

Fig. 3. Soil texture classification based on the USDA textural triangle.

3.2. Salting out extraction procedure

Average recoveries obtained from the extraction of neonicotinoids in soils with various salts are shown in Table 3. Good recoveries of analytes (77.5–96.8%; RSD \leq 9.0) were obtained for all salts at the 80 $\mu\text{g kg}^{-1}$ fortification level without the need for clean-up sorbents. However analyte recoveries at the 8 $\mu\text{g kg}^{-1}$ fortification level were relatively low with higher relative standard deviations (48.6 to 87.4%; RSD \leq 17.7) especially when citrate salts were used for extraction. Better yields were obtained from both NaCl & MgSO_4 (76.5–87.4; RSD \leq 14.2) and NaOAc & MgSO_4 (64.9–86.3; RSD \leq 16.2) extracts for all analytes. The low recoveries with high relative standard deviations for analytes at the lower fortification level may have been due to a higher influence of matrix at that low concentration of analytes.

Differences in recoveries for the three salts were minimal as shown in Fig. 4 for the 80 $\mu\text{g kg}^{-1}$ level of fortification. However the differences were found to be statistically significant in almost all analytes except thiamethoxam, based on a one way analysis of variance (ANOVA) at a 95% confidence interval. Bonferroni post hoc comparisons suggest the choice of NaCl & MgSO_4 for extraction resulted in significantly higher yields in almost all analytes compared to the other two salt mixtures. The peculiarity in the results of thiamethoxam may have been due to the high variability among replicates which led to higher values of RSDs.

Besides inducing phase separation, the type of salts applied in extracting analytes under the QuEChERS procedure may impact pH, matrix polarity and matrix constitution thereby influencing recovery. Although the addition of the various salts impacted on the pH of the matrix (citrate, chloride and acetate salts in increasing order of pH), its influence on analytes as well as matrix components is unclear in the current study. It was however observed that soil pH had less of an influence on the final pH of the matrix after extraction.

In general, the important differences in the various salts used under the QuEChERS procedure are the resulting pH of the medium and the presence or absence of buffering. The original salting-out procedure employed the use of NaCl & MgSO_4 in acetonitrile (Anastassiades et al., 2003). However, low recoveries of some pH sensitive analytes prompted the need for a buffering medium. The result of the application of sodium acetate and sodium citrate in response to this need increased recoveries remarkably leading to the adoption of both as official methods (AOAC 2007.01 and EN 15662 respectively) (Lehotay et al., 2005).

The results obtained in this study however, do not suggest a need for a buffering medium for neonicotinoids in the matrix being studied. Despite the minimal differences, NaCl & MgSO_4 were chosen as optimum for extraction due to the higher yields and lower RSDs compared to the other salts. Shi et al. (2010) and Dong et al. (2009) have reported similar salt choices in acetonitrile extraction of oxadiazyl and metaflumizone respectively from soils without the need for buffering. While acetate salts have been used in some studies (Rashid et al., 2010), the use of citrate salts appears to be the most commonly reported in literature for soil matrix (Lesueur et al., 2008; Asensio-Ramos et al., 2010; Yang et al., 2010b; Qiao et al., 2011). It is quite obvious that the important considerations in the choice of a particular salting out procedure are largely based on the nature of analytes and matrix under study (Lehotay et al., 2005).

3.3. Clean-up procedure

Recovery of analytes following d-SPE ranged from 78.5 to 104.8% and 48.6 to 87.2% at 80 and 8 $\mu\text{g kg}^{-1}$ fortification levels respectively (Table 4). Yields were improved after clean-up, in almost all analytes and sorbent types particularly at the 80 $\mu\text{g kg}^{-1}$ level of fortification.

Table 3

Percentage recoveries of neonicotinoids with varying salting out extraction procedures at two fortification levels ($\mu\text{g kg}^{-1}$) (n = 6 for each treatment).

Salts	Fortification	Imidacloprid	Thiacloprid	Thiamethoxam	Acetamiprid	Clothianidin
NaOAc & MgSO_4	8	71.0 \pm 9.6	64.9 \pm 16.2	86.3 \pm 10.9	72.0 \pm 10.7	76.5 \pm 11.4
	80	83.8 \pm 3.3	80.8 \pm 2.8	84.9 \pm 6.7	92.4 \pm 2.4	88.9 \pm 1.3
NaCl & MgSO_4	8	77.0 \pm 13.8	76.3 \pm 8.9	87.4 \pm 14.2	76.5 \pm 6.0	77.3 \pm 14.0
	80	91.2 \pm 5.0	89.3 \pm 3.5	86.1 \pm 6.9	93.9 \pm 4.2	96.8 \pm 1.3
NaCitrate & MgSO_4	8	53.6 \pm 17.7	48.3 \pm 11.4	72.9 \pm 8.6	55.0 \pm 10.4	65.4 \pm 14.4
	80	85.2 \pm 2.7	77.5 \pm 5.5	90.1 \pm 9.0	86.7 \pm 3.6	90.1 \pm 8.0

