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ORIGINAL RESEARCH

Agro-environmental assessment of recycling abattoir blood meal powder as an organic fertilizer using soil quality index and hazard quotient

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Abstract

Purpose This study assessed the fertilizing potential and environmental impacts of recycling the blood meal powder (BMP) produced in the abattoir as an organic fertilizer in agriculture.

Method In this study, a 70-day laboratory incubation experiment was conducted using a clayey calcareous soil to study the effects of adding abattoir BMP at three rates (1.5, 3.0, and 6.0 ton ha^{-1}). At the end of the incubation period, the pH value, total C and N, inorganic N, and availability of macro- and micronutrient minerals (P, K, Cu, Fe, Mn, Ni, and Zn) were measured. Soil basal respiration, substrate-induced respiration, the abundance of culturable bacteria, fungi, and *azotobacter*, and dehydrogenase, alkaline and acid phosphomonoesterase, cellulase, invertase, protease, and urease enzymes activities were also determined as biochemical indicators of soil fertility.

Results The results showed that the BMP has potential as fertilizer because it increased C, N, P, and Zn as compared to the control soil. Furthermore, the abundance of culturable microorganisms and dehydrogenase activity increased in the amended soil, whereas the other soil enzyme activities and basal respiration did not show an increase. The calculation of the Hazard Quotient (HQ) and the soil quality index (SQI) indicated that 3.0-ton BMP ha^{-1} is an appropriate treatment to improve soil quality without environmental hazards.

Conclusion The results indicate that abattoir BMP application increased the fertility status of calcareous soil without environmental threats.

Keywords Blood meal powder, Enzyme activities, Fertilizing potential, Soil nutrients, Soil quality

Introduction

Each year approximately 40 Mt of organic waste is produced worldwide (Kumar et al. 2013). Proper management and maximization of recycling of such large amounts of waste to prevent adverse effects on human health and the environment are one of the biggest challenges facing the world (Zamotaev et al. 2018). Abattoirs aim at optimizing the recovery of edible meat parts for human consumption, but slaughtering operations generate a large amount of waste organic materials rich in proteins and minerals (Steffen and Kirsten 1989). From the perspective of the circular economy and of the sustainable management of organic waste, the best option for organic wastes is their recycling in agriculture as fertilizers, which can at the same time reduce the use of raw materials and close the natural nutrient cycles in the agro-ecosystems (Mondini et al. 2008; Londhe and Bhosale 2015). Blood is among the most abundant by-products of abattoirs. Fresh blood has a protein content of up to 18%

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(Ockerman and Hansen 2000), it is generally dried and bulked into blood powder by various thermo-chemical processes, and used in animal feed (Aniebo et al. 2009). Blood meal can contain up to 80% crude protein, and on average 13% N, 1.0% P, 0.6% K, 0.2% Fe, 160 mg kg^{-1} Mg, and 100 mg kg^{-1} Ca (Kikafunda and Sserumaga 2005). Yunta et al. (2013) reported that blood meal by providing an N source together with the capability to keep the Fe bound to porphyrin compounds makes it a good candidate to be used as Fe fertilizer in organic farming. Ciavatta et al. (1997) reported that blood meal incubated into the soil for one year increased total and mineral N, the Fe availability, and the soil organic matter humification degree. In a comparative study between straw and cotton residues, and meat bone meal and blood meal, it was found that the animal residues induced more CO_2-C evolution and N mineralization, whereas crop residues caused N immobilization (Cayuela et al. 2009). In a comparative study testing six conventional N fertilizers and blood meal, Hirzel et al. (2018) reported that conventional fertilizers affected soil pH value, and the availability of N, P, K, Ca, Mg, and S. In contrast blood meal had the least impact on the soil physico-chemical properties. Najjari and Ghasemi (2018) showed that the 5% and 10% (w/w) addition of powdered blood meal to sawdust vermicompost significantly accelerated its maturation and stabilization, and enrich the compost in N, P, K, Fe, Zn, and Mn. Though blood and other rendered animal residues (e.g. meat and bone meals) have been utilized as animal feed for a long time, there is still a concern in the public and regulating agencies that their use may lead to environmental contamination with the pathogenic bacteria. For example, Kinley et al. (2010) analyzed a large number of animal-based products, including blood meals, and reported that more than 80% contained *Enterococcus* spp., less than 10% were positive for Salmonella spp., mainly due to the post-processing contamination, but no *E. coli* were detected in the finished products. The results indicate that sterilization processes of animal tissues are generally effective in readily inactivating or destroying potential pathogens of human and animal health relevance. Although the blood meal's high fertilizing potential is well established, knowledge of its impacts on soil quality (SQ) and biological activities is still limited. The SQ is the capacity of soil to function within ecosystems to support primary production, maintain environmental quality, and promote plant and animal health (Doran and Parkin 1994). Organic amendments influence the soil environment through positive effects on soil physical, chemical, and biological properties, responsible for SQ, and the magnitude of such changes can be assessed by the soil quality index (SQI) (Qiu et al. 2019). The SQI can be calculated by a minimum data set (MDS) of relevant indicators (Andrews and Carroll 2001; Muñoz-Rojas et al. 2016), which can be selected by principal components analysis (PCA) (Andrews et al. 2002).

