.4 $g \cdot kg^{-1}$) for intensive cattle and 0.8–1.4 $g \cdot kg^{-1}$ ($\overline{x}$ = 1.1 $g \cdot kg^{-1}$) for extensive cattle.

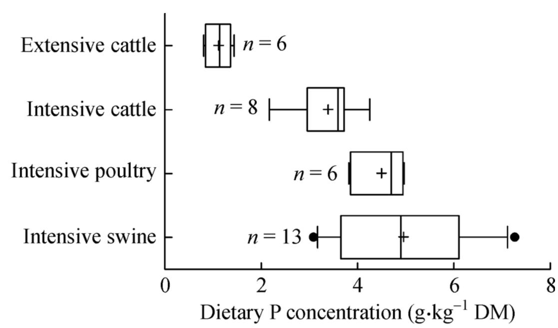

Fig. 1 Box plot showing the range in the concentration of dietary P concentration ($g \cdot kg^{-1}$ DM) for different animals. In plots, the horizontal bars represent the 10th and 90th percentiles, the outer edges of the boxes represent the 25th and 75th percentiles, and the vertical line and the plus symbol within the boxes represent the medians and means. Circles indicate outliers and n is number of the samples.

3.2 Relationship between manure P and dietary P

The relationships between manure P and dietary P ($g \cdot kg^{-1}$ DM) is shown in Fig. 2. There was a significant linear correlation for swine manure; for each unit increase in swine dietary P there was a 2.2 unit increases in total manure P. However, there were no significant linear correlations between dietary P and total manure P for poultry and cattle manures.

3.3 Comparison of P fractions extracted by sequential fractionation procedure

For the sequential fractionation procedure, the concentration of P in animal manures extracted by H_2O, $NaHCO_3$,

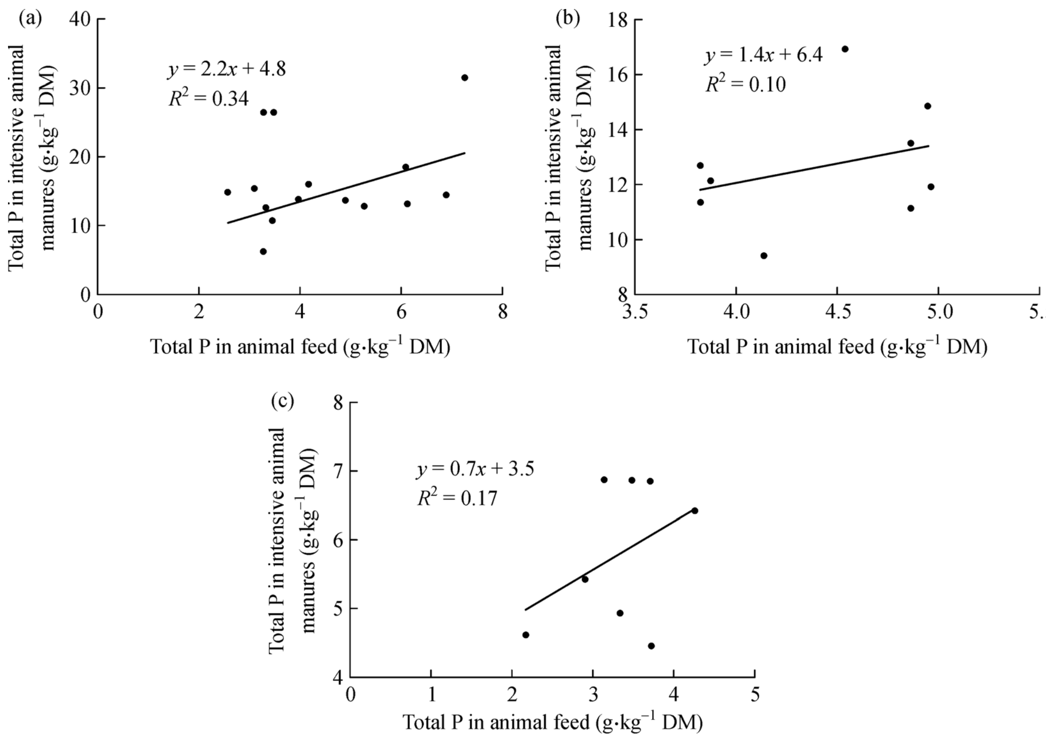

Fig. 2 Relationship between dietary P concentration for intensive swine (a), poultry (b) and cattle (c) and total manure P concentration.