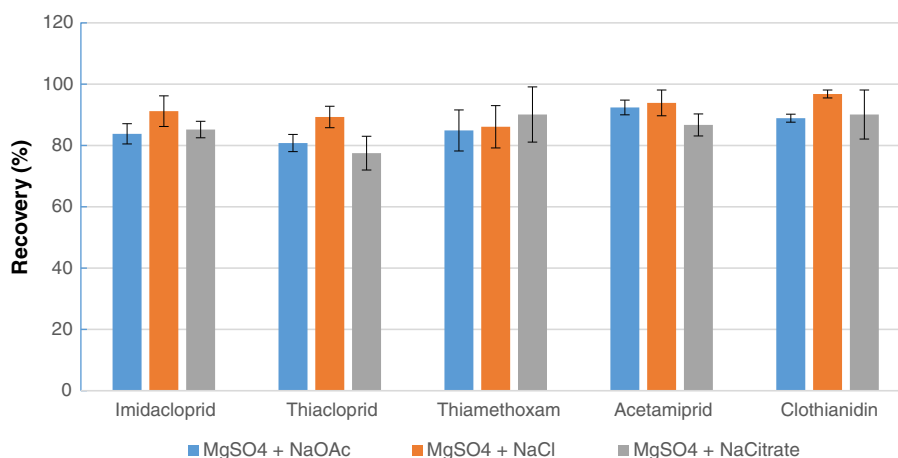


Fig. 4. Percentage recoveries of neonicotinoids showing RSDs after extraction with different salts at $80 \mu\text{g kg}^{-1}$ level of fortification ($n = 6$).

Recoveries were highest with PSA and appeared to decrease upon addition of C-18 and GCB. With the exception of thiamethoxam, analysis of variance indicated higher yields of statistical significance for all analytes upon PSA clean-up at the $80 \mu\text{g kg}^{-1}$ level of fortification. The apparent effectiveness in the use of PSA as sorbent was evident in all salt matrixes. However analytes appeared less amenable to d-SPE clean-up at the relatively low concentration of $8 \mu\text{g kg}^{-1}$ (Table 4). This may probably have been due to a relative increase in matrix influence at the low concentrations of analytes, closer to their limits of quantification as suggested in Section 3.2.

The QuEChERS procedure as originally developed by Anastassiades et al. (2003) comprises a salting out extraction followed by a d-SPE clean-up procedure. The addition of the clean-up step was important in removing most matrix co-extracts. Soil as a matrix is very complex and diverse. This complexity is further enhanced by high levels of organic matter which have been reported to decrease extraction efficiency (Bragança et al., 2012; Correia-Sá et al., 2012) often warranting the use of a clean-up procedure. PSA has been employed often in the removal of organic acids, fatty acids and sugars from diverse sample matrixes. The presence of primary and secondary amines in its structure helps in the retention of many polar compounds including sugars, organic acids and fatty acids which may have been components in the soil matrix studied. Unlike PSA, the addition of C-18 commonly used

in the removal of fats and other non-polar compounds as well as GCB often used in the removal of pigments tends to decrease the recovery of analytes suggesting perhaps the absence of these as matrix components and a possible adsorption of analytes by these sorbents.

By far the choice of sorbent in clean-up extraction of various analytes from soils has been PSA (Lesueur et al., 2008; Dong et al., 2009; Asensio-Ramos et al., 2010; Yang et al., 2010b). Reported recoveries however vary, perhaps based on analytes and matrix composition. The use of PSA & C-18 as sorbents has been reported by Drozdzyński and Kowalska with recoveries ranging from 83–104% (Drozdzyński and Kowalska, 2009). However not all studies have employed sorbents. Shi et al. (2010) have reported recoveries ranging from 82.9 to 112% for oxadiargyl residues in soils after a salting out extraction with MgSO₄ and NaCl without the need for clean-up. Similarly Caldas et al. (2011) have reported recoveries of 70.3 to 120% in the QuEChERS extraction of multiple classes of pesticides from soils without clean-up sorbents. In this study, the importance of a clean-up procedure was particularly evident at higher concentrations ($80 \mu\text{g kg}^{-1}$) of analyte and less so at lower concentrations ($8 \mu\text{g kg}^{-1}$). Although PSA was found to be the best sorbent for the clean-up of analytes in the matrix studied, the choice of a clean-up procedure will be largely based on the degree of precision required. In the current study, a clean-up procedure using PSA as sorbent was chosen for the reasons stated above.

Table 4

Percentage recoveries of neonicotinoids from varying d-SPE clean-up conditions ($n = 6$ for each treatment).