The livestock industrial abattoir of Sanandaj (Kurdistan Province, Iran) produces about 1 t blood meal powder (BMP) per month, which is mainly recycled locally as organic fertilizer. In blood meal, nutrients are mainly in organic forms, and information on the main mechanisms and timing of the release of the different nutrients is still scarce. This study aimed to analyze the short-term release of nutrients in soil amended with BMP to improve its fertilizing value. In the present work, we illustrate an approach for selecting an MDS of soil parameters using PCA to calculate SQI. We tested this approach by analyzing soil after the amendment with BMP. We also calculated the Hazard Quotient (HQ) to assess the BMP impact on soil quality and also the potential environmental risks related to the use of BMP as fertilizer.

Materials and methods

Site description and soil and blood powder sampling method and preparation

The BMP was obtained by the industrial livestock abattoir of Sanandaj (Kurdistan Province, Iran). The area of this abattoir is 7150 m^2, and it has the capacity to slaughter 1000 light and 100 heavy livestock per day and produces about 1 t blood powder per month. For the present experiments, according to the soil sampling recommendations (Gholami et al. 2020), a composite sample of 5 kg BMP was prepared with five subsamples of 1 kg each. The soil used for the incubation experiment in this study was sampled from a rainfed conventional wheat farm located in Saral Agricultural Research Station, Kurdistan Province, as a composite sample of 10 kg (with five sub-samples each 2 kg) from a 0-25 cm depth. The climate of the area is semi-arid, with the mean annual temperature and precipitation 10.0 °C and 330.6 mm, respectively. In the laboratory, soil, and BMP were sieved at 2.0 mm before analysis, and their main soil physico-chemical properties are reported in Table 1.

Table 1 Values of sand, silt, clay, pH, equivalent calcium carbonate (ECC), and the total concentration of organic carbon and nitrogen and available concentration of K, Fe, Cu, Mn, and Zn of soil used in the study[1]

Properties	**Value**
Sand (%)	43.30±0.46
Silt (%)	7.01±0.65
Clay (%)	49.70±0.23
pH	7.76±0.01
Equivalent calcium carbonate (ECC) (%)	13.1±3.07
Organic carbon (%)	0.81±0.02
Total nitrogen (%)	0.16±0.01
Available Fe (mg kg^{-1})	2.71±0.12
Available Cu (mg kg^{-1})	2.78±0.07
Available Mn (mg kg^{-1})	14.30±0.67
Available Zn (mg kg^{-1})	0.34±0.05
Available K (mg kg^{-1})	135.00±2.05

[1]Values are means of three replicates ± the standard error.

Experimental setup and BMP and soil samples analyses

To perform the test, 1 kg of air-dried soil (≤ 2 mm) was poured into clean plastic containers (15 x 10 x 8 cm). Then the BMP was mixed evenly with the soil samples at the rate of 0.0, 1.5, 3.0, and 6.0 ton ha^{-1} in the randomized complete block with three replications per treatment. To calculate the rates of BMP for the tested soil (1 kg), the weight (2750 ton ha^{-1}) of 1 ha of used soil with a bulk density of 1.1 g cm^{-3} and 25 cm depth was calculated. Thus, 0.54, 1.09, and 2.18 g of BMP per 1 kg soil were obtained to achieve 1.5, 3.0, and 6.0 ton BMP ha^{-1}, respectively. The amended soils were brought to 70% field capacity moisture content with sterile deionized water and incubated at 28 °C for 70 days. Soil water content was adjusted daily to the corresponding soil moisture level if needed by adding sterile deionized water. At the end of the incubation period, the soil of each replicate was divided into two parts: one soil sub-samples was used fresh for the biological analyses, and the other sub-samples were air-dried at room temperature for the analysis of chemical properties. The soil particle-size distribution was determined using the hydrometer method, as reported by Gee and Bauder (1986). The pH value was measured in a 1:2 soil:water (w:v) and 1:10 BMP:water (w:v) ratio using a pH meter (Metrohm Pty Ltd., Herisau, Switzerland). The soil equivalent calcium carbonate (ECC) was measured by back titration with acid (Loeppert and Suarez 1996). Soil total organic carbon was measured by the Walkley and Black (1934) method, whereas the total organic matter content of the blood powder was determined by loss on ignition in muffle (Nelson and Sommers 1996). The total organic carbon content was calculated as TOC% = TOM% × 0.58 (Broadbent 1953). Total N in both soil and BMP was determined by the Kjeldahl method (Bremner 1960), and the soil NO_3^- and NH_4^+ concentrations were quantified by steam distillation of the

2M KCl extract using Devarda's alloy and MgO as catalysts (Bremner and Keeney 1966). The soil available P was extracted by 0.5M $NaHCO_3$ buffered at pH 8.5, and quantified by a spectrophotometer (Cary 50, Varian Australia Pty Ltd. Mulgrave, Victoria), according to Murphy and Riley (1962). The soil available K was extracted by 1M NH_4COOH buffered at pH 7.0, whereas soil available Fe, Mn, Zn, Cu, and Ni were extracted by ammonium-bicarbonate diethylene triaminepentaacetic acid (AB-DTPA). Blood powder was analyzed for K, Fe, Zn, Cu, Cd, Pb, Mn, and Ni total concentrations using the dry ashing method (Jones 2001). The K concentration was determined by a flame photometer (Model BWB-1, Technology, UK Ltd.), whereas Fe, Zn, Cu, Cd, Pb, Mn, and Ni concentrations were determined by atomic absorption spectrophotometry (Varian SpectrAA 220, Varian Australia Pty Ltd. Mulgrave, Victoria).