NaOH, HCl and concentrated sulfuric acid are shown in Fig. 3. Substantial variation was found in the concentration of P in the fractions from different manures. For intensive swine manure (Fig. 3(a)), the mean concentration of P_i and P_o extracted by sequential procedure was 5.5 and 1.4 g·kg^{-1} (H_2O-P_i and -P_o), 2.9 and 1.1 g· kg^{-1} ($NaHCO_3$-P_i and -P_o), 0.5 and 1.2 g·kg^{-1} (NaOH-P_i and -P_o), 1.1 and 1.1 g·kg^{-1} (HCl-P_i and -P_o), and 0.05 g·kg^{-1} (residual-P), respectively. Corresponding P_i and P_o fractions for intensive poultry manure were 5.5 and 0.8 g·kg^{-1} for H_2O extracted, 1.5 and 2.1 g·kg^{-1} for $NaHCO_3$, 0.4 and 0.8 g·kg^{-1} for NaOH, and 1.3 and 1.0 g·kg^{-1} for HCl, respectively, with a residual-P of 0.02 g·kg^{-1} (Fig. 3(b)). The concentration of P in the fractions in intensive and extensive cattle manures (Fig. 3(c) and Fig. 3(d)) were 1.9 and 0.5 g·kg^{-1} for H_2O-P_i and -P_o in intensive cattle manure vs 1.6 and 0.6 g·kg^{-1} in extensive cattle manure, 1.2 and 1.0 g·kg^{-1} for $NaHCO_3$-P_i and -P_o vs 0.2 and 0.8 g·kg^{-1}, 0.1 and 0.5 g·kg^{-1} for NaOH-P_i and -P_o vs 0.02 and 0.5 g·kg^{-1}, 0.2 and 0.3 g·kg^{-1} for HCl-P_i and -P_o vs 0.03 and 0.2 g·kg^{-1}, and 0.2 g·kg^{-1} residual-P vs 0.1 g·kg^{-1}, respectively, which were much lower than the corresponding values in intensive swine and poultry manures.

The concentration of total P in animal manures determined by the sequential fractionation procedure ranged from 6.2 to 31.4 g·kg^{-1} ($\overline{x}$= 14.9 g·kg^{-1}) in intensive swine manure, 11.1–16.9 g·kg^{-1} ($\overline{x}$= 13.4 g·kg^{-1}) in intensive poultry manure, 4.4–6.9 g·kg^{-1} ($\overline{x}$= 5.8 g·kg^{-1}) in intensive cattle manure and 2.3–4.9 g·kg^{-1} ($\overline{x}$= 4.1 g·kg^{-1}) in extensive cattle manure. In intensive swine and poultry manures, the mean concentration of P_o were 4.8 and 4.7 g·kg^{-1}, respectively, compared with 2.3 g·kg^{-1} in intensive cattle manure and 2.1 g·kg^{-1} in extensive cattle manure. The percent of cumulative bioavailable P fractions (sum of H_2O-P_t and $NaHCO_3$-P_t) determined by the sequential fractionation procedure was 73% in intensive swine manure, 74% in intensive poultry manure, 79% in intensive cattle manure and 78% in extensive cattle manure.

3.4 Comparison of P fractions by two-step fractionation procedure

For the simplified two-step fractionation procedure, the concentration of P_i and P_o in manures extracted by $NaHCO_3$-NaOH/EDTA are given in Fig. 4. The concentration of P_i and P_o extracted by $NaHCO_3$ from intensive swine manure ranged from 2.8 to 7.3 g·kg^{-1} ($\overline{x}$= 4.9 g·kg^{-1}) and from 0.2 to 1.9 g·kg^{-1} ($\overline{x}$= 0.6 g·kg^{-1}), while P fractions extracted by NaOH/EDTA ranged from 1.5 to 8.4 g·kg^{-1} for P_i ($\overline{x}$= 4.7 g·kg^{-1}) and from 0.6 to 19 g·kg^{-1} for P_o ($\overline{x}$= 4.2 g·kg^{-1}), respectively (Fig. 4(a)). For intensive poultry manure, the P_i and P_o fractions extracted by $NaHCO_3$ ranged from 3.5 to 6.5 g·kg^{-1} ($\overline{x}$= 4.9 g·kg^{-1}) and from 0.6 to 3.5 g·kg^{-1} ($\overline{x}$= 1.9 g·kg^{-1}), respectively, and for NaOH/EDTA fractions P_i ranged between 2.8 and 5.6 g·kg^{-1} ($\overline{x}$= 3.6 g·kg^{-1}), and P_o varied