Level	Salts	Sorbent	Imidacloprid	Thiacloprid	Thiamethoxam	Acetamiprid	Clothianidin	
$80 \mu\text{g kg}^{-1}$	NaOAc & MgSO ₄	PSA	97.8 ± 2.7	92.3 ± 1.2	100.5 ± 2.5	101.2 ± 2.4	100.0 ± 5.6	
		PSA + C18	93.2 ± 3.5	89.3 ± 3.9	92.5 ± 9.3	96.7 ± 2.6	98.3 ± 1.8	
		PSA + GCB	92.8 ± 2.7	84.5 ± 2.8	87.9 ± 12.9	94.3 ± 1.6	94.0 ± 1.9	
	NaCl & MgSO ₄	PSA	98.6 ± 3.1	97.1 ± 1.9	94.0 ± 9.0	102.9 ± 1.7	104.8 ± 2.1	
		PSA + C18	96.3 ± 1.9	95.5 ± 3.5	92.4 ± 10.3	99.6 ± 3.9	98.6 ± 3.9	
		PSA + GCB	94.5 ± 1.6	91.7 ± 3.3	85.3 ± 6.9	96.5 ± 1.1	97.3 ± 4.3	
	NaCitrate & MgSO ₄	PSA	89.8 ± 5.3	81.0 ± 6.9	92.2 ± 5.5	93.4 ± 4.5	89.3 ± 6.7	
		PSA + C18	86.5 ± 3.7	80.3 ± 5.6	84.8 ± 11.7	89.6 ± 4.4	91.8 ± 2.8	
		PSA + GCB	84.7 ± 3.7	78.5 ± 5.5	87.4 ± 9.6	88.9 ± 2.9	88.4 ± 9.5	
	$8 \mu\text{g kg}^{-1}$	NaOAc & MgSO ₄	PSA	65.2 ± 13.5	63.8 ± 19.1	76.1 ± 14.1	72.0 ± 12.9	70.6 ± 13.7
			PSA + C18	67.0 ± 20.9	63.8 ± 16.5	80.2 ± 13.3	70.7 ± 13.0	74.1 ± 18.6
			PSA + GCB	60.2 ± 19.4	60.9 ± 17.2	82.3 ± 10.3	69.1 ± 12.4	68.0 ± 18.8
NaCl & MgSO ₄		PSA	79.3 ± 11.3	79.6 ± 8.7	85.8 ± 15.0	81.3 ± 4.8	72.0 ± 14.4	
		PSA + C18	78.5 ± 17.2	77.4 ± 6.8	86.4 ± 9.1	76.7 ± 7.3	69.3 ± 13.9	
		PSA + GCB	87.2 ± 7.5	74.2 ± 6.5	85.2 ± 11.0	75.3 ± 5.0	74.5 ± 13.2	
NaCitrate & MgSO ₄		PSA	58.2 ± 13.4	51.0 ± 8.8	75.2 ± 5.9	56.8 ± 8.5	60.7 ± 6.3	
		PSA + C18	62.5 ± 13.6	50.6 ± 11.1	74.6 ± 9.9	55.0 ± 9.1	63.9 ± 20.8	
		PSA + GCB	48.6 ± 17.7	48.8 ± 8.6	75.4 ± 8.4	54.3 ± 6.1	49.8 ± 21.9	

contamination, due to the extensive use and multiple application rates. In previous studies, we have demonstrated that, applied neonicotinoids (in particular imidacloprid) in cocoa farms may enter and persist in soils for several months following application (Dankyi et al., 2014). Unfortunately the fate of these insecticides in cocoa beans, which is the main product consumed, is not yet known. In the literature, much of the current knowledge on residues of neonicotinoids has been restricted to honey and honey bees due to the perceived detrimental health effects of this class of insecticides on pollinators (Blacquière et al., 2012; Jovanov et al., 2015; Laycock et al., 2012; Tanner and Czerwenka, 2011). Although some research on fruit and vegetables exist, they are mainly restricted to periodic market surveys or the application of newly developed procedures, often in multi-class pesticides analysis (Bakirci et al., 2014; Garrido Frenich et al., 2008; Gilbert-López et al., 2010; Obana et al., 2002; Wang et al., 2012; Xie et al., 2011; Zhang et al., 2012). A recent study by Chen et al. (2014) has demonstrated the widespread presence of neonicotinoid insecticide residues in foods, including fruit and vegetables (Chen et al., 2014). However knowledge in tropical foods, such as cocoa beans, and of actual sources of contamination remains low. Although neonicotinoids have been used extensively in the production of cocoa and other crops in Ghana and other West African countries for several years, their levels in food is not yet known.

Whereas some research exists on pesticide residues in cocoa beans, they have been restricted to other classes of pesticides, including organochlorines, organophosphates and carbamates, which are less readily used in cocoa production in recent years, particularly in Ghana (K.S. Frimpong et al., 2012a, 2012b; Frimpong et al., 2012; Owusu-Ansah et al., 2010). With the coming into force of market regulations seeking to limit pesticide usage in food crops, such as the new European Union (EU) Regulation 396/2005/EC on “maximum residue levels of pesticides in or on food and feed of plant and animal origin” (European Commission, 2008), the need for knowledge on pesticide levels in produce such as cocoa beans mainly produced for export to European and other markets cannot be overemphasized.

In literature, conventional methods that are widely used for the extraction of pesticides from fatty matrixes include solid-phase micro-extraction (SPME), matrix solid-phase extraction (MSPD), gel permeation chromatography (GPC) and supercritical fluid extraction (SFE) (Gilbert-López et al., 2009).

It is quite evident that the effective management of these insecticides in food from developing countries will require reliable quantitative and qualitative assessment based on simple, efficient and less expensive techniques to ensure food safety. The QuEChERS procedure does not only offer this simplicity, reliability and effectiveness but also a high flexibility for application to a wide range of analytes and matrixes (Lehotay et al., 2010).