Soil enzyme activities and analysis of culturable microbial community

Concerning soil biological properties, the cellulase and invertase activities were determined according to the method of Schinner and von Mersi (1990), the alkaline and acid phosphomonoesterase activities were determined by the method of Tabatabai and Bremner (1969), the urease activity was determined according to Tabatabai and Bremner (1972), the dehydrogenase activity was determined by the method of Thalmann (1968), and the protease activity was determined by the method of Ladd and Butler (1972). The total abundance of culturable bacteria, fungi, and *azotobacter* was determined using the plate count method by inoculating the appropriate serial dilutions on nutrient agar (NA), potato dextrose agar (PDA), and LG media, respectively (Alef and Nannipieri 1995). Soil basal respiration and substrate-induced respiration were determined by titration of alkali against HCl (Jaggi 1976). The substrate-induced respiration was induced by 50 g soil amendment with 0.25g of dry powdered glucose.

Hazard quotient (HQ)

The hazard quotient (HQ) was calculated by the ratio of the concentration of each parameter to the corresponding reference concentration (RfC) (Sharifi and Renella 2015; Ahadi et al. 2020), according to the guidelines set by the Canadian Council of Ministers of the Environment (CCME 2005) for compost category A (for agricultural proposes), using the Eq. 1:

$$HQ = \frac{Fraction\ concentration}{Rfc} \quad (1)$$

For HQ > 1.0, adverse environmental effects are expected to occur for each selected parameter. The American screening values for soils mainly correspond to a risk level of 10^{-6} for carcinogenic substances and HQ = 1 for noncarcinogenic ones (Semenkov and Koroleva 2022).

Determination of the minimum data set (MDS) of soil parameters

The total dataset comprised the following soil properties: pH, P, K, total N, NH_4^+, NO_3^-, total organic carbon, Ni, Cu, Zn, Fe, Mn, basal respiration, substrate-induced respiration, invertase, urease, cellulase, dehydrogenase, protease, alkaline, and acid phosphomonoesterase activities, and total abundance of culturable bacteria, fungi, and *azotobacter*. A principal component analysis (PCA) was applied to select soil quality indicators of chemical and biological properties for determining the MDS. In the PCA method, eigenvalues indicate which PCs account the most for the variability of the data. The selection of the MDS based on the work of Andrews et al. (2002) was done in a way that only PCs with eigenvalues ≥1 were considered. Then within each PC, the indicators within 10% of the highest factor loading were selected for use in MDS. When more than one parameter was retained under a single factor, a Pearson correlation analysis was used to check whether other indicators should be removed.

If the highly loaded factors were not correlated, then each was considered important, and thus, retained for the SQI calculation. Among the well-correlated variables (> 0.70), the variable with the highest factor loading was chosen for the SQI (Andrews and Carroll 2001; Masto et al. 2008).

For the indicators retained in the MDS, a standard scoring function (SSF) was used to normalize soil parameters by assigning scores ranging between 0 and 1 using the linear scoring method. The selected indicators in the MDS were considered “more is better” for indicators that their higher values had a positive effect on the soil quality (Eq. 2), and “less is better” for the indicators that their lower values had a positive effect on the soil quality (Eq. 3), (Andrews et al. 2002; Askari and Holden 2014).

$$L\,(Y) = \frac{X - L}{U - L} \quad (2)$$

$$L\,(Y) = 1 - \frac{X - L}{U - L} \quad (3)$$

where L (Y) is the linear score varying from 0 to 1, X is the soil indicator value, L is the minimum value, and U is the maximum value of each soil indicator observed between different treatments (Masto et al. 2008).

Soil quality indicators' weight in the MDS was calculated using the factor analysis (FA) approach based on the communality of each indicator. Communality describes the proportion of variance of each MDS indicator data in each component of the PCA model. Weight values for every indicator were calculated from the proportion of communality of each indicator to the sum of communalities examined in the MDS method (Johnson and Wichern 2002).

Calculation of the soil quality index (SQI)

After the remaining indicators in MDS were scored and weighted, the soil quality index was calculated using Eq. 4 (Doran and Parkin 1994):

$$SQI = \sum_{i=1}^{n} Wi \times Ni \quad (4)$$

where Wi is the weighing value of each indicator, Ni is the indicator score, and n is the number of indicators in the MDS.

Data analysis

One-way ANOVA and Duncan's multiple range tests were used to determine significant differences of means between treatments ($P < 0.05$) by SAS (9.1) software. For statistical PCA, correlations, and scoring functions analyses, the SPSS version 16.0 was used.

Results and discussion

BMP properties and assessment of fertilizing potential

The main chemical properties of BMP are reported in Table 2. The BMP pH value was within the permissible limits for organic fertilizer category A (5.3 < 8). The BMP total organic carbon and total N concentrations were 51.9% and 5.1%, respectively, resulting in a C:N ratio value of 10.1, all values in the respective recommended ranges (total organic carbon > 16%, total N ≥ 0.5%, and C:N ratio < 20).

Total concentration (mg kg^{-1}) of the analyzed elements was K (7333.0) > Fe (6378.0) > Zn (76.0) > Pb (12.0) > Cu (8.6) > Mn (3.4), whereas the total Cd and Ni concentrations were below the instrumental detection limit (0.002 mg kg^{-1}).

The results of potential environmental risk analysis by hazard quotient (HQ) showed that HQ values for all the studied elements < 1, indicating that the BMP could be used as fertilizer with no risk to human health (Table 2).