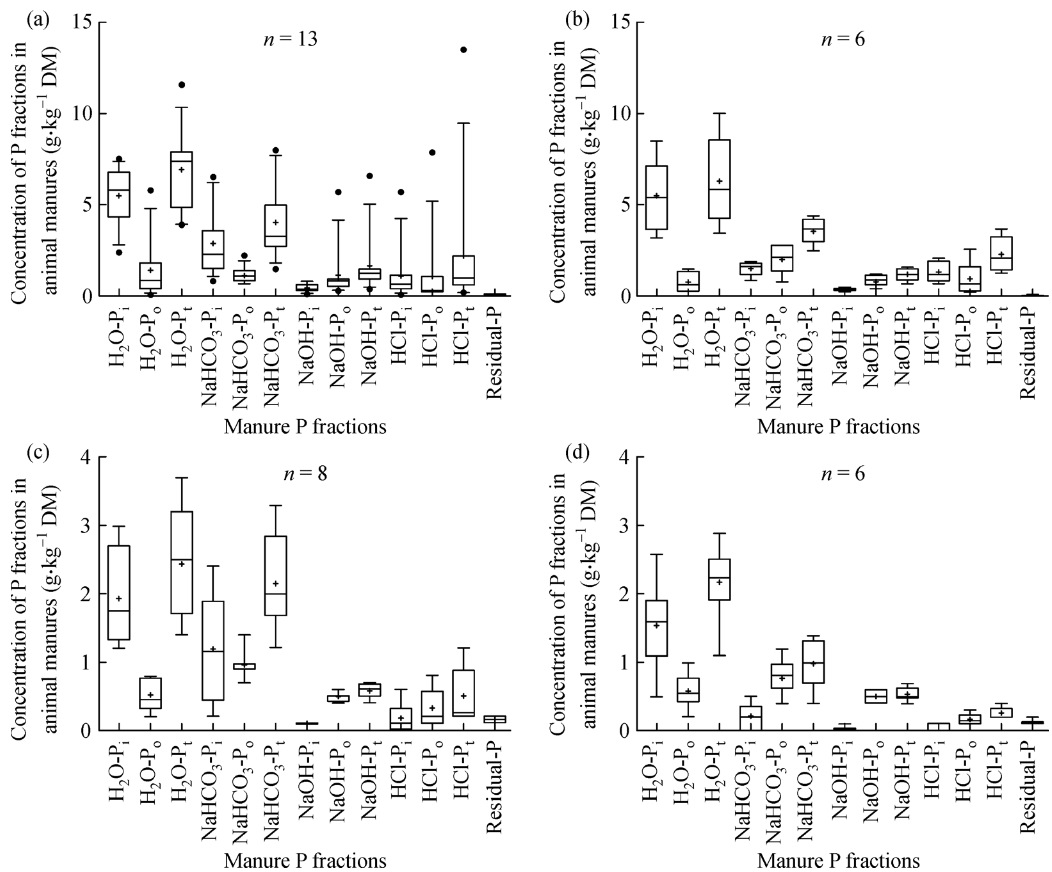

Fig. 3 Box plots showing the range in the concentration of P in the fractions extracted from intensive swine (a), poultry (b), cattle (c) manures and extensive cattle manure (d), respectively, by the sequential procedure. The manures were collected from Inner Mongolia, Beijing, Hebei and Shandong. The vertical bars represent the 10th and 90th percentiles, the outer edges of the boxes represent the 25th and 75th percentiles, and the horizontal line and the plus symbol within the boxes represent the medians and means. Circles indicate outliers and *n* is the number of samples.

from 2.1 to 7.3 $g \cdot kg^{-1}$ ($\bar{x}$ = 4.3 $g \cdot kg^{-1}$) (Fig. 4(a)). Relatively low values of the corresponding P fractions were detected in intensive cattle manure; $NaHCO_3$-P_i and P_o varied between 1.8 and 4.0 $g \cdot kg^{-1}$ ($\bar{x}$ = 2.8 $g \cdot kg^{-1}$) and 0.3–0.8 $g \cdot kg^{-1}$ ($\bar{x}$ = 0.5 $g \cdot kg^{-1}$); while NaOH/EDTA-P_i and P_o ranged from 0.2 to 1.0 $g \cdot kg^{-1}$ ($\bar{x}$ = 0.6 $g \cdot kg^{-1}$) and 0.6 to 3.0 $g \cdot kg^{-1}$ ($\bar{x}$ = 1.9 $g \cdot kg^{-1}$), respectively (Fig. 4(c)). The mean residual-P fractions in intensive swine, poultry and cattle manures were 2.9, 2.4 and 1.0 $g \cdot kg^{-1}$, respectively (Fig. 4). The total P of intensive cattle, poultry and swine manure measured by the sequential fractionation and $NaHCO_3$-NaOH/EDTA procedures was similar (Table 2).

4 Discussion

4.1 Dietary and manure P

The dietary P is very important for growing animals and is normally used for soft and hard tissue formation and body maintenance[21]. A low level of dietary P may result in a reduced growth rate and bone mineralization[22,23]. Animal manure P is a combination of unabsorbed dietary P and P excreted into the gastrointestinal tract[24], and mainly depends on the level of P intake[6,25–27]. However, the establishment of P requirement for intensive swine production is often confounded by the interactions of P with other nutrients, particularly with Ca, and the response criteria selected to establish the P requirement[28]. Normally, recommendations are largely based on optimization of swine growth. Currently, the recommendation of dietary P concentration by Chinese Standard, GB 8471-87-"Feeding standard for lean-type pigs" are 4.9 and 4.6 $g \cdot kg^{-1}$ for pregnant and lactating sow, respectively[29], which is similar to the mean of 5.0 $g \cdot kg^{-1}$ (2.6–7.3 $g \cdot kg^{-1}$) found in this study. However, there was considerable variation in dietary P concentration in the current study, and several values were far higher than the recommendations due to the excess supply of mineral phosphate additives in the diets. The excess of total P in swine diets has resulted in the high P-concentration of swine manure and the significant linear correlation between dietary and manure P for swine demonstrated that the excess P intake above the daily P demands for body maintenance is passed