However, the QuEChERS method was originally designed for low-fat food matrixes; application to high fat matrixes often presents a challenge due to high lipid co-extractives that may not only adversely affect extraction and chromatographic efficiency but also instrumentation (Chamkasem et al., 2013). In recent years, the method has been applied to matrixes of medium ($\approx 15\%$) to high fat ($>40\%$) content, including nuts, avocado, fish and animal foods with varying degrees of success (Chamkasem et al., 2013; Choi et al., 2015; Koesukwiwat et al., 2010; Lozano et al., 2014; Luzardo et al., 2013; Rajska et al., 2013; Sobhanzadeh et al., 2012). Lehotay et al. (2005) have observed that the QuEChERS method compares favourably with established methods such as MSPD in the analysis of polar and semi-polar pesticides from low fat food matrixes but performed poorly with nonpolar pesticides (Lehotay et al., 2005). Various modifications of the method have been performed to enhance the efficiency of QuEChERS extraction in high fat matrixes, including the use of a freeze-out step (Koesukwiwat et al., 2010),

higher solvent-sample ratios (Chamkasem et al., 2013), and zirconium sorbents (Lozano et al., 2014; Rajska et al., 2013; Sapozhnikova and Lehotay, 2013).

The challenge in the application of the QuEChERS method to the cocoa matrix is not only due to its high fat content ($>40\%$) but also its highly pigmented nature (Torres-Moreno et al., 2015). In the current study, the QuEChERS procedure was explored in the extraction and clean-up of residues of five neonicotinoid insecticides: imidacloprid, acetamiprid, thiamethoxam, clothianidin and thiacloprid in cocoa beans and shells acquired from Ghana. An optimized procedure was then used to assess the levels of residues of neonicotinoid insecticides resulting from their use in the country's cocoa production. Cocoa shells comprise the thin outer covering (husk) of the beans, which are usually removed during the processing of cocoa. In our study, the shells were analyzed separately from the de-shelled beans (cocoa nibs) to examine insecticide distribution.

2. Materials and methods

2.1. Chemicals and reagents

Insecticide standards of thiamethoxam (99.6%), clothianidin (99.9%) and imidacloprid (99.9%) as well as labelled internal standards (IS) of imidacloprid- d_4 (99.9%) and thiamethoxam- d_3 (98%) were all purchased from Sigma-Aldrich (Steinheim, Germany). Acetamiprid (98.1%) and thiacloprid (98.0%) were purchased from Dr. Ehrenstorfer (Augsburg, Germany).

Prepackaged 12-mL tubes containing one of the following: (a) 4 g of $MgSO_4$ & 1 g of NaCl; (b) 6 g of $MgSO_4$ & 1.5 g of sodium acetate (NaOAc); (c) 4 g $MgSO_4$, 1 g of NaCl, 0.5 g sodium citrate dibasic sesquihydrate (SCDS), 1 g of sodium citrate tribasic dehydrate (SCTD) were purchased from Supel QuE product lines, Sigma-Aldrich. Dispersive solid phase clean-up sorbent tubes (2 mL) containing 150 mg of $MgSO_4$ together with one of the following sorbents: (a) 50 mg PSA only; (b) 50 mg PSA & 50 mg C-18; (c) 50 mg PSA, 50 mg C-18 & 50 mg GCB; (d) 50 mg Z-Sep+ were all purchased from Sigma-Aldrich. Sodium hydrogen carbonate ($NaHCO_3$) and ammonium acetate (NH_4Ac) were obtained from Merck (Darmstadt, Germany). HPLC-grade acetonitrile and glacial acetic acid were obtained from Rathburn (Walkerburn, Scotland) and VWR (Fontenay-sous-Bois, France), respectively. Deionized water was prepared using a Merck Millipore Milli-Q advantage A10 ultrapure water purification system (Darmstadt, Germany).

2.2. LC-MS/MS instrumentation

Chromatographic separation of analytes was performed on an Agilent 1260 Infinity HPLC system (Santa Clara, CA) equipped with a BDS Hypersil reversed-phase C-18 column (250 mm \times 2.1 mm; 5 μm ; Thermo Electron Co., Waltham, MA) at a temperature of 30 °C. Mobile phases **A** and **B** consisted of 5 mM ammonium acetate and 95% acetonitrile in 5 mM ammonium acetate, respectively. The gradient was run at 300 $\mu L min^{-1}$ for 16 min as follows: 10% **B** increased linearly to 100% in 8 min; held constant for 2 min; decreased back to 10% **B** in 1 min; and maintained for an equilibration time of 5 min. The injection volume was 5 μL . The mass spectrometer used was a 3200 QTRAP (AB Sciex, Foster City, CA) equipped with electrospray ionization (ESI). The MS determination of all analytes was performed in positive mode with multiple reaction monitoring (MRM) of the two most intense precursor-product ion transitions for each analyte, one used for quantification and the other for confirmation. The source parameters employed were: curtain gas (CUR) of 20 psi, collision gas (CAD) of medium pressure, ion spray voltage of 4500 V, source

A.3 Papers Accepted for conferences:

1. Neonicotinoid insecticide residues in soils and cocoa beans following multiple applications in cocoa farming
2. Neonicotinoid application in Ghana's mass cocoa spraying exercise- Implications for research and education on policy and environmental sustainability.