Table 2 Values of pH, C:N ratio and the total concentration of organic carbon, nitrogen, K, Fe, Cu, Mn, Pb, Ni, Cd, and Zn and their hazard quotient values of BMP used in the experiments

Properties	**Value**	**Standard limit**	**Calculated HQ**
pH	5.33±0.01	≤ 8.2	0.65
Organic carbon (%)	51.90±0.17	≥ 16	-
Total nitrogen (%)	5.09±0.06	≥ 0.5	-
C:N	10.20±0.15	≤ 20	0.5
Total Fe (mg kg^{-1})	6378.00±135.00	-	-
Total Cu (mg kg^{-1})	8.63±0.80	400	0.02
Total Mn (mg kg^{-1})	3.45±0.24	-	-
Total Zn (mg kg^{-1})	76.5±26.50	700	0.1
Total K (mg kg^{-1})	7333.00±80.60	≥ 8300	-
Total Pb (mg kg^{-1})	12.78±2.47	150	0.08
Total Ni (mg kg^{-1})	BDL[1]	62	NC[2]
Total Cd (mg kg^{-1})	BDL[1]	3	NC[2]

[1]Below detection limit and [2]Not calculated. Values are means of three replicates ± the standard error

Soil pH

Significant (P < 0.05) acidification of the soil amended with BMP at all application rates as compared to the control soil was observed (Table 3). Soil acidification after the amendment of calcareous soils by blood powder was previously reported (Koenig and Johnson 1999), and it could be due to the N mineralization and nitrification as well as the release of organic acids from decomposing blood matrix by microbial activity (Garg et al. 2006; Lazcano et al. 2008; Suthar 2009).

Table 3 Values of pH, the concentration of total organic carbon (TOC), total nitrogen (TN), NH_4^+, NO_3^-, available K and P values of control and blood meal powder (BMP) amended soils after 70 days of incubation[1]

		BMP treatments		
Parameter	**Control**	**1.5 (ton ha^{-1})**	**3.0 (ton ha^{-1})**	**6.0 (ton ha^{-1})**
pH	$7.85±0.02^{a}$	$7.75±0.02^{b}$	$7.71±0.01^{b}$	$7.59±0.02^{c}$
Available P (mg kg^{-1})	$4.67±0.44^{bc}$	$3.67±0.22^{c}$	$6.81±0.44^{a}$	$5.52±0.54^{ab}$
TOC (%)	$0.85±0.01^{c}$	$0.91±0.02^{b}$	$0.92±0.01^{b}$	$0.98±0.01^{a}$
Available K (mg kg^{-1})	$290±2.31^{a}$	$248.00±2.85^{b}$	$249±9.42^{b}$	$248.00±1.53^{b}$
TN (%)	$0.33±0.01^{b}$	$0.28±0.02^{bc}$	$0.24±0.02^{c}$	$0.71±0.02^{a}$
NH_4^+ (mg kg^{-1})	$15.20±1.34^{a}$	$9.33±0.67^{b}$	$7.38±1.02^{b}$	$2.13±0.19^{c}$
NO_3^- (mg kg^{-1})	$49.70±1.71^{d}$	$96.20±2.71^{c}$	$121.20±5.84^{b}$	$222.90±4.62^{a}$

[1]Values are the means of three replicates ± the standard error. Mean values with different superscripts were significantly different (P < 0.05) according to Duncan's multiple-range test.

Total organic carbon and nitrogen

The total soil organic carbon (SOC) content increased in all treatments compared to control soil proportionally to the amendment rate (Table 3), whereas the total

N only significantly increased by 115% at the highest amendment rate. These results were in agreement with those of Cayuela et al. (2009) and Ciavatta et al. (1997). SOC content, as the measurable component of soil organic matter, influences many soil characteristics including cation exchange capacity, nutrient and moisture holding capacity, structural stability, air, and water infiltration ability, and biological activity (Bot and Benites 2005; Lehmann and Kleber 2015). Its preservation and propagation are key factors for the health, fertility, and usability of soil (Travnikova et al. 2002). Given these cases, BMP with the potential to increase SOC can be a very good option for organic carbon management in sustainable agriculture.

Available concentration of macronutrients

Compared to the control soil, P availability increased in the soil amended at the rates of 3.0 and 6.0 tons BMP ha^{-1} (Table 3). This result was interesting because BMP was able to increase the P solubility in the studied calcareous soil in which P generally from insoluble forms (Bertrand et al. 1999), likely due to the BMP richness in organic ligands that could outcompete the soil P adsorption sites. Manure application to calcareous soils can increase P phytoavailability due to P dissolution and by prevention of P sorption and precipitation on calcite by the released of organic acids during organic matter decomposition and complexation of multivalent cations (Schneider and Haderlein 2016; Weyers et al. 2017; Gerdelidani and Hosseini 2017; Safian et al. 2020). Perassi and Borgnino (2014) reported that the humic acid resulting from the decomposition of organic matter could decrease the P adsorption in soil, as both ions compete for the adsorption onto the Ca-montmorillonite surface sites. The BMP amendment significantly decreased the NH_4^+-N and significantly increased the NO_3^--N concentrations compared to the control soil. These results could be explained by the depletion of N-rich proteinaceous moiety (Said-Pullicino et al. 2007) and ammonia nitrification (Khwairakpam et al. 2009). These results parallel the previously reported fast N mineralization in blood powder (Hartz and Johnstone 2006; Mondini et al. 2008), with transient ammonia increase lasting 1-2 weeks after soil amendment (Koller et al. 2002; Agehara and Warncke 2005; Hartz and Johnstone 2006). Carabassa et al. (2018) reported that NH_4^+-N undergoes rapid nitrification of organic matter decomposition in calcareous soils. Generally, the application of chemical and organic fertilizers stimulates the activity and increases the abundance of ammonia-oxidizing bacteria activity (Norton and Ouyang 2019).