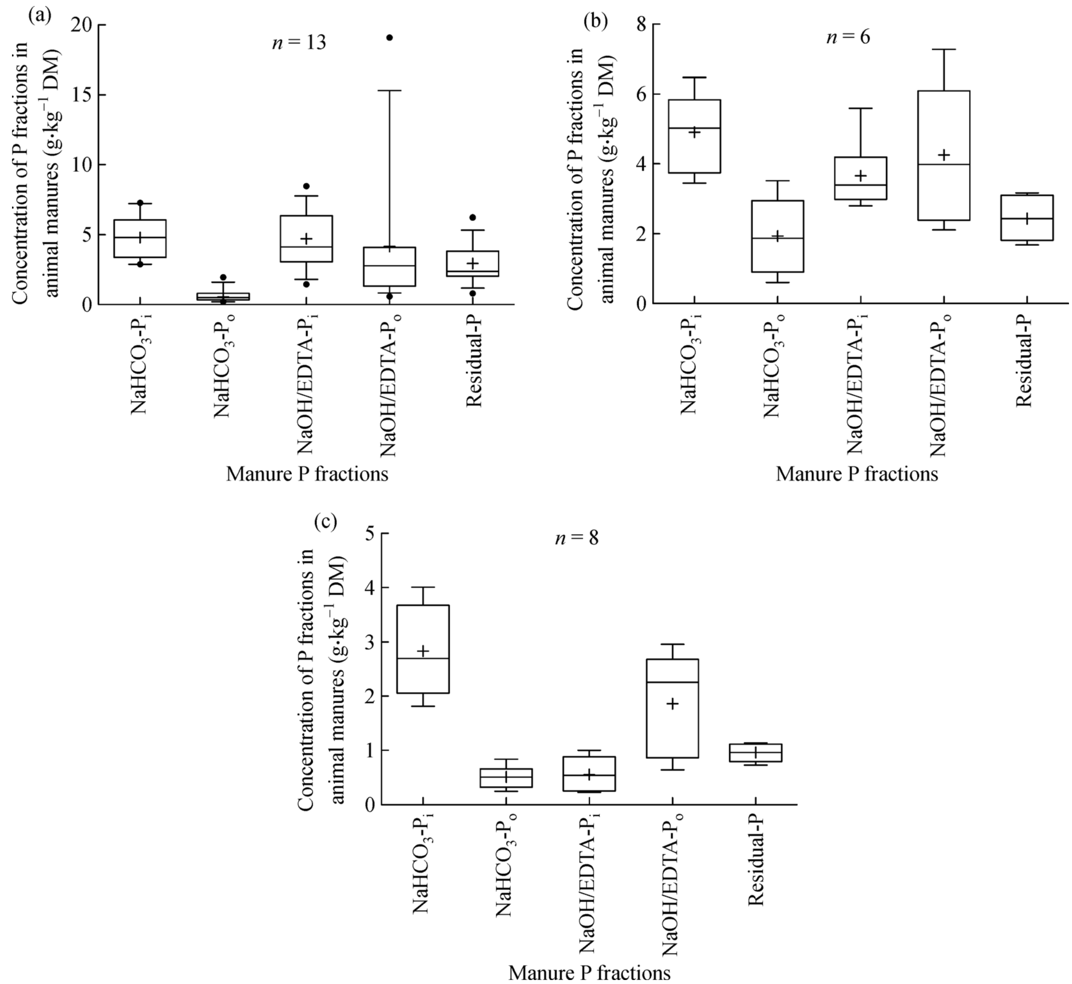

Fig. 4 Box plots showing the range in the concentration of P in the fractions extracted from intensive swine (a), poultry (b) and cattle (c) manures by the two-step fractionation ($NaHCO_3$-NaOH/EDTA). The manures were collected from Inner Mongolia, Beijing, Hebei and Shandong. In the plots, the vertical bars represent the 10th and 90th percentiles, the outer edges of the boxes represent the 25th and 75th percentiles, and the horizontal line and the plus symbol within the boxes represent the medians and means. Circles indicate outliers and *n* is the number of samples.

Table 2 Total phosphorus concentration in animal manures ($g \cdot kg^{-1}$ DM)

Procedure	Cattle	Poultry	Swine
Sequential fractionation	5.80±1.06 b	13.40±2.21 a	14.85±5.75 a
$NaHCO_3$-NaOH/EDTA	6.76±1.27 b	16.26±2.12 a	16.98±6.77 a

Note: Values represent the mean of replicates±SD; there were 8 replicates for cattle, 6 (sequential fractionation) or 9 ($NaHCO_3$-NaOH/EDTA) for poultry and 13 for swine. Different letters in the same line denote significant differences at $P \leqslant 0.05$.

in manure. The slope of the linear regression indicated that each unit increase in dietary P resulted in a 2.2 unit increases of manure P. Management to reduce overfeeding in order to minimize excess dietary P and addition of feed additives, such as phytase, is necessary to reduce the P concentration in swine manure, which will reduce the risk of P losses to the environment with field application of manures[11], although the P losses is not avoided.