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 45th World Chemistry Congress August 9-14, 2015
 BEXCO, Busan, Korea
www.iupac2015.org


Neonicotinoid insecticide residues in soils and cocoa beans following multiple applications in cocoa farming

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According to the international cocoa organization (ICCO), up to 40% of global annual cocoa production is lost to insect pests and diseases. In Ghana, the second largest producer of cocoa beans, pests and diseases account for significant losses in yields. To curtail this decline, the government of Ghana through the Ghana Cocoa Board introduced the Cocoa Diseases and Pests Control (CODAPEC) program geared towards the mass application of insecticides on cocoa farms across the country at no financial cost to farmers. Neonicotinoids (particularly imidacloprid) are the most widely used class of insecticides in cocoa production and are extensively applied under the program. Notwithstanding the increasing yields in cocoa production as a result of the free insecticide application program, the tendency for build-up of insecticides in the environment and in crops has been heightened. In our study, we examined the extent of environmental and food contamination by assessing the concentration and distribution of neonicotinoid insecticides in soils and in cocoa beans. The QuEChERS procedure was employed in both analysis in soils and in cocoa beans due to its flexibility and adaptability. Analytes were quantified using LC-MS/MS. Our findings suggest that, neonicotinoid insecticides may persist in soils for several months after application. In cocoa beans, neonicotinoids were found to selectively accumulate in shells to relatively high concentrations.

1. Dankyi E, Gordon C, Carboo D, Fomsgaard I S (2014). Quantification of neonicotinoid insecticide residues in soils from cocoa plantations using a QuEChERS extraction procedure and LC-MS/MS. *Sci Total Environ.* 499: 276-283
2. ICCO (2013). Regional workshop on integrated management of cocoa pests and pathogens in Africa: Controlling indigenous pests and diseases and preventing the introduction of exogenous ones; Accra, Ghana.

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**Neonicotinoid application in Ghana's mass cocoa spraying exercise
- Implication for research and education on policy and
environmental sustainability**

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Based on projections by the international cocoa organization (ICCO), pests and diseases account for about 40% of yields losses in cocoa production worldwide. Yield losses are similarly high in Ghana, the second largest cocoa producer. Due in parts to the importance of the cocoa sector to the economy of the country as well as the commitment to ensure the use of only approved pesticides, the government of Ghana introduced the cocoa mass spraying exercise over a decade ago to address the decline from pests. Under the program, pesticides are applied in cocoa farms, about four times each year, at virtually no financial cost to farmers. Neonicotinoids, currently banned in Europe due to reported detrimental effects on bee health, are perhaps the most widely used class of insecticides under the mass spraying program and has contributed to significant increases in yields among other interventions. However the scale, volume and intensity of application have invariably increased the possibility of exposure of large quantities of these chemicals in the environment. In our study, we assessed the extent of exposure of neonicotinoid insecticides in soils from cocoa farms across Ghana. Analytes were quantified by means of LC-tandem MS after extraction using the QuEChERS procedure. Our results suggest the occurrence of neonicotinoids in soils several months after application, with concentrations above 4 µg/Kg in more than 50% of samples studied. Based on our findings with its possible implications for persistence and potential mobility, as well as systemic activity and application rates of neonicotinoids in the Ghanaian environment, we suggest a critical role for research and education in ensuring environmental sustainability in pesticide application in Ghana.

Appendix B.1

Table B.1 Soil properties (soil organic carbon, pH and texture classification) of cocoa growing soils