The K availability was significantly lower in all the BMP-amended soils than in the control soil (Table 3). Potassium pools in soil are mainly controlled by clay mineralogy, texture, moisture level, cation exchange capacity, and pH value, and in agricultural soils by fertilization and cropping regimes (Simonsson et al. 2007; Samadi 2010). The leveling of K availability to similar concentrations in the studied K-depleted soil regardless of the BMP amendment rate aggress with previous results of increased K fixation in K-depleted clayey soils (Kansal and Sekhon 1976; Sparks 1987; Samadi 2010) due to stronger K forcing in inner lattice positions (Graham and Lopez 1969).

Micronutrient availability

The availability of Cu significantly decreased by 13% only in the 6.0-ton ha^{-1} BMP treatment compared to the control soil (Table 4), likely due to the combined effects of the high clay content (Alvarez-Ayuso and Garcıa-Sánchez 2003), Cu precipitation a carbonate in this calcareous soil (Kumpiene et al. 2008), and high Cu affinity for the by soil organic matter (Agbenin 2010). An abundant organic matter-bound Cu fraction has been previously reported (Balasoiu et al. 2001). The Mn and Fe availability slightly increased in all treatments compared to the control soil, though not significantly (Table 4).

Table 4 Available concentrations (mg kg^{-1}) of Cu, Mn, Fe, Zn, and Ni values of control and blood meal powder (BMP) amended soils after 70 days of incubation

		BMP treatments		
Parameter	**Control**	**1.5 (ton ha^{-1})**	**3 (ton ha^{-1})**	**6 (ton ha^{-1})**
Available Cu	2.15±0.11^{a}	2.17±0.07^{a}	2.11±0.01^{ab}	1.87±0.05^{b}
Available Mn	4.77±0.41^{a}	6.22±0.06^{a}	6.18±0.19^{a}	5.44±0.86^{a}
Available Fe	2.61±0.15^{a}	2.97±0.18^{a}	2.91±0.11^{a}	2.71±0.25^{a}
Available Zn	0.15±0.01^{b}	0.18±0.01^{ab}	0.21±0.01^{a}	0.18±0.01^{ab}
Available Ni	0.12±0.01^{c}	0.26±0.02^{a}	0.18±0.01^{b}	0.21±0.01^{b}

Values are the means of three replicates ± the standard error. Mean values with different superscripts were significantly different ($P < 0.05$) according to Duncan's multiple-range test.

While the Mn availability reflected its low Mn concentration in the BMP (Table 2), the lack of effects on Fe availability could depend on the high concentrations of Ca^{2+} and bicarbonate in the studied calcareous soils (Schenkeveld and Kraemer 2018). The Fe in BMP is mainly present as Fe^{2+} in the porphyrin rings of hemoglobin, and it is rapidly oxidized to Fe^{3+} after entering the soil solution. Therefore a transient increase of Fe availability after soil amendment before its stabilization in inorganic mineral phases cannot be excluded (Lindsay 1979). Kalbasi and Shariatmadari (1993) reported an increase in Fe availability shortly after the BMP amendment of soil. The Zn and Ni availability increased in all treatments compared to control soil, with the highest increase for the 3.0 and 1.5 ton ha^{-1} treatments, respectively (Table 4). This result could be ascribed to the increased concentration of organic ligands, which can lead to the formation of soluble Zn organic complexes (Smith 2009; Al Chami et al. 2013). The BMP is rich in low molecular weight organic compounds rich in amine, carboxyl, and phenolic functional groups with stronger chelating abilities towards Zn solubilization (de la Fuente et al. 2011) than stable humified organic matter (Smith 2009). Madrid et al. (2007) reported an increase in DTPA-extractable Ni after the application of municipal solid waste compost at a rate of 2.1, 2.1, and 1.8 kg m^{-2} to three consecutive tomato, zucchini, and green pepper crops are grown on sandy soil.

Soil respiration, soil enzyme activities, and culturable microbial community

The soil respiration was not significantly changed by soil amendment with BMP, whereas the substrate-induced respiration increased in amended soils compared to the control soil, significant only for the 3.0 ton ha^{-1} treatment (Table 5). The differences observed between soil basal respiration and substrate-induced respiration towards BMP amendment could be explained by the fact that while basal respiration depends on the availability of soil organic carbon, substrate-induced respiration indicates the activity of soil microorganisms responding to glucose addition (Ghosh et al. 2004). Overall, the basal respiration results were in agreement with previous findings on soil respiration during the decomposition of labile C (Wang et al. 2011; Kittredge et al. 2018; Marzi et al. 2020), and with those of Koenig and Johnson (1999) who reported that BMP incorporated into the soil was rapidly decomposed by the bacteria in calcareous soils.

The result showed there is no clear relationship between basal respiration and microbial population. In the study, soil respiration was measured at the end of the experiment (70th day).

Table 5 Values of basal respiration (BR), substrate-induced respiration (SIR), enzyme activities, and abundance of microorganisms values of the control and blood meal powder (BMP) amended soils after 70 days of incubation[1]