The mean concentration of dietary P for intensive cattle in the current study was 3.4 $g \cdot kg^{-1}$, which is similar to the recommended value[30] of 3.3 $g \cdot kg^{-1}$ for the mean milk yield (27.9 $kg \cdot d^{-1}$ per cow)[30,31]. Dou et al.[27] also reported that the range of dietary P concentration for dairy cattle was from 3.0 to > 5.0 $g \cdot kg^{-1}$ at any given sampling time on over 90 farms. However, as reported by Toor et al.[32,33] and Dou et al.[31], a significant linear correlation existed between dietary P and manure P. All studies reported that for each unit increase in dietary P ($g \cdot kg^{-1}$, DM) there was an increase in manure P of 1.0–2.1 units, which was higher than the value 0.7 found in current study. The reason may be that the dietary and manure samples in the previous studies were collected from the mature milking cows. The P intake by cows is allocated to milk production, body maintenance, urine and feces[6,27]. Normally, P used for milk production and body maintenance is almost constant at the mature stage, while the urinary P is relatively small. Excess dietary P is mainly passed in manure. However, in the current study, the dietary and manure samples were collected from both milk cows and calves. The majority of dietary P for calves is used for the body growth, and less is passed in manure, which reduced the slope of the linear regression equation.

The dietary P concentration in intensive poultry in the current study was 4.5 $g \cdot kg^{-1}$, which is in the reported range of 3.1–6.7 $g \cdot kg^{-1}$[34]. The slope of the linear regression indicated that for each unit increase in dietary P there was a 1.4 unit increases of manure P. Many reports indicate that dietary P modification in poultry, such as by adding phytase enzymes, could aid the digestion of phytate P in diets, which would be an efficient way to reduce P excretion without reducing productivity[35–37]. As shown in Fig. 2, the relative low R^2 (0.10–0.34) for the linear regression equations indicate that the total P concentration in animal manures is not only determined by dietary P, but also may be influenced by many other factors, such as the housing or penning system or animal growth stage.

The total P concentrations in intensive animal manures determined in the current study were significantly higher than those reported in China in the 1990s (i.e., 8.5–9.5 $g \cdot kg^{-1}$ in swine manure, 8.7–9.9 $g \cdot kg^{-1}$ in poultry manure and 4.1–4.5 $g \cdot kg^{-1}$ in cattle manure)[11]. This significant increase is mainly due to a change in animal production from family-based animal production (extensive) to intensive animal feeding operations with greater inputs of protein and energy in animal diets. However, there was no significant change in the dietary P for extensive cattle compared to the previous values. The similar concentration of total P in extensive cattle manure determined in the current study to the values reported in the 1990s is consistent with this explanation.

4.2 Sequential fractionation and $NaHCO_3$-NaOH/EDTA procedures

In this study, the sequential fractionation and $NaHCO_3$-NaOH/EDTA procedures were both adopted to analyze manure samples. The former method can divide manure P into five fractions, but it is a little complicated and time-consuming. The latter simplified method reduces the extraction procedures to two steps. It saves much sample preparation time prior to analysis and has been adopted widely in manure P fraction analysis.

Water-soluble P (sum of H_2O-P_t and $NaHCO_3$-P_t) in animal manure is the most vulnerable fraction losses by runoff[31]. In the current study, more than 73% of total P in intensive animal manures is water-soluble P, which demonstrates that there is an increased risk of P losses when manures are surface applied, especially in the rainy season. The relative environmental risk from high to low for water-soluble P is swine then poultry and cattle manure.

Compared to poultry and cattle manures, the highest concentration of total P and greater variation in HCl extractable P fractions (HCl-P_i, HCl-P_o and HCl-P_t) was observed in swine manure. The reason for the highest P concentration in swine manure may be that the dietary P in the feeding practice is imprecise and in excess of the actual requirements of the swine, possibly as a result of farmers being risk-adverse for reduced animal performance due to the low dietary P. As a consequence, the excess dietary P is passed in manure[38]. A large variation of HCl extractable P fractions in swine manure is probably due to the amount of feed additives, such as dicalcium phosphate, in swine diets varying with swine age and growth performance, particularly in the finishing stages. Therefore, Ca-P extracted by HCl is the primary compound in swine manure resulting from excess feed additives and contributes to the large variation in acid-soluble P fractions[9,17,39].