Sample	SOC (%)	Clay (%)	Sand (%)	Silt (%)	pH (CaCl ₂)	Texture class
ADU	2.33	17.88	69.34	12.77	6.27	sandy-loam
ADW	2.52	17.91	61.62	20.47	6.03	sandy-loam
AGA	1.89	27.81	27.69	44.49	5.12	silt-loam
AGO	1.69	15.73	63.3	20.97	6.34	sandy-loam
AKY	1.89	12.6	69.76	17.64	5.1	sandy-loam
ANT	1.43	31.14	29.92	38.93	6.63	clay-loam
ANY	3.62	13.6	64.44	21.88	6.05	sandy-loam
ASA	3.57	18.99	32.16	48.85	5.69	loam
ASB	1.89	12.89	74.22	12.89	6.18	loam
ASF	3.34	16.79	66.41	16.79	5.56	sandy-loam
ASI	4.21	12.69	36.65	50.76	6.37	silt-loam
ASK	2.32	12.6	72.28	15.12	6.15	sandy-loam
ASM	4.24	20.26	44.29	50.76	7.54	loam
ASR	2.54	21.38	54.18	24.43	6.78	sandy-clay-loam
BEK	5.68	16.96	43.47	39.57	7.37	loam
BRE	2.32	37.41	26.06	34.53	6.76	clay-loam
DOR	3.56	49.51	29.71	33.99	7.53	clay
DZA	3.57	15.59	41.41	40.11	5.68	loam
ASS	1.63	23.68	57.91	28.94	4.97	sandy-clay-loam
GOA	2.7	16.76	59.30	23.94	6.67	sandy-loam
HWI	4.43	18.00	60.00	22.00	6.00	sandy-loam
JAS	1.89	22.76	44.36	32.88	5.37	loam
KAD	2.67	25.92	55.94	18.14	6.61	sandy-clay-loam
KAJ	2.88	26.69	46.62	26.69	5.67	sandy-clay-loam
KAS	2.48	16.77	52.46	30.76	5.77	loam
KON	2.68	15.89	54.98	52.99	5.77	sandy-loam
KPA	3.62	20.21	48.01	31.77	5.49	loam
LEA	4.72	12.60	69.76	17.64	5.87	sandy-loam
LEK	1.55	20.00	64.00	12.00	5.97	sandy-loam
MAN	3.92	17.3	45.62	37.07	5.58	loam
MEE	2.72	12.6	69.76	17.64	6.37	sandy-loam
MEH	5.56	16.07	46.42	37.5	7.46	Loam
MIF	2.56	20.04	31.29	48.67	7.47	Loam
MIM	3.87	33.24	31.72	42.02	7.44	clay-loam
ASU	2.79	12.35	47.51	40.14	6.2	Loam
NKA	4.46	29.93	50.12	19.95	6.94	sandy-clay-loam

Table B.1 Soil properties (soil organic carbon, pH and texture classification) of cocoa growing soils continued.

Sample	SOC (%)	Clay (%)	Sand (%)	Silt (%)	pH (CaCl ₂)	Texture class
NKE	3.52	21.42	67.89	10.7	6.9	sandy-clay-loam
NKI	2.33	13.97	58.10	27.93	6.73	sandy-loam
NKR	1.98	20.39	38.84	40.77	7.17	Loam
NKW	2.59	26.84	38.27	34.89	6.28	loam
NSO	7.43	15.6	81.8	2.6	4.88	sandy-loam
OBU	2.22	15.34	43.72	40.93	6.83	loam
ODA	1.89	21.8	70.02	8.17	4.62	sandy-clay-loam
NED	2.11	20.23	50.87	28.9	5.45	sandy-clay-loam
OFF	2.56	21.1	49.35	29.54	6.84	loam
OFO	1.51	24.83	67.72	7.45	5.79	sandy-clay-loam
SAE	3.79	26.11	55.61	18.28	7.21	sandy-clay-loam
SAN	2.83	27.19	33.25	39.56	6.56	clay-loam
SUN	2.79	19.42	27.86	52.72	6.48	silt-loam
TAF	3.13	24.12	68.64	7.23	4.46	sandy-clay-loam
TEP	1.89	20.16	72.26	7.58	4.62	sandy-clay-loam
TWH	2.48	15.14	69.72	15.14	6.16	sandy-loam
TWP	1.28	15.43	69.14	15.43	6.62	sandy-loam
AVERAGE	2.94	20.41	52.94	27.80	6.20	

Appendix B.2 Analysis of Variance

EFFECT OF EXTRACTION SALTS

. bysort pesticide: oneway recovery salts, bonferroni tabulate

-> pesticide = imida

Salts	Summary of Recovery		
	Mean	Std. Dev.	Freq.
acetate	83.791667	2.8697416	6
unbuffere	91.25	4.7355042	6
citrate	85.166667	2.148643	6
Total	86.736111	4.6363908	18

Source	Analysis of Variance			F	Prob > F
	SS	df	MS		
Between groups	189.048611	2	94.5243056	8.04	0.0042
Within groups	176.385417	15	11.7590278		
Total	365.434028	17	21.4961193		

Bartlett's test for equal variances: $\chi^2(2) = 2.9644$ Prob> $\chi^2 = 0.227$

Comparison of Recovery by Salts (Bonferroni)		
Row Mean- Col Mean	acetate	unbuffer
unbuffer	7.45833 0.006	
citrate	1.375 1.000	-6.08333 0.023

-> pesticide = thiac

Salts	Summary of Recovery		
	Mean	Std. Dev.	Freq.
acetate	80.791667	2.4208297	6
unbuffere	89.291667	3.171816	6
citrate	77.5	3.9749214	6
Total	82.527778	5.955033	18

Source	Analysis of Variance			F	Prob > F
	SS	df	MS		
Between groups	444.256944	2	222.128472	21.01	0.0000
Within groups	158.604167	15	10.5736111		
Total	602.861111	17	35.4624183		

Bartlett's test for equal variances: $\chi^2(2) = 1.0940$ Prob> $\chi^2 = 0.579$

Row Mean- Col Mean	Comparison of Recovery by Salts (Bonferroni)	
	acetate	unbuffer
unbuffer	8.5 0.001	
citrate	-3.29167 0.300	-11.7917 0.000

-> pesticide = thiam

Salts	Summary of Recovery		
	Mean	Std. Dev.	Freq.
acetate	84.916667	17.14181	6
unbuffere	86.083333	5.9595861	6
citrate	90.083333	7.3223402	6
Total	87.027778	10.854617	18