Parameters	Control	BMP treatments 1.5 (ton ha^{-1})	3 (ton ha^{-1})	6 (ton ha^{-1})
BR (mg CO_2-C kg^{-1} 24 h^{-1})	101±6.77^{a}	107±6.80^{a}	107±9.0^{a}	101±5.90^{a}
SIR (mg CO_2-C kg^{-1} 24 h^{-1})	706±46^{b}	671±47^{b}	1025±77^{a}	777±77^{b}
Invertase (µg glucose g^{-1} soil h^{-1})	425±10^{b}	412±24^{b}	501±24^{a}	415±24^{b}
Cellulase (µg glucose g^{-1} soil 24 h^{-1})	90±0.82^{b}	93±4.10^{ab}	93±2.90^{ab}	100±1.85^{a}
Protease (µg tyrosine g^{-1} soil h^{-1})	298±2.80^{a}	226±24^{a}	256±22^{a}	242±29^{a}
Alkaline phosphomonoesterase[1] (µg P.N.P g^{-1}soil h^{-1})	254±11^{a}	251±11^{a}	262±11^{a}	252±0.55^{a}
Acid phosphomonoesterase[1] (µg P.N.P g^{-1}soil h^{-1})	102±5.10^{a}	101±8.80^{a}	124±10^{a}	101±5.90^{a}
Urease (µg NH_4-N g^{-1} soil h^{-1})	7.2±0.61^{ab}	6.5±0.46^{b}	8.6±0.46^{a}	6.4±0.42^{b}
Dehydrogenase[2] (µg TPF g^{-1} soil 24 h^{-1})	0.87±0.05^{c}	1.2±0.09^{b}	2.0±0.11^{a}	1.8±0.05^{a}
Fungi[3] (CFU g^{-1} dry soil^{-1})×10 ^{3}	23±3.30^{d}	63.3±6.70^{c}	100±10^{b}	160±17^{a}
Bacteria[3] (CFU g^{-1} dry soil^{-1})×10 ^{3}	860±32^{d}	1600±113^{c}	3233±145^{b}	8260±74^{a}
Azotobacter[3] (CFU g^{-1} dry soil^{-1})×10 ^{3}	2750±75^{c}	3927±49^{b}	5127±332^{a}	3320±327^{bc}

[1]Values are the means of three replicates ± the standard error. Mean values with different superscripts were significantly different ($P < 0.05$) according to Duncan's multiple-range test, [1]P-N.P indicates para-nitrophenyl phosphate; [2]TPF indicates triphenyl formazan; [3]CFU indicates colony forming units.

Research in the past have been showed that the C mineralization trends could be divided into two phases: A first C-dominated phase with a high respiration rate that lasted about a month, and a second phase in which the decomposition continued at a slower rate until the respiration rate returned to the basal values. (Koenig and Johnson 1999; Wang et al. 2011; Kittredge et al. 2018; Marzi et al. 2020). Another reason for the no clear relationship between basal respiration and microbial population in this study can be due to signalizing optimal carbon consumption by the soil microbes and their adaptation to the soil conditions at the end of the experiment. Soil microbes in suitable conditions assimilate more carbon and thus produce less carbon dioxide in their respiration process. In unsuitable soils, they require more energy to survive, so more carbon is liberated in the form of carbon dioxide, as a smaller portion is integrated into the biomass (Feketeová et al. 2021). Furthermore, the culturability of soil microorganisms generally increases after the addition of nutrients. However, the culturable fraction of soil microorganisms represents only 1-5% of total soil microorganisms. No correlation between culturable microorganisms and soil respiration has been reported for different soils (e.g. Johnston and Sibly 2018; Kaneda et al. 2019; Smith et al. 2021). The increase of the substrate-induced respiration with increasing BMP amendment rates indicated that the microbial activity was limited by available C in soil, and it was stimulated by the added fresh energy substrates. This result paralleled the significant increase of the soil dehydrogenase activity in all the amended soils also in function of the amendment rate (Table 5). This result confirmed the link between soil dehydrogenase activity, organic C availability, and microbial activity in soil (Chodak and Niklińska 2010; Moeskops et al. 2010; Romero et al. 2010). Another factor that also explains the dehydrogenase response could be the fact that the studied soil was calcareous. Zhang et al. (2010) reported that as well the dehydrogenase activity was strongly correlated to carbonate content and organic C

availability, and Ros et al. (2003) reported that the optimum pH value for dehydrogenase activity in soil is in the neutral sub-alkaline range. The total culturable bacterial and fungal biomass significantly increased in all the amended soils compared to the control (Table 5), consistently with the BMP application rates. The *azotobacter* abundance significantly increased in all treatments compared to the control soil, particularly in the 1.5- and 3-tons BMP ha^{-1} treatments (Table 5). *Azotobacter* is a non-symbiotic, gram-negative, heterotrophic, aerobic, and free-living bacterium, capable of N_2 fixation, and producers of plant biostimulants such as hormones, vitamins, etc. (Singh et al. 2017). The results suggest that the BMP-derived labile organic C stimulated *azotobacter* population as previously reported by Sinegani and Sharifi (2004). *Azotobacter* proliferation could also have been favored by the sub-alkaline pH value of the soil (Lenart 2012), and the lower *azotobacter* abundance in the 6-ton BMP ha^{-1} treatment compared to the control can be attributed to the significant soil acidification in this treatment (Table 3). This finding indicates that BMP can be a good choice to increase the biological nitrogen fixation by *azotobacter* via increasing their population in the soil. These results confirm previous findings on the increase of microbial biomass soil following organic amendments (Rawls et al. 2003; Carter 2007) and parallel the increase of substrate-induced respiration values and soil dehydrogenase activity. Positive significant correlations ($P < 0.01$) of dehydrogenase activity with culturable fungi, bacteria, and *azotobacter* populations found in this study, are in agreement with previous studies (Pascual et al. 2000) and confirmed that fresh additions of labile organic matter activate soil microorganisms that accelerate the soil organic matter decomposition. Concerning the soil enzyme activities involved in C decomposition and N and P mineralization, minor changes were observed at the end of the incubation period, except for invertase and urease activities in the 3.0 ton BMP ha^{-1} treatment, and for cellulase activity in 6.0 ton BMP ha^{-1} treatment, which was significantly higher than in control soil (Table 5). The fact that no generalized increase of most of the measured enzyme activities was observed at the end of the incubation period, could be explained by the fact that being an energy-demanding process, the synthesis of enzymes by soil microorganisms after fresh addition of labile C is transient and rapidly revert after exhaustion of the energy substrates (Renella et al. 2007), and by the fact that the BMP organic matrix consists mainly of easily degradable protein and simple sugars. According to the economic theory of enzyme production in soil (Allison and Vitousek 2004), the significant increases of cellulase and invertase could be due to the large N availability in BMP-amended soils, whereas the significantly higher urease activity observed in the 3.0 ton BMP per ha treatment could be due to the formation of urea pool upon protein and heme-group degradation. The insignificant change in phosphomonoesterase activity could be due to enzyme feedback inhibition caused by the high inorganic P availability (Olander and Vitousek 2000).