The reason for the lower organic P concentration in cattle manure than in swine and poultry manures may be that dairy cattle being ruminants can secrete phytases and other phosphatases from their gut to increase the hydrolysis of organic P in their diets[36,40]. In contrast, monogastric animals such as swine do not have phytase enzymes in quantities that would allow them to utilize phytate in diets as a source of P and, as a consequence, the majority of phytate P in diets is passed in manure[41]. Another reason may be that the majority of dietary P for non-ruminant animals (such as swine and poultry) is in the indigestible forms (especially phytate P)[42]. Most grains used in swine diets, such as corn, soybean and wheat, store as much as 80%–90% of the total P in the form of inositol hexa-phosphate (phytate). Swine cannot digest and absorb phytate P, so the manure P is inevitable high[36,40]. There is usually less phytate in the forage and silage for dairy cattle. Considerable variation in total P concentrations in animal manures was observed in the current study. Such variation is mainly a result of factors including feedstock composition[43–48], protein intake[49], feed additives[34,40,50], energy level, animal growth stage[51], P intake[31,50], and Ca:P ratio in diets. All of these and other physiological factors contribute simultaneously to the variation in manure P composition. In summary, the sequential fractionation procedure enabled comprehensive evaluation of manure P composition that can be used to better understand manure P dynamics and further enhance the capacity to manage manure P to reduce environmental risks.

The simplified $NaHCO_3$-NaOH/EDTA procedure developed by Turner and Leytem[15] successfully indicated the environmental risk of different types of animal manure by separating readily soluble and poorly soluble P into two convenient extracts in the current study. Compared with the HCl extract in the sequential fractionation procedure, the use of alkaline solution (NaOH/EDTA) to extract animal manure not only improved organic P recovery, but also avoided hydrolyzing some organic phosphate compounds during the extraction[15]. The means of the readily soluble P fractions extracted by $NaHCO_3$-NaOH/EDTA from intensive swine and poultry manures were higher than those in intensive cattle manure. This demonstrates that for surface application or shallow incorporation of swine and poultry manures, the runoff and leachate of soluble P fractions should be considered carefully. A much higher concentration of organic P (NaOH/EDTA-P_t) in intensive swine and poultry manures also indicates a much higher environmental risk, given that this soluble organic P is sorbed weakly in soil and is mobile in the soil profile. This is especially important as relatively small concentrations of this organic P can cause serious environmental harm[52].

5 Conclusions

In the current study, a considerable variation in the total P concentration in animal manures was detected. The mean of total P concentration of the manures determined by a sequential fractionation procedure was 14.9 $g \cdot kg^{-1}$ in intensive swine production, 13.4 $g \cdot kg^{-1}$ in intensive poultry production, 5.8 $g \cdot kg^{-1}$ in intensive cattle production and 4.4 $g \cdot kg^{-1}$ in extensive cattle production. About 73% of total P in intensive swine manure, 74% in intensive poultry manure, 79% in intensive cattle manure and 78% in extensive cattle manure was found to be bioavailable P (P_t extracted by H_2O and $NaHCO_3$). The $NaHCO_3$-NaOH/EDTA procedure indicated that the relative environmental risk from high to low was swine manure, then poultry manure and cattle manure. Taking into account the P composition in these different types of animal manure, several alternate strategies, such as diet modification and better operation of manure collection and storage are necessary to reduce nutrient losses that can negatively impact surface and ground water quality.

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Compliance with ethics guidelines Guohua Li, Qian Liu, Haigang Li, and Fusuo Zhang declare that they have no conflicts of interest or financial conflicts to disclose.