Source	Analysis of Variance			F	Prob > F
	SS	df	MS		
Between groups	88.1111111	2	44.0555556	0.35	0.7136
Within groups	1914.875	15	127.658333		
Total	2002.98611	17	117.822712		

Bartlett's test for equal variances: $\chi^2(2) = 6.0299$ Prob> $\chi^2 = 0.049$

Comparison of Recovery by Salts
(Bonferroni)

Row Mean- Col Mean	acetate	unbuffer
unbuffer	1.16667 1.000	
citrate	5.16667 1.000	4 1.000

-> pesticide = aceta

Salts	Summary of Recovery		
	Mean	Std. Dev.	Freq.
acetate	92.375	2.2513885	6
unbuffere	93.875	3.1809983	6
citrate	86.666667	2.8838631	6
Total	90.972222	4.1381376	18

Source	Analysis of Variance			F	Prob > F
	SS	df	MS		
Between groups	173.590278	2	86.7951389	11.08	0.0011
Within groups	117.520833	15	7.83472222		
Total	291.111111	17	17.124183		

Bartlett's test for equal variances: $\chi^2(2) = 0.5508$ Prob> $\chi^2 = 0.759$

Comparison of Recovery by Salts
(Bonferroni)

Row Mean- Col Mean	acetate	unbuffer
unbuffer	1.5 1.000	
citrate	-5.70833 0.009	-7.20833 0.001

-> pesticide = clothi

Salts	Summary of Recovery		
	Mean	Std. Dev.	Freq.
acetate	88.875	3.8103478	6
unbuffered	96.791667	1.4354152	6
citrate	90.166667	6.4858821	6
Total	91.944444	5.4755845	18

Source	Analysis of Variance			F	Prob > F
	SS	df	MS		
Between groups	216.465278	2	108.232639	5.54	0.0158
Within groups	293.229167	15	19.5486111		
Total	509.694444	17	29.9820261		

Bartlett's test for equal variances: $\chi^2(2) = 8.1786$ Prob> $\chi^2 = 0.017$

Comparison of Recovery by Salts (Bonferroni)		
Row Mean- Col Mean	acetate	unbuffered
unbuffered	7.91667 0.022	
citrate	1.29167 1.000	-6.625 0.061

EFFECT OF D-SPE CLEAN-UP SORBENTS

name: <unnamed>
 log: C:\Users\Eric\Dropbox\Enok_Eric\e1.smcl
 log type: smcl
 opened on: 6 Jun 2014, 22:42:39

. bysort pesticide: oneway recovery sorbent, bonferroni scheffe sidak tabulate

-> pesticide = Imidac

Sorbent	Summary of Recovery		Freq.
	Mean	Std. Dev.	
none	91.25	4.7355042	6
psa	98.625	3.204489	6
c-18	96.333333	1.921371	6
gcb	94.5	1.5652476	6
Total	95.177083	4.0043992	24

Source	Analysis of Variance			F	Prob > F
	SS	df	MS		
Between groups	174.632812	3	58.2109375	6.00	0.0044
Within groups	194.177083	20	9.70885417		
Total	368.809896	23	16.0352129		

Bartlett's test for equal variances: $\chi^2(3) = 6.6956$ Prob> $\chi^2 = 0.082$

**Comparison of Recovery by Sorbent
(Bonferroni)**

Row Mean- Col Mean	none	psa	c-18
psa	7.375 0.003		
c-18	5.08333 0.063	-2.29167 1.000	
gcb	3.25 0.515	-4.125 0.197	-1.83333 1.000

Comparison of Recovery by Sorbent
(Scheffe)

Row Mean- Col Mean	none	psa	c-18
psa	7.375 0.006		
c-18	5.08333 0.076	-2.29167 0.660	
gcb	3.25 0.377	-4.125 0.189	-1.83333 0.792

Comparison of Recovery by Sorbent
(Sidak)

Row Mean- Col Mean	none	psa	c-18
psa	7.375 0.003		
c-18	5.08333 0.061	-2.29167 0.770	
gcb	3.25 0.417	-4.125 0.181	-1.83333 0.901

-> pesticide = Thiaclo

Sorbent	Summary of Recovery		Freq.
	Mean	Std. Dev.	
none	89.291667	3.171816	6
psa	97.125	1.9348773	6
c-18	95.541667	3.3668111	6
gcb	91.666667	3.0808549	6
Total	93.40625	4.1870741	24

Source	Analysis of Variance			F	Prob > F
	SS	df	MS		
Between groups	230.070313	3	76.6901042	8.86	0.0006
Within groups	173.15625	20	8.6578125		
Total	403.226563	23	17.5315897		

Bartlett's test for equal variances: $\chi^2(3) = 1.5083$ Prob> $\chi^2 = 0.680$

Comparison of Recovery by Sorbent
(Bonferroni)

Row Mean- Col Mean	none	psa	c-18
psa	7.83333 0.001		
c-18	6.25 0.009	-1.58333 1.000	
gcb	2.375 1.000	-5.45833 0.026	-3.875 0.202