Soil quality index (SQI)

The relationship between the eigenvalue and the principal components (PCs) is shown in Fig. 1. With an increase in PC, there is a corresponding decrease in eigenvalue. As shown in Table 6, five PCs had an eigenvalue >1 with a cumulative variation of 87.6%, with the eigenvalue and variance explained decreased from PC_1 to PC_5. The high loading factors in the first principal component (PC_1) were NH_4^+ (-0.941), with NO_3^-, pH, total organic carbon, dehydrogenase activity, the abundance of fungi, and bacteria had a loading value within 10% of the highest factor. The NH_4^+ concentration and pH value showed a significant positive correlation with each other. Thus, NH_4^+ was selected in PC_1 because of its highest loading factor (Table 6).

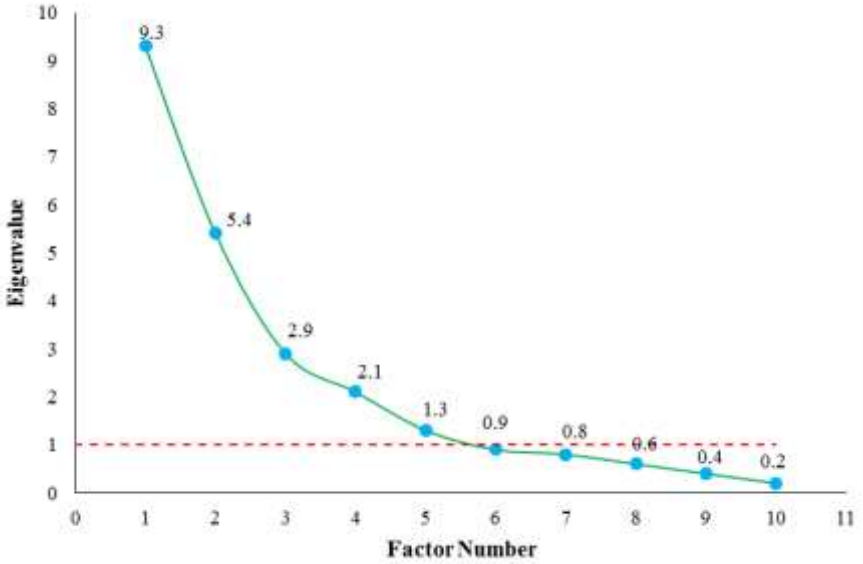

Fig. 1 Relationship between eigenvalue and principal components (scree plot)

Table 6 Results of principal component analysis (PCA) of soil biology and chemical properties under different treatments[1]

Properties	PC1	PC2	PC3	PC4	PC5
pH	**-0.930**	0.245	0.032	0.020	0.122
P	0.479	0.464	0.602	-0.05s1	-0.069
K	-0.784	-0.179	0.436	-0.122	0.271
TN	0.585	-0.688	0.182	0.148	0.256
NH_4^+	**-0.941**	0.210	0.052	0.100	0.061
NO_3^-	**0.923**	-0.330	0.074	0.072	0.060
TOC	**0.896**	-0.126	-0.065	-0.062	0.284
Ni	0.509	0.041	**-0.719**	0.079	0.073
Cu	-0.605	0.478	-0.411	-0.039	0.291
Zn	0.625	0.468	-0.188	-0.363	-0.152
Fe	0.172	0.512	-0.524	-0.249	**0.517**
Mn	0.375	0.546	-0.455	-0.299	0.297
BR	0.004	0.237	-0.239	**0.898**	-0.187
SIR	0.368	0.607	0.529	-0.294	-0.050
Invertase	0.214	**0.843**	0.281	-0.041	0.106
Alk. Phos.	0.057	0.492	0.023	0.804	0.235
Acid Phos.	0.263	**0.813**	0.182	0.089	-0.003
Urease	-0.086	0.667	0.399	0.151	-0.041
Cellulase	0.717	-0.141	0.064	0.399	0.365
Dehydrogenase	**0.891**	0.382	0.224	0.014	-0.013
Protease	-0.444	0.003	0.579	0.026	**0.520**
Fungi	**0.912**	-0.233	0.148	-0.079	-0.005
Bacteria	**0.872**	-0.395	0.214	0.081	0.091
Azotobacter	0.389	**0.760**	-0.176	0.089	-0.316
Eigen value	9.29	50.40	2.90	2.10	1.33
Variation (%)	38.70	22.48	12.08	8.75	5.56
Cumulative variation (%)	38.70	61.19	73.27	82.02	87.56

[1]Bold factor loadings are considered highly weighted and underlined bold loadings represent the minimum dataset for calculating the soil quality index (SQI). TN = total nitrogen; TOC = total organic carbon; BR = basal respiration; SIR = substrate-induced respiration.