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References

1. Sharpley A N, Chapra S C, Wedepohl R, Sims J T, Daniel T C, Reddy K R. Managing agricultural phosphorus for protection of surface waters: issues and options. *Journal of Environmental Quality*, 1994, **23**(3): 437–451
2. Sims J T, Edwards A C, Schoumans O F, Simard R R. Integrating soil phosphorus testing into environmentally based agricultural management practices. *Journal of Environmental Quality*, 2000, **29**(1): 60–71
3. Sharpley A, Moyer B. Phosphorus forms in manure and compost and their release during simulated rainfall. *Journal of Environmental Quality*, 2000, **29**(5): 1462–1469
4. He Z, Griffin T S, Honeycutt C W. Phosphorus distribution in dairy manures. *Journal of Environmental Quality*, 2004, **33**(4): 1528–1534
5. Bromfield S. Sheep faeces in relation to the phosphorus cycle under pastures. *Australian Journal of Agricultural Research*, 1961, **12**(1): 111–123
6. Dou Z, Knowlton K F, Kohn R A, Wu Z, Satter L D, Zhang G, Toth J D, Ferguson J D. Phosphorus characteristics of dairy feces affected by diets. *Journal of Environmental Quality*, 2002, **31**(6): 2058–2065
7. Barnett G M. Phosphorus forms in animal manure. *Bioresource Technology*, 1994, **49**(2): 139–147
8. He Z, Honeycutt C W, Griffin T S, Cade-Menun B J, Pellechia P J, Dou Z. Phosphorus forms in conventional and organic dairy manure identified by solution and solid state p-31 NMR spectroscopy. *Journal of Environmental Quality*, 2009, **38**(5): 1909–1918
9. He Z, Zhang H, Toor G S, Dou Z, Honeycutt C W, Haggard B E, Reiter M S. Phosphorus distribution in sequentially extracted fractions of biosolids, poultry litter, and granulated products. *Soil Science*, 2010, **175**(4): 154–161
10. Gerritse R G, Zugec I. The phosphorus cycle in pig slurry measured from $^{32}PO_4$ distribution rates. *Journal of Agricultural Science*, 1977, **88**(1): 101–109
11. Li G, Li H, Leffelaar P A, Shen J, Zhang F. Characterization of phosphorus in animal manures collected from three (dairy, swine, and broiler) farms in China. *PLoS One*, 2014, **9**(7): e102698
12. Hedley M J, Stewart J W B, Chauhan B S. Changes in inorganic and organic soil phosphorus fractions induced by cultivation practices and by laboratory incubations1. *Soil Science Society of America Journal*, 1982, **46**(5): 970–976
13. Leinweber P, Haumaier L, Zech W. Sequential extractions and 31P-NMR spectroscopy of phosphorus forms in animal manures, whole soils and particle-size separates from a densely populated livestock area in northwest Germany. *Biology and Fertility of Soils*, 1997, **25**(1): 89–94
14. Dou Z, Toth J D, Galligan D T, Ramberg C F, Ferguson J D. Laboratory procedures for characterizing manure phosphorus. *Journal of Environmental Quality*, 2000, **29**(2): 508–514
15. Turner B L, Leytem A B. Phosphorus compounds in sequential extracts of animal manures: chemical speciation and a novel fractionation procedure. *Environmental Science & Technology*, 2004, **38**(22): 6101–6108
16. Ajiboye B, Akinremi O O, Racz G J. Laboratory characterization of phosphorus in fresh and oven-dried organic amendments. *Journal of Environmental Quality*, 2004, **33**(3): 1062–1069
17. He Z, Honeycutt C W, Griffin T S. Comparative investigation of sequentially extracted phosphorus fractions in a sandy loam soil and a swine manure. *Communications in Soil Science and Plant Analysis*, 2003, **34**(11–12): 1729–1742