Comparison of Recovery by Sorbent
(Scheffe)

Row Mean- Col Mean	none	psa	c-18
psa	7.83333 0.002		
c-18	6.25 0.014	-1.58333 0.832	
gcb	2.375 0.591	-5.45833 0.036	-3.875 0.192

Comparison of Recovery by Sorbent
(Sidak)

Row Mean- Col Mean	none	psa	c-18
psa	7.83333 0.001		
c-18	6.25 0.009	-1.58333 0.933	
gcb	2.375 0.690	-5.45833 0.026	-3.875 0.186

-> pesticide = Thiame

Sorbent	Summary of Recovery		
	Mean	Std. Dev.	Freq.
none	86.083333	5.9595861	6
psa	93.958333	8.5738216	6
c-18	92.375	9.5691562	6
gcb	85.291667	6.0444534	6
Total	89.427083	8.1579113	24

Source	Analysis of Variance				
	SS	df	MS	F	Prob > F
Between groups	345.028646	3	115.009549	1.94	0.1557
Within groups	1185.65625	20	59.2828125		
Total	1530.6849	23	66.5515172		

Bartlett's test for equal variances: $\chi^2(3) = 1.5991$ Prob> $\chi^2 = 0.660$

Comparison of Recovery by Sorbent
(Bonferroni)

Row Mean- Col Mean	none	psa	c-18
psa	7.875 0.550		
c-18	6.29167 1.000	-1.58333 1.000	
gcb	-.791667 1.000	-8.66667 0.392	-7.08333 0.760

Comparison of Recovery by Sorbent
(Scheffe)

Row Mean- Col Mean	none	psa	c-18
psa	7.875 0.394		
c-18	6.29167 0.582	-1.58333 0.988	
gcb	-.791667 0.998	-8.66667 0.313	-7.08333 0.485

Comparison of Recovery by Sorbent
(Sidak)

Row Mean- Col Mean	none	psa	c-18
psa	7.875 0.439		
c-18	6.29167 0.679	-1.58333 1.000	
gcb	-.791667 1.000	-8.66667 0.333	-7.08333 0.557

-> pesticide = Acetam

Sorbent	Summary of Recovery		
	Mean	Std. Dev.	Freq.
none	93.875	3.1809983	6
psa	102.875	1.8957189	6
c-18	99.583333	4.0331956	6
gcb	96.458333	1.2289901	6
Total	98.197917	4.326347	24

Source	Analysis of Variance				F	Prob > F
	SS	df	MS			
Between groups	273.049479	3	91.0164931	11.56	0.0001	
Within groups	157.447917	20	7.87239583			
Total	430.497396	23	18.7172781			

Bartlett's test for equal variances: $\chi^2(3) = 6.7309$ Prob> $\chi^2 = 0.081$

Comparison of Recovery by Sorbent (Bonferroni)				
Row Mean- Col Mean	none	psa	c-18	
psa	9 0.000			
c-18	5.70833 0.013	-3.29167 0.334		
gcb	2.58333 0.759	-6.41667 0.005	-3.125 0.408	

Comparison of Recovery by Sorbent
(Scheffe)

Row Mean- Col Mean	none	psa	c-18
psa	9 0.000		
c-18	5.70833 0.020	-3.29167 0.279	
gcb	2.58333 0.484	-6.41667 0.008	-3.125 0.321

Comparison of Recovery by Sorbent
(Sidak)

Row Mean- Col Mean	none	psa	c-18
psa	9 0.000		
c-18	5.70833 0.013	-3.29167 0.291	
gcb	2.58333 0.556	-6.41667 0.005	-3.125 0.345

-> pesticide = Clothian

Sorbent	Summary of Recovery		Freq.
	Mean	Std. Dev.	
none	96.791667	1.4354152	6
psa	104.79167	2.2990034	6
c-18	98.625	3.8818488	6
gcb	97.333333	4.3001938	6
Total	99.385417	4.4183284	24

Source	Analysis of Variance			F	Prob > F
	SS	df	MS		
Between groups	244.466146	3	81.4887153	7.97	0.0011
Within groups	204.53125	20	10.2265625		
Total	448.997396	23	19.5216259		

Bartlett's test for equal variances: $\chi^2(3) = 5.9176$ Prob> $\chi^2 = 0.116$

Comparison of Recovery by Sorbent
(Bonferroni)

Row Mean- Col Mean	none	psa	c-18
psa	8 0.002		
c-18	1.83333 1.000	-6.16667 0.020	
gcb	.541667 1.000	-7.45833 0.004	-1.29167 1.000

Comparison of Recovery by Sorbent
(Scheffe)

Row Mean- Col Mean	none	psa	c-18
psa	8 0.004		
c-18	1.83333 0.805	-6.16667 0.028	
gcb	.541667 0.993	-7.45833 0.007	-1.29167 0.920

Comparison of Recovery by Sorbent
(Sidak)

Row Mean- Col Mean	none	psa	c-18
psa	8 0.002		
c-18	1.83333 0.912	-6.16667 0.019	
gcb	.541667 1.000	-7.45833 0.004	-1.29167 0.983