Similarly, invertase, acid phosphatase, and *azotobacter* were highly weighted under PC_2, and the three variables were significantly correlated with each other. Thus, invertase (highest loading factor) was retained under PC_2. The Ni concentration and basal respiration rate were highly weighted variables under PC_3 and PC_4, respectively, and were both retained. The high loading factor in PC_5 was protease and Fe had values within 10% of this value, but protease activity was not correlated with Fe concentration, therefore both protease activity, and Fe concentration were kept in the MDS under PC_5. Therefore, the variables retained in MDS for evaluating soil quality were: NH_4^+, NO_3^-, total organic carbon, fungal and bacterial abundance, dehydrogenase, invertase and protease activities, Ni and Fe concentrations, and basal respiration rate. All parameters in the MDS were considered "more is better" and were calculated with linear scoring functions (Eq. 2). The communalities and weights of the MDS showed that invertase activity had the highest and BR had the lowest contribution (Table 7).

Table 7 Results of estimated communality and weight of soil quality indicators

Properties	COM	Weight
TOC	0.907	0.092
NH_4^+	0.901	0.092
NO_3^-	0.980	0.100
Fe	0.947	0.097
Ni	0.908	0.093
BR	0.581	0.059
Bacteria	0.948	0.097
Fungi	0.945	0.096
Dehydrogenase	0.969	0.099
Invertase	0.993	0.101
Protease	0.731	0.074

1COM = means communality of each soil attribute; TOC = total organic carbon; BR = basal respiration.

For the comprehensive evaluation of SQI, the calculation of SQI is given by the following equation (5) in each treatment.

$$SQI = 0.101S_{Invertase} + 0.100S_{NO3} + 0.099S_{Dehydrogenase} + 0.097S_{Bacteria} + 0.097S_{Fe} + 0.096S_{Fungi} + 0.093S_{Ni} + 0.092S_{NH4} + 0.092S_{Total\ organic\ carbon} + 0.074S_{Protease} + 0.059S_{Basal\ respiration} \quad (5$$

The SQI value in the studied BMP treatments showed the following values: 0.59 (6.0 ton ha^{-1}) ≈ 0.53 (3.0 ton ha^{-1}) > 0.39 for the 1.5 ton ha^{-1} ≈ 0.26 for the blank (Fig. 2).

This result shows that the use of BMP in agricultural soil can have a positive effect on soil quality.

This study showed that it is possible to define a minimum dataset for the calculation of SQI using a PCA approach. In our opinion, the proposed methodology for the identification of the parameters is relatively related to soil quality and simple to be measured that could be useful for the assessment of the effects and optimization of the use of novel fertilizers such as BMP.

Conclusion

Our study confirmed that BMP produced by abattoirs has high fertilizing potential. The results showed that

BMP stimulated the proliferation of bacteria and fungi and increased cellulase, invertase, and urease activities. An increase in microbial activity and enzymatic activities involved in C and N mineralization can improve the overall soil quality by increasing the soil organic matter stability. The methodology used for selecting an MDS for the calculation of the soil quality index, allowed us to identify soil parameters that are relevant to the functionality of soil in the agro-ecosystems.

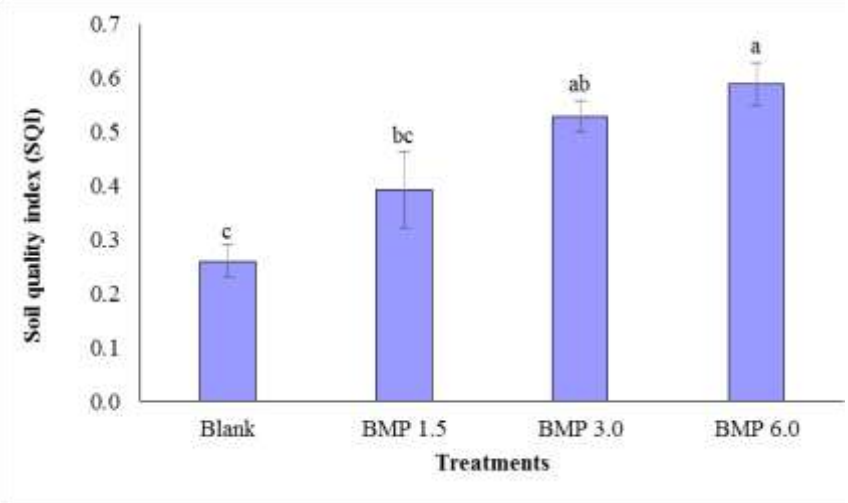

Fig. 2 Soil quality index under different treatments

Different superscript letters indicate significant differences at $P < 0.05$ level according to Duncan's multiple-range test

The SQI based on the MDS of simple and inexpensive indicators indicated that a significant increase of BMP in the studied soil was achieved at 3.0 tons BMP per ha^{-1} treatment, a dose very optimal for use in fields and degraded lands without environmental hazards. Although the BMP undergoes sterilization during the production stage, the persistence of human and animal pathogens should be always tested in the finished BMP before its use as fertilizer to prevent any potential infection or impact on the environmental biological quality in the long term. Furthermore, the effects of the waste in terms of improving soil fertility, environmental health, and crop productivity should be also evaluated in large-scale field experiments over time.

Author contributions Mehran Gholami conducted the experiments, interpreted the data, contributed to the writing of discussion and compiled the manuscript. Zahed Sharifi interpreted the data, included writing of discussion, edited the manuscript, and supervised the work. Giancarlo Renella contributed to the discussions and advised the work.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest associated with this study.

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