18. Bai Z H, Ma L, Oenema O, Chen Q, Zhang F S. Nitrogen and phosphorus use efficiencies in dairy production in china. *Journal of Environmental Quality*, 2013, **42**(4): 990–1001
19. Knowlton K F, Kohn R. Feeding management to reduce phosphorus losses from dairy farms. *Proceedings of the Mid Atlantic Dairy Management Conference*, 1999: 94–108
20. Walter W G. Standard methods for the examination of water and wastewaterb (11^{th} ed). Washington D.C.: *American Public Health Association*, 1961, **51**(6): 940
21. Reinhart G A, Mahan D C. Effect of various calcium: phosphorus ratios at low and high dietary phosphorus for starter, grower and finishing swine. *Journal of Animal Science*, 1986, **63**(2): 457–466
22. Miller E R, Ullrey D E, Zutaut C L, Baltzer B V, Schmidt D A, Hoefer J A, Luecke R W. Phosphorus requirement of the baby pig. *Journal of Nutrition*, 1964, **82**(1): 34–40
23. Koch M E, Mahan D C, Corley J R. An evaluation of various biological characteristics in assessing low phosphorus intake in weanling swine. *Journal of Animal Science*, 1984, **59**(6): 1546–1556
24. McDowell L R. Minerals in animal and human nutrition. London: *Academic Press*, 1992
25. Ternouth J H. Endogenous losses of phosphorus by sheep. *Journal of Agricultural Science*, 1989, **113**(3): 291–297
26. Khorasani G R, Janzen R A, McGill W B, Kennelly J J. Site and extent of mineral absorption in lactating cows fed whole-crop cereal grain silage of alfalfa silage. *Journal of Animal Science*, 1997, **75**(1): 239–248
27. Dou Z, Ramberg C F Jr, Chapuis-Lardy L, Toth J D, Wu Z, Chase L E, Kohn R A, Knowlton K F, Ferguson J D. A fecal test for assessing phosphorus overfeeding on dairy farms: evaluation using extensive farm data. *Journal of Dairy Science*, 2010, **93**(2): 830–839
28. Crenshaw T D. Reliability of dietary Ca and P levels and bone mineral content as predictors of bone mechanical properties at various time periods in growing swine. *Journal of Nutrition*, 1986, **116**(11): 2155–2170
29. Huaitalla R M, Gallmann E, Liu X, Hartung E. Nutrients and trace elements in a pig farm in Beijing: Chinese and German recommendations. *Journal of Agricultural Science and Technology A*, 2012, **2**(2): 191–208
30. Council N R. Nutrient requirements of dairy cattle: 2001 Washington D.C.. *National Academies Press*, 2001
31. Dou Z, Ferguson J D, Fiorini J, Toth J D, Alexander S M, Chase L E, Ryan C M, Knowlton K F, Kohn R A, Peterson A B, Sims J T, Wu Z. Phosphorus feeding levels and critical control points on dairy farms. *Journal of Dairy Science*, 2003, **86**(11): 3787–3795
32. Toor G S, Cade-Menun B J, Sims J T. Establishing a linkage between phosphorus forms in dairy diets, feces, and manures. *Journal of Environmental Quality*, 2005, **34**(4): 1380–1391
33. Toor G S, Sims J T, Dou Z. Reducing phosphorus in dairy diets improves farm nutrient balances and decreases the risk of nonpoint pollution of surface and ground waters. *Agriculture, Ecosystems & Environment*, 2005, **105**(1–2): 401–411
34. Maguire R O, Dou Z, Sims J T, Brake J, Joern B C. Dietary strategies for reduced phosphorus excretion and improved water quality. *Journal of Environmental Quality*, 2005, **34**(6): 2093–2103
35. Maguire R O, Sims J T, McGrath J M, Angel C R. Effect of phytase and vitamin D metabolite (25OH-D_3) in turkey diets on phosphorus solubility in manure-amended soils. *Soil Science*, 2003, **168**(6): 421–433
36. Maguire R O, Sims J T, Saylor W W, Turner B L, Angel R, Applegate T J. Influence of phytase addition to poultry diets on phosphorus forms and solubility in litters and amended soils. *Journal of Environmental Quality*, 2004, **33**(6): 2306–2316
37. McGrath J M, Sims J T, Maguire R O, Saylor W W, Angel C R, Turner B L. Broiler diet modification and litter storage: impacts on phosphorus in litters, soils, and runoff. *Journal of Environmental Quality*, 2005, **34**(5): 1896–1909
38. Dou Z, Ramberg C F Jr, Chapuis-Lardy L, Toth J D, Wang Y, Munson R J, Wu Z, Chase L E, Kohn R A, Knowlton K F, Ferguson J D. A novel test for measuring and managing potential phosphorus loss from dairy cattle feces. *Environmental Science & Technology*, 2007, **41**(12): 4361–4366
39. Kuo S, Hummel R L, Jellum E J, Winters D. Solubility and leachability of fishwaste compost phosphorus in soilless growing media. *Journal of Environmental Quality*, 1999, **28**(1): 164–169
40. Maguire R O, Sims J T, Applegate T J. Phytase supplementation and reduced-phosphorus turkey diets reduce phosphorus loss in runoff following litter application. *Journal of Environmental Quality*, 2005, **34**(1): 359–369
41. Pointillart A, Fontaine N, Thomasset M, Jay M E. Phosphorus utilization, intestinal phosphatases and hormonal control of calcium metabolism in pigs fed phytic phosphorus: soyabean or rapeseed diets. *Nutrition Reports International*, 1985, **32**(1): 155–167
42. Poulsen H D. Phosphorus utilization and excretion in pig production. *Journal of Environmental Quality*, 2000, **29**(1): 24–27
43. Church D C. Digestive physiology and nutrition of ruminants. Volume 2. Nutrition. Corvallis, USA: *O & B Books, Inc*., 1979
44. Shaw D T, Rozeboom D W, Hill G M, Booren A M, Link J E. Impact of vitamin and mineral supplement withdrawal and wheat middling inclusion on finishing pig growth performance, fecal mineral concentration, carcass characteristics, and the nutrient content and oxidative stability of pork. *Journal of Animal Science*, 2002, **80**(11): 2920–2930
45. Miller E R, Ullrey D E, Zutaut C I, Hoefer J A, Luecke R W. Mineral balance studies with the baby pig: effects of dietary magnesium level upon calcium, phosphorus and magnesium balance. *Journal of Nutrition*, 1965, **86**(2): 209–212
46. Miller E R, Ullrey D E, Zutaut C L, Hoefer J A, Luecke R W. Mineral balance studies with the baby pig: effects of dietary vitamin D2 level upon calcium, phosphorus and magnesium balance. *Journal of Nutrition*, 1965, **85**(3): 255–259
47. Bagheri S, Guéguen L, Camus P. Effect of wheat bran and pectin on the absorption and retention of phosphorus, calcium, magnesium and zinc by the growing pig. *Reproduction, Nutrition, Development*, 1985, **25**(4A): 705–716
48. Dourmad J Y, Jondreville C. Impact of nutrition on nitrogen, phosphorus, Cu and Zn in pig manure, and on emissions of ammonia and odours. *Livestock Science*, 2007, **112**(3): 192–198
49. Braithwaite G D. Calcium and phosphorus metabolism in ruminants with special reference to parturient paresis. *Journal of Dairy Research*, 1976, **43**(3): 501–520

50. Maguire R O, Plumstead P W, Brake J. Impact of diet, moisture, location, and storage on soluble phosphorus in broiler breeder manure. *Journal of Environmental Quality*, 2006, **35**(3): 858–865
51. Peirce C J, Smernik R, McBeath T. Phosphorus availability in chicken manure is lower with increased stockpiling period, despite a larger orthophosphate content. *Plant and Soil*, 2013, **373**(1–2): 359–372
52. Turner B L, Frossard E, Baldwin D S, Turner B L, Frossard E, Baldwin D S. Organic phosphorus in the environment. Wallingford, USA: *CABI Publishing*, 